RESEARCH ARTICLE

Exploratory Investigation of Genetic Associations with Basal Cell Carcinoma Risk: Genome-Wide Association Study in Jeju Island, Korea

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Abstract

 Aim: Little is known about the genetic associations with Basal cell carcinoma (BCC) risk in non-Caucasian populations, in which BCC is rare, as in Korea. We here conducted a pilot genome-wide association study (GWAS) in 12 patients and 48 standard controls. Method: A total of 263,511 SNPs were analyzed with the Illumina HumanOmni1 Quad v1.0 DNA Analysis BeadChip for cases and Korean HapMap 570K for controls. Results: SNP-based analyses, based on the allele genetic model with adjustment for sex and age showed suggestive associations with BCC risk for 6 SNPs with a P-value (P < 0.0005). However, these associations were not statistically significant after Bonferroni correction: rs1040503, rs2216491, rs13407683, rs4751072, rs9891263, and rs1368474. In addition, results from gene-based analyses showed suggestive associations with BCC risk for 33 candidate genes with a P-value (P <0.0005). Consistent with previous GWAS and replication studies in Caucasian populations, *PADI6, RHOU* and *SLC45A2* were identified as having null associations with BCC (P > 0.05), likely due to the smaller sample size. Conclusions: Although this was a small-scale negative study, to our knowledge, we have conducted the first GWAS for BCC risk in an Asian population. Further large studies in non-Caucasian populations are required to achieve statistical significance and confirm these findings.

Keywords: Basal cell carcinoma - genome-wide association study - Korea

Introduction

 Basal cell carcinoma (BCC), also called basalioma, basal cell epithelioma, rodent ulcer and Jacobs' ulcer, is the most common cancer among Caucasians (Madan, 2010). BCC can grow aggressively, causing extensive tissue destruction, if left untreated. Metastasis to lymph nodes, lung, liver and bone is very rare (<0.1%) (von Domarus et al., 1984; Elghissassi et al., 2009).

 Recent insights into the molecular etiology and pathogenesis of BCC have uncovered environmental and other genetic factors that could account for the development of BCC. Genome-wide association studies (GWAS) have been used in the identification of loci associated with BCC in Caucasian populations. Recent meta-analyses of GWAS results on pigmentation and skin cancers have conclusively demonstrated and identified the genetic loci associated with skin cancer, mostly BCC in Caucasians (Gerstenblith et al., 2010). However, little is known about the genetic loci in non- Caucasian populations with lower BCC incidence.

 Although BCC has become the most common type of skin cancer today, only 2,140 newly diagnosed cases were reported in 2011, from the Korean population of approximately 50 million people (KCCR, 2014). In the present study we conducted a GWAS, as an exploratory investigation, to identify the genetic association with BCC risk in Jeju Island, Korea.

Materials and Methods

Subjects

 A total of 12 cases were recruited from patients who had been diagnosed with histologically confirmed BCC at the Jeju National University Hospital, from May 2010 to April 2011. The province of Jeju is an island located apart from the southwest corner of the Korean Peninsula. It's the number of population was 57 thousands in 2011. A total of 48 control subjects were obtained from Korean HapMap Project (http://sysbio.kribb.re.kr:8080/khapmap/index.jsp), matched to cases by sex and age. The subject pools are shown in Table 1.

 The GWAS of BCC protocol was approved by the Institutional Review Board of the Jeju National University

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Hospital. Informed consent was obtained from all patients. The study was conducted according to the Declaration of Helsinki Principles.

Genotyping

Genotyping was performed on DNA extracted from 12 cases using the Illumina HumanOmni1 Quad v1.0 DNA Analysis Bead Chip array containing 1,140,419 SNP loci with a marker distance of 1.2 kb (median) and a call rate of 99.79% (Illumina, Inc., San Diego, CA, USA). However, Korean HapMap 570K was used for 48 controls with a call rate of 99.53%.

Genomic DNA quantitation

DNA samples were checked the quality using NanoDrop® ND-1000 UV-Vis Spectrophotometer. Then samples were electrophoresed on agarose gels and samples with intact genomic DNA showing no smearing on agarose gel electrophoresis were selected for experiment. Intact genomic DNA was diluted to 50 ng/µl concentration based on Quant-iT Picogreen (Invitrogen) quantitation. Concentrations were adjusted based on these results. All prepared samples were hybridized to Illumina Infinium Human-1 microarrays according to the manufacturer's protocols.

Sample amplification and hybridization for BeadChips

The whole-genome amplification process requires 200 ng of input gDNA and creates a sufficient quantity of DNA (1000X amplification) to be used on a single BeadChip in the Infinium assay (Illumina MP1 and AMM). After amplification, the product is fragmented using a proprietary reagent (FRG), precipitated with 2-propanol (plus precipitating reagent; PM1), and resuspended in formamide-containing hybridization buffer (RA1). The DNA samples are denatured at 95°C for 20 min, then placed in a humidified container for a minimum of 16 h at 48°C allowing SNP loci to hybridize to the 50mer capture probes.

