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A population-based study of hedgehog pathway gene variants in relation to the dual risk of basal cell carcinoma plus another cancer

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Abstract

Introduction—A personal history of basal cell carcinoma (BCC) is associated with increased risk of other malignancies, but the reason is unknown. The hedgehog pathway is critical to the etiology of BCC, and is also believed to contribute to susceptibility to other cancers. This study

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tested the hypothesis that hedgehog pathway and pathway-related gene variants contribute to the increased risk of subsequent cancers among those with a history of BCC.

Methods—The study was nested within the ongoing CLUE II cohort study, established in 1989 in Washington County, Maryland, USA. The study consisted of a cancer-free control group (n=2,296) compared to three different groups of cancer cases ascertained through 2007, those diagnosed with: 1) Other (non-BCC) cancer only (n=2,349); 2) BCC only (n=534); and 3) BCC plus other cancer (n=446). The frequencies of variant alleles were compared among these four groups for 20 single nucleotide polymorphisms (SNPs) in 6 hedgehog pathway genes (*SHH*, *IHH*, *PTCH2*, *SMO*, *GLI1*, *SUFU*), and also 22 SNPs in *VDR* and 8 SNPs in *FAS*, which have cross-talk with the hedgehog pathway.

Results—Comparing those with both BCC and other cancer versus those with no cancer, no significant associations were observed for any of the hedgehog pathway SNPs, or for the *FAS* SNPs. One *VDR* SNP was nominally significantly associated with the BCC cancer-prone phenotype, rs11574085 [per minor allele odds ratio (OR) 1.38, 95% confidence interval (CI) 1.05–1.82; p-value=0.02].

Conclusion—The hedgehog pathway gene SNPs studied, along with the *VDR* and *FAS* SNPs studied, are not strongly associated with the BCC cancer-prone phenotype.

Keywords

skin cancer; genetics; polymorphisms; hedgehog; vitamin D receptor; fas

INTRODUCTION

Basal cell carcinoma of the skin (BCC) is the most common cancer [1]. BCC is usually treated by local excision and is rarely fatal, but a personal history of BCC is associated with increased risk of other malignancies. A prior BCC diagnosis was associated with a doubling in the risk of other malignancies [2]. In a meta-analysis of prospective studies, a prior BCC diagnosis was associated with a significantly greater risk of developing another type of cancer [3]. BCC was associated with a broad spectrum of noncutaneous malignancies [2,3]. Thus, a personal history of BCC may be a marker of a cancer-prone phenotype, but it is not known why.

The hedgehog pathway is important in regulating cell growth and differentiation in many tissues, particularly the basal stem cells of the skin. Somatic mutations in hedgehog genes are linked with sporadic BCC [4–6]. Germline mutations in *PTCH1*, which encodes for a membrane receptor protein essential for pathway regulation, causes nevoid basal cell carcinoma syndrome (Gorlin's syndrome), a disease characterized by excess risk of early onset BCC, medullo-blastomas, and other cancers [7]. The hedgehog pathway may contribute to carcinogenesis in other organs, such as the brain, prostate, lung, breast, and gastrointestinal tract [8,9].

The hedgehog pathway is activated by a ligand known as the hedgehog protein (Hh); in humans, this takes the form of sonic hedgehog (Shh), Indian hedgehog (Ihh), or desert hedgehog (Dhh) [8]. Hh binds to the transmembrane receptor Patched1 (Ptch1) or Patched2 (Ptch2) [8,9]. In the absence of Shh, Ptch1 inhibits the transmembrane protein Smoothened (Smo), but upon Shh binding to Ptch1, Smo is released into the cytoplasm, initiating a signaling cascade that activates the glioma-associated (Gli) family of transcription factors. The three Gli proteins include Gli1, a nuclear transcription factor that leads to transcription of hedgehog target genes to activate the Ras-Erk and other pathways, which in turn leads to increased cell proliferation [10]. Suppressor of fused [Su(Fu)] down-regulates the hedgehog

pathway by binding to Gli, blocking transcription of hedgehog target genes. Stimulation of the hedgehog pathway promotes carcinogenesis; pathway activators such as Shh, Smo, and Gli are oncogenic in animal models. Conversely, pathway suppressors such as Ptch1 and Sufu are anti-carcinogenic.