Single-base extension and staining on BeadChips

Following hybridization, the BeadChip/Te-Flow chamber assembly was placed on the temperature-controlled Tecan Flowthrough Rack, and all subsequent washing, extension and staining were performed by addition of reagents to the Te-Flow chamber.

For the single-base extension (SBE) (Infinium II) assay, primers were extended with a polymerase and labeled nucleotide mix (TEM), and stained with repeated application of STM (staining reagent) and ASM (anti-staining reagent). After staining was complete, the slides were washed with low salt wash buffer (PB1), immediately coated with XC4, and then imaged on the Illumina BeadArray Reader.

Imaging the BeadChip and data analysis

The Illumina BeadArray Reader is a two-color (543 nm/643 nm) confocal fluorescent scanner with 0.84-μm pixel resolution. The scanner excites the fluorophors generated during signal amplification/ staining of the allele-specific (one color) extension products on the BeadChips. The image intensities are extracted using Illumina's BeadScan software. SNPs were called using Illumina's GenomeStudio V2009 (Genotyping Module 1.19) (Illumina, Inc.).

Quality control

The number of identical SNPs was 970,342 with Illumina HumanOmni1 Quad v1.0 from cases, 576,292 with Korean HapMap 570K from controls and 346,710 as the overlapping contents between them. About 36% (124,744 SNPs) of these SNPs were located in introns, 30% (104,444 SNPs) in flanking 5'UTR, 30% (102,710 SNPs) in flanking 3'UTR, and the rest were functionally located in coding, 3'UTR, 5'UTR, and UTR. Out of the initial full set of 346,710 SNPs, we discarded 8,091 SNPs in the case and 1,809 SNPs in the control with SNP-wised call rate <95%, 564 SNPs solely in the control with allele frequencies deviating from Hardy-Weinberg equilibrium (HWE) (p<10-6), and 59,925 SNPs in the case and 40,883 SNPs in the control with minor allele frequency (MAF) <0.01. Therefore, the final SNP set for the genetic association analyses contained 263,511 SNPs. The Results and Procedure of Quality Control (QC) in Subjects and SNPs on the GWAS are summarized in Table 2.

Statistical analyses

We calculated genotype frequencies for each individual polymorphism and evaluated Hardy-Weinberg equilibrium to check the data quality and genotype error using 'genetics' and 'dgc.genetics' packages in the R program. Fisher's exact test statistic was used to compare the observed numbers of each genotype with those expected following chi-square distribution with a one degree of freedom (Weir, 1996).

Association analysis was performed for 263,511 common SNPs that passed QC criteria for cases and

Table 1. The Subject Pools

	Cases		Controls	
	Male	Female	Male	Female
Number of subjects	4	8	12	12
Age (mean, yr.)	63	62	60	62

Table 2. The results and procedure of quality control (QC) in subjects and SNPs on the GWAS

	No. of cases or SNPs in case group	No. of controls or SNPs in control group
QC procedure in subjects		
Subjects at the start of QC	12	90
Exclusion of subjects by		
Gender inconsistency	0	0
Low call rate (Averaged call rate)	0 (99.79%)	0 (99.53%)
Final subjects	12	48
QC in SNPs		
SNPs at the start of QC in each group	346,710	346,710
Exclusion of SNPs by		
HWE <10-6		564
Call rate <95%	8,091	1,809
MAF <0.01	59,925	40,883
Final SNPs in total subjects	263,511	

*Not mutually exclusive

Table 3. Individual SNP-based Analyses: Associations between Skin Basal Cell Cancer and SNPs with a p-value ($<5\times10^{-4}$) from a Genetic Model

SNP	Chromosome	Gene	Location	*OR (95% CI)	p-value
rs1040503	1	ATP1B1	intron	9.82 (3.12-30.90)	9.4×10^{-5}
rs2216491	2	LOC344371	flanking_5UTR	8.62 (2.70-27.50)	2.7×10^{-4}
rs13407683	2	LOC344371	flanking_5UTR	8.62 (2.70-27.50)	2.7×10^{-4}
rs4751072	10	MGMT	flanking_5UTR	10.18 (3.54-29.27)	1.7×10^{-5}
rs9891263	17	TOM1L1	flanking_5UTR	10.29 (3.26-32.50)	7.0×10^{-5}
rs1368474	19	LPHN1	flanking_5UTR	9.79 (3.01-31.86)	1.5×10^{-4}

*Allele OR adjusting for age and sex

Table 4. Individual Gene-based Analyses: Associations between Skin Basal Cell Cancer and SNPs with a p-value ($<5\times10^{-4}$)