The present study was carried out to test the hypothesis that a common hedgehog-based mechanism of carcinogenesis contributes both to BCC and to the risk of other malignancies in BCC patients. This hypothesis was tested by measuring the associations of 20 single nucleotide polymorphisms (SNPs) in hedgehog pathway genes that encode for Hh (*SHH*, *IHH*), Patched (*PTCH2*), Smo (*SMO*), Gli1 (*GLI1*), and Su(Fu) (*SUFU*) in relation to the cancer-prone phenotype characterized by the diagnosis of both BCC and another type of cancer in the same individual.

The study was also expanded to include genes that encode proteins that cross-talk with the hedgehog pathway. *Vitamin D Receptor (VDR)* SNPs were studied due to the role of vitamin D signaling in mediating hedgehog pathway activation. In the mouse model, VDR knockout results in greater Shh expression in keratinocytes and enhanced susceptibility to skin carcinogenesis [11]. Further, Gli leads to activation of the Ras-Erk signaling cascade, resulting in cell proliferation [10]. Shunting of the Ras-Erk signaling cascade to Fas leads to apoptosis [10]. Hence, SNPs from the *FAS* gene were also investigated.

METHODS

This prospective, population-based study was embedded within the CLUE II (named after the campaign slogan of “Give us a clue to cancer and heart disease”) cohort. CLUE II is a community-based cohort established in Washington County, Maryland in 1989 [12]. In the present study, the independent variables were single nucleotide polymorphisms (SNPs) in hedgehog genes. The dependent variable was the occurrence of cancer classified according to the joint outcome of BCC and other cancer, with a cancer-free group to compare to those with cancer other than BCC, BCC only, and both BCC plus other cancer.

Study population

The CLUE II cohort was established when baseline data were collected from May through November 1989 from volunteers who were mostly residents of Washington County, Maryland, USA [12]. The campaign was designed to collect blood samples from as many adult residents as possible in the Washington County and surrounding (30-mile radius) area. Brief medical histories and blood pressures were taken, and 20 ml of blood was drawn into heparinized Vacutainers®. Specimens were refrigerated at once, and processed within 24 hours. Buffy coats were placed in storage at -70°C . During the baseline data collection, participants completed a questionnaire that included information on age, race, sex, cigarette smoking, height, weight, and years of schooling. This study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and the Medical University of South Carolina.

From the entire cohort of 30,726 participants, $n=6,589$ were selected for inclusion in the present study. This included all those with a confirmed cancer diagnosis as of 9/30/2007 (plus $n=96$ cases added 4/1/2008) and a cancer-free comparison group that was a 10% age-stratified random sample of adult CLUE II participants, plus $n=250$ controls added for a lung cancer sub-study. Once selected, BCC or other cancer diagnoses that occurred by 12/31/10 contributed to the categorization of study endpoints. This original group was selected for a study of nonmelanoma skin cancer (NMSC), but those with an NMSC diagnosis that was limited only to squamous cell carcinoma of the skin were excluded from the present study.

Genotyping was attempted for 6,227 subjects. Of these, n=263 (4.1%) with 5% genotyping failures were excluded. A further n=291 persons who were diagnosed solely with squamous cell carcinoma of the skin (SCC) were excluded from this investigation because of the central relevance of the hedgehog pathway to BCC. Among the remaining subjects, genetic heterogeneity was assessed using principal components. Comparison with the three HapMap2 populations identified n=98 (1.6%) subjects of non-Caucasian ethnic ancestry, who were excluded due to concerns about population stratification and differential risks of NMSC. To account for any residual ancestral differences, all analyses were adjusted for the first three principal components. The final study population of n=5,625 was classified into four categories: 1) cancer-free group (n=2,296) for comparison to those with a pathologically confirmed diagnosis of 2) any cancer other than BCC ("Other cancer only", n=2,349); 3) BCC with no other cancer diagnosis ("BCC only", n=534); and 4) BCC plus another type of cancer ("BCC plus other cancer", n=446).

Cancer diagnoses were ascertained via linkage to the Washington County Cancer Registry, augmented (except for NMSC) by linking to the Maryland Cancer Registry. Pathologically-confirmed NMSC diagnoses were ascertained by the Washington County Cancer Registry, including NMSC cases diagnosed both before and after study baseline in 1989.

SNP Selection

From the hedgehog pathway genes selected for study, all known non-synonymous SNPs with were selected for genotyping without regard for MAF, and tagging SNPs were selected using a minor allele frequency (MAF) cut-off of 0.05. The CEPH population (European Caucasians) data from the HapMap (CEU) was used to estimate SNP frequencies and linkage disequilibrium, as the Clue II cohort was comprised largely of Caucasians of northern European descent. R^2 was set to be >0.80 , and SNPs were excluded if they had a genotyping platform design score <0.60 . HapMap data were accessed using TaggerTM software [13], with 97 hedgehog and hedgehog-related gene SNPs identified that met these criteria.