Gene	No. of SNPs per gene	flanking_5'UTR	5'UTR	intron	coding	3'UTR	flanking_3'UTR	p-value[a]
LOC727819	31	16	0	1	0	0	14	2.94×10^{-10}
ATOH1	33	9	0	0	0	0	24	3.32×10^{-9}
LOC644681	71	38	0	0	0	0	33	9.62×10^{-9}
C20orf23	52	15	0	36	1	0	0	6.49×10^{-8}
LOC440337	43	4	0	0	2	0	37	3.63×10^{-7}
KRT8P21	30	15	0	0	0	0	15	5.89×10^{-7}
CYP3A7	11	0	0	7	0	1	3	9.98×10^{-7}
HLA-DQB1	48	43	0	0	0	2	3	1.31×10^{-6}
GPC6	156	28	0	126	1	0	1	2.48×10^{-6}
ABCC2	23	0	1	18	0	0	4	5.64×10^{-6}
FBN2	35	8	0	21	4	0	2	1.07×10^{-5}
LOC645174	36	16	0	0	0	0	20	1.39×10^{-5}
C10orf107	26	13	0	8	0	0	5	1.48×10^{-5}
XCL2	10	4	0	0	0	0	6	2.36×10^{-5}
C8orf34	95	8	1	47	1	0	38	2.41×10^{-5}
CALD1	29	7	0	20	1	0	1	3.48×10^{-5}
CDH13	430	71	0	358	0	0	1	3.79×10^{-5}
TYRP1	135	129	0	3	0	0	3	3.90×10^{-5}
LRP12	20	19	0	1	0	0	0	4.50×10^{-5}
C9orf66	12	7	0	0	1	0	4	5.91×10^{-5}
ELL2	12	2	0	8	0	0	2	5.91×10^{-5}
TANC1	26	4	0	21	0	1	0	6.25×10^{-5}
LOC728048	50	12	0	0	0	0	38	8.30×10^{-5}
TMEM26	22	3	0	3	0	1	15	8.40×10^{-5}
LOC132817	64	37	0	2	0	0	25	1.03×10^{-4}
CLCN4	23	0	0	12	0	3	8	1.12×10^{-4}
BARHL2	34	3	0	0	0	0	31	1.20×10^{-4}
TFEC	34	3	0	7	0	1	23	1.20×10^{-4}
MAPRE2	19	5	0	8	0	1	5	1.49×10^{-4}
RNF130	19	1	0	13	0	0	5	1.49×10^{-4}
LOC344371	134	129	0	0	0	0	5	1.84×10^{-4}
ABO	15	3	0	5	3	0	4	1.87×10^{-4}
PGRMC2	25	24	0	0	0	0	1	1.94×10^{-4}

[a]Fisher's exact test

controls under an allele genetic model (one degree of freedom) of logistic regression with adjustment for age and gender in 12 cases and 48 controls. And we controlled false discovery rate (FDR) to deal with multiple testing problem (Sorin, 2003). We adopted the conservative Bonferroni correction, which is the most commonly used method to adjust type1 error, α, treats each single-SNP test as an independent test. The Bonferroni threshold for significance of association was 1.89×10^{-7} (α=0.05/263,511).

Results

The results for SNP-based analyses through allele genetic model with adjustment for sex and age showed suggestive associations with BCC risk for 6 SNPs with a p-value ($p<5\times10^{-4}$) in Table 3. However, these were not statistically significant after Bonferroni correction: rs1040503 (OR, 9.82;95% CI, 3.12-30.90; p=9.4×10^{-5}),

rs2216491 (A>G) (odds ratio (OR), 8.62; 95% confidence interval(CI), 2.70-27.50; p=2.7×10^{-4}), rs13407683 (A>G) (OR, 8.62;95% CI, 2.70-27.50; p=2.7×10^{-4}), rs4751072 (OR, 10.18;95% CI, 3.54-29.27; p=1.7×10^{-5}), rs9891263 (OR, 10.29;95% CI, 3.26-32.50; p=7.0×10^{-5}) and rs1368474 (OR, 9.79; 95% CI, 3.01-31.86; p=1.5×10^{-4}). And the results for gene-based analyses showed suggestive associations with BCC risk for 33 suspected genes with a p-value ($p<5\times10^{-4}$) in Table 4.

Discussion

Using large populations with mostly Caucasian individuals, GWAS have produced sufficient evidence of the genetic associations with BCC risk (Stokowski et al., 2007; Sulem et al., 2007; Brown et al., 2008; Han et al., 2008; Stacey et al., 2008; Sulem et al., 2008; Falchi et al., 2009; Gudbjartsson et al., 2009; Nan et al., 2009a; Nan et

al., 2009b; Rafnar et al., 2009; Stacey et al., 2009; Duffy et al., 2010). However, a paucity of studies based on data from non-Caucasian deals with the genetic associations with BCC risk (Cho et al., 2001; Kim et al., 2002; Kang et al., 2007). This may be because BCC is rare among African, Asians, and Hispanic populations (Yakubu et al., 1993; Diepgen et al., 2002). Although in Korea there is a very low incidence of BCC, we conducted a pilot GWAS in patients with histology-confirmed BCC in Jeju Island, Korea.

We performed a case control study comparing the frequency of SNP alleles between the Korean BCCs and the unaffected standard controls from the Korean HapMap Project. Since the controls were collected from the same ethnic background and geographical area as the case subjects, we could assume a good match between the genetic and environmental background of cases and controls (Lewis, 2002).

Single-SNP association tests were performed with logistic regression including sex and age as covariates. There were suggestive associations of the top six SNPs having a p-value ($p<5 \times 10^{-4}$) with BCC. These associations, however, were not significant after Bonferroni correction, our pilot GWAS was thus halted before the stage of replication. On the other hand, 33 putative genes showed suggestive associations with BCC risk from gene-based analyses. Although any of the ten genes identified as BCC risk factors by several previous GWAS and replication studies in Caucasian populations (PADI6, RHOU, SLC45A2, TERT/CLPTM1L, KLF14, CDKN2A/ B, TYR, KRT5, MC1R and ASIP) (Gerstenblith et al., 2010) were not included among our results of 33 genes, PADI6, RHOU and SLC45A2 were consistently identified notwithstanding that they had null associations with BCC (p>0.05), perhaps due to the smaller sample size.

The gene PADI6, peptidylarginine deiminase type six, carrying rs7538876 and the gene RHOU, ras homolog gene family member u, carrying rs801114 both are located in short arm 1p36 and long arm 1q42 chromosome 1, respectively (Stacey et al., 2008). The mechanisms by which these two loci increase the risk for BCC are unknown (Gerstenblith et al., 2010). The gene SLC45A2, solute carrier family 45, member 2, carrying rs16891982 is located in short arm 5p13.3 chromosome 5. This is a potassium dependent sodium/calcium exchanger (Fernandez et al., 2008; Guedj et al., 2008). It has been reported that the SLC45A2 SNP rs16891982 has a significant association with subjectively measured skin color in a population of European ancestry (Graf et al., 2007).

GWASs have investigated that BCC develops by at least two pathways: one pigmentation-dependent and one pigmentation-independent. SLC45A2, TYR, MC1R and ASIP suggest that BCC and melanoma share a common development pathway via pigmentation (Fernandez et al., 2008; Nan et al., 2008; Gudbjartsson et al., 2009). An alternative possibility is they do not themselves bring to BCC development but predispose to BCC. Since replication studies adjusted for pigmentation did not show consistent associations for some SNPs on TYRP1, TYR, and ASIP genes with BCC (Nan et al., 2009a; Duffy et al.,

2010), these genes should be investigated later whether subjects carrying these SNPs are associated with the risk of BCC via pigmentation alone or via pathways independent of pigmentation.

Epidemiological studies also suggested that BCC tumorigenesis may not unique genetic alteration. Disparities in clinic-pathologic characteristics were shown between sun-exposed head/neck BCC and less-exposed truncal BCC (Pelucchi et al., 2007; Betti et al., 2010). And a direct relation between UV exposure and BCC development was found for head/neck BCC only (Ramos et al., 2004). In addition, the lower mean age at occurrence of truncal BCC appears to support that BCC of sun-protected anatomic sites may related with decreased DNA repair capacity (Wei et al., 1993).

In the near future, surgical excision resulting in mutilating defects may no longer be the first treatment option for BCC. A good substitute for it will exist with the availability of limited primary excision followed by adjuvant therapy using an immunomodulator, a proapoptotic agent and/or a cell-signaling modulator (Tilli et al., 2005; Sekulic et al., 2012; Tang et al., 2012; Macha et al., 2013). This better and novel treatments and tailored drug therapy is regarded the most likely short-term benefit from genetic association studies (Hirschhorn et al., 2002).

We explored the genetic loci showing a putative association with BCC risk. To our knowledge, this is the first GWAS for BCC risk in Asian populations. However, the sample size was too small to achieve any statistical significance. Although we conducted a null study, this might contribute to avoid publication bias in meta-analyses later convincingly confirmed or refuted of all similar studies. Further larger studies in non-Caucasian populations are required to achieve statistical significance and to confirm the findings as independent replications (Zhang et al., 2012; Malik et al., 2014). And several important variables concerning gene should be considered such as family history of skin cancers and all other cancers, occupational sun exposure, smoking, and radiotherapy.

Acknowledgements

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