Genomic DNA Extraction and Genotyping

Buffy coats were stored at -70°C from collection until DNA extraction. Genomic DNA was extracted from buffy coats using standard phenol/chloroform extraction procedures, followed by ethanol precipitation. The precipitated DNA was re-suspended in low-salt buffer to uniform DNA concentrations ($50\mu\text{g/mL}$) for genotyping. When previously extracted DNA was available, that DNA was ethanol precipitated and re-suspended in low-salt buffer to $50\mu\text{g/mL}$. Genotyping was attempted for n= 97 selected hedgehog gene SNPs using Illumina GoldenGate® arrays customized by the manufacturer. Among these, SNPs were excluded for the following reasons: 8 genotyping failure in 5% samples, 30 with $\text{MAF}<0.05$ (4 of these were monomorphic or quasi-monomorphic), and 9 deviated from Hardy-Weinberg equilibrium (HWE) at $p<0.05$. This left a total of 50 SNPs for analyses, 20 from hedgehog genes, 22 from *VDR*, 8 from *FAS*.

Statistical Analyses

For each SNP, an exact test was used to test whether genotypic frequencies in the cancer-free controls departed from Hardy-Weinberg equilibrium (HWE) [14]. Logistic regression was used to estimate odds ratios, confidence intervals, and p-values for the associations between each SNP and cancer risk. Odds ratios were estimated using the cancer-free group as the referent category. The statistical hypothesis tested was that minor alleles in hedgehog genes would be most prevalent in the BCC plus other cancer group, and least prevalent in the cancer-free comparison group. Given this hypothesis, the SNP screening strategy focused on the BCC plus other cancer group. The additive genetic model (SNPs coded as

having 0, 1, or 2 copies of the minor allele) was used to screen the associations with p-values < 0.05 in the BCC plus other cancer category. Analyses were carried out in the statistical environment R (<http://cran.r-project.org/>). All statistical tests were two-sided.

RESULTS

Baseline descriptive characteristics of the four study groups are summarized in Table 1. The cancer-free group was significantly younger (average age of 43 years) than the three groups that included cancer, whose average ages ranged from 57 to 62 years. The BCC plus other cancer group had a higher proportion of males (55%) compared to the other study groups (42–47%). The prevalence of ever-smokers was highest (56%) in the other cancer only group, and current smoking was less prevalent in study groups that included BCC.

Table 2 shows the additive model results for the 20 hedgehog gene SNPs, ordered by p-value for the cancer-prone phenotype (“Both BCC plus other cancer”) versus the “No cancer” comparison. All the p-values for the “Both BCC plus other cancer” versus the “No cancer” comparison were 0.18 or greater. Not only were no significant associations observed for the cancer-prone phenotype, none of the SNPs were significantly associated with the risk of only BCC or the risk of only another type of cancer.

Table 3 lists the additive model results for the 22 *VDR* SNPs, ordered by p-value for the cancer-prone phenotype (“Both BCC plus other cancer”) versus the “No cancer” comparison. The only p-value < 0.05 for the “Both BCC plus other cancer” versus the “No cancer” comparison was for rs11574085 (per minor allele odds ratio (OR) 1.38, 95% confidence limits (CL) 1.05, 1.82; p-value = 0.02). *VDR* SNP rs11574085 was not significantly associated with “Other cancer only” (per minor allele OR 1.08, 95% CL 0.91, 1.28) or “BCC only” (per minor allele OR 1.20, 95% CL 0.92, 1.57), but the ORs were consistently in the risk direction. Both rs10875695 and rs2239186 were nominally significantly associated with both the “Other cancer only” and “BCC only” groups, but not in the cancer-prone phenotype group. Both rs4516035 and rs11168287 were nominally significantly associated with the “Other cancer only” group alone.

Table 4 lists the additive model results for the 8 *FAS* SNPs, ordered by p-value for the cancer-prone phenotype (“Both BCC plus other cancer”) versus the “No cancer” comparison. Compared to the group with no cancer, the p-values for the associations with the “Both BCC plus other cancer” group were all 0.27 or greater. Similarly, no statistically significant associations were observed for the comparison of the cancer-free group to the “BCC only” and “Other cancer only” groups, respectively.

DISCUSSION

SNPs were genotyped in multiple hedgehog pathway genes, including *Hh* (*SHH*, *IHH*), patched (*PTCH2*), smoothened (*SMO*), glioma-associated (*GLI1*), and suppressor of fused (*SUFU*), in a population-based study to determine whether germ-line polymorphisms were associated with susceptibility to developing both BCC plus another type of cancer. This is a novel study question with a strong scientific rationale. However, the almost uniformly null results provide evidence against the hypothesis. SNPs were also assessed in genes that interact with the hedgehog pathway, *VDR* and *FAS*, and most of these associations were also null.

Somatic mutations of hedgehog pathway genes have been extensively studied in tumors, but there is less evidence on the associations between germline polymorphisms and risk of BCC or other cancers. Most of the available evidence focuses on *PTCH1* polymorphisms. Germ-line *PTCH1* polymorphisms have previously been studied in relation to BCC risk in three

studies [15–17], with evidence of associations between individual SNPs and BCC risk in two of these studies [15,16]. Both identified the same nonsynonymous SNP (codon 1315; rs357564) as potentially being associated with BCC. In a few studies, *PTCH* SNPs have been studied in relation to noncutaneous malignancies, such as breast [18] and colorectal [19] cancer, with mixed results. The only other hedgehog pathway gene that has been investigated for association with cancer risk is *GLII*, with the results indicating no association between either of the two studied SNPs and BCC [20].

A major limitation of the present study is that no SNPs were successfully genotyped in *PTCH1*, a key gene in the hedgehog pathway, leaving the coverage of relevant genes incomplete. Further, even in the genes that were included in the present study the coverage of allelic variants within the genes was not complete. For example, of the 20 SNPs studied in hedgehog pathway genes, the numbers of SNPs per gene were seven from *SUFU*, four from *SHH*, three from *PTCH2*, three from *SMO*, two from *GLII*, and one from *IHH*. This relatively low density sampling of SNPs leaves open the possibility that genuine associations between genetic markers in hedgehog pathway genes and cancer risk may have been missed. Thus, the relative absence of signal observed in the present study between common genetic variants in hedgehog pathway and hedgehog pathway-related genes and risk of the BCC cancer-prone phenotype could be due to incomplete pathway coverage. The results of the present study are therefore best viewed as a first step in testing this question, and future studies with more thorough pathway coverage will help to overcome this issue. Additionally, even within a cohort of more than 30,000 followed for almost two decades, the inferences for the primary hypothesis center on the 446 individuals with both BCC plus another cancer. The statistical precision would have been strengthened by a greater number of cases in this group. However, the results were not only not statistically significant for the SNPs in the hedgehog pathway genes, the observed odds ratios were in fact uniformly very near the null value of unity, indicating that if there is truly an underlying association for any of these SNPs and the BCC cancer-prone phenotype, the association with cancer risk is likely to be very weak.

A unique strength of the present investigation is the study of SNPs from multiple genes from throughout the hedgehog pathway, as opposed to one or a few genes. To our knowledge, only one previous epidemiologic study has taken a similar approach [21], and that study had a clinical endpoint (therapeutic outcomes among patients with bladder cancer) rather than risk of developing cancer. Additional strengths of the present study were that it addressed a novel question within a soundly-designed study embedded within a well-established community-based cohort. Further, genetic heterogeneity within the study population was well controlled by limiting the study to Caucasians and adjusting for potential residual ethnic differences.

The *VDR* gene encodes for the vitamin D receptor, which interacts with the hedgehog pathway [11] and has been independently proposed to have a role in skin cancer risk [22]. Thus, a *VDR* role in carcinogenesis could potentially be mediated by hedgehog signaling. The gene was well covered with tagging SNPs, which should have allowed detection of any potential associations across the entire *VDR* gene. One SNP (rs11574085) was associated with the risk of BCC plus another cancer (p-value<0.02), but even this association is not compelling given the role of chance can still not be ruled out because 22 *VDR* SNPs were genotyped. A recent study of three *VDR* gene SNPs that were not included in the present study did not observed significant associations between *VDR* SNPs and BCC [23].

In conclusion, the results of a community-based epidemiologic study did not support the hypothesis that common variants in hedgehog pathway genes may affect the increased overall cancer risk experienced by individuals with a personal history of BCC.

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Table 1

Baseline characteristics and cancer outcomes during follow-up according to a personal history of cancer by the end of follow-up: CLUE II Cohort, Washington County, MD (1989–2010).

Characteristic	No cancer		Other cancer only		BCC only		BCC plus other cancer		Total		P
	2296	100%	2349	100%	534	100%	446	100%	5625	100%	
Total											
Mean age (25 th ,75 th percentile)	43.1	(30,57)	56.7	(48,67)	59.7	(49,70)	62.5	(57,70)	51.9	(40,65)	<0.0001
Sex											
Male	963	41.9%	1041	44.3%	252	47.2%	247	55.4%	2503	44.5%	<0.0001
Female	1333	58.1%	1308	55.7%	282	52.8%	199	44.6%	3122	55.5%	
Mean BMI (25 th ,75 th percentile)	25.7	(22.0,28.4)	26.8	(23.6,29.3)	25.8	(22.9,27.9)	26.1	(23.3,28.1)	26.2	(22.8,28.7)	<0.0001
Education											
<12 y	597	26.0%	681	29.0%	148	27.7%	115	25.8%	1541	27.4%	0.24
12 y	998	43.5%	991	42.2%	215	40.3%	191	42.8%	2395	42.6%	
>12 y	701	30.5%	674	28.7%	171	32.0%	140	31.4%	1686	30.0%	
Cigarette smoking											
Never	1161	50.6%	1034	44.0%	274	51.4%	222	49.8%	2691	47.9%	<0.0001
Former	561	24.4%	830	35.3%	196	36.8%	170	38.1%	1757	31.2%	
Current	574	25.0%	485	20.6%	63	11.8%	54	12.1%	1176	20.9%	
Type of Cancer	Not applicable				Not applicable				n=2793		
Lung			294	12.5			27	6.1	321	11.5%	
Colorectal			227	9.7			49	11.0	276	9.9%	
Breast			434	18.5			71	15.9	505	18.1%	
Prostate			364	15.5			91	20.4	455	16.3%	
Melanoma			138	5.9			49	11.0	187	6.7%	
Pancreas			62	2.6			8	1.8	70	2.5%	
Bladder			93	4.0			28	6.3	121	4.3%	
Other			737	31.4			123	27.6	858	30.7%	

Column totals may vary due to missing data and column percentage may not sum exactly to 100% due to rounding.

Table 2

Odds ratios (ORs) and 95% confidence intervals (CI) for the association between n=20 hedgehog gene SNPs and risk of 1) Other Cancer Only; 2) BCC only; and 3) Both BCC plus another cancer. SNPs are ordered by p-value for the allelic trend test for the “BCC plus other cancer” versus “No cancer” comparison.

Rank	SNP	Gene	pHWE ^a	MAF ^b	Other Cancer Only		BCC Only		BCC plus Other Cancer		
					OR	95 % CL	OR	95% CL	OR	95% CL	P-value
1	rs1475335	<i>SUFU</i>	0.40	0.33	0.98	(0.89,1.06)	1.06	(0.92,1.22)	0.90	(0.77,1.05)	0.18
2	rs2242578	<i>GLII</i>	0.34	0.34	0.92	(0.84,1.01)	0.97	(0.84,1.12)	0.91	(0.78,1.06)	0.24
3	rs17393012	<i>PTCH2</i>	0.75	0.14	1.05	(0.93,1.18)	1.01	(0.83,1.22)	0.88	(0.71,1.09)	0.25
4	rs12540568	<i>SMO</i>	0.93	0.36	0.93	(0.86,1.02)	1.03	(0.90,1.18)	1.08	(0.93,1.25)	0.32
5	rs10954231	<i>SMO</i>	0.70	0.47	0.91	(0.84,0.99)	1.05	(0.92,1.20)	1.07	(0.93,1.24)	0.35
6	rs2228224	<i>GLII</i>	0.24	0.36	0.89	(0.82,0.97)	1.01	(0.88,1.16)	0.93	(0.80,1.09)	0.38
7	rs111191355	<i>SUFU</i>	0.15	0.16	1.06	(0.94,1.18)	1.03	(0.85,1.24)	1.09	(0.90,1.33)	0.39
8	rs7519592	<i>PTCH2</i>	0.72	0.18	1.00	(0.90,1.11)	0.88	(0.74,1.05)	0.93	(0.77,1.12)	0.44
9	rs111191325	<i>SUFU</i>	0.67	0.32	0.99	(0.90,1.08)	0.93	(0.80,1.07)	0.96	(0.82,1.12)	0.57
10	rs11596235	<i>SUFU</i>	0.54	0.30	1.00	(0.92,1.09)	1.04	(0.90,1.20)	0.96	(0.82,1.12)	0.59
11	rs7799059	<i>SHH</i>	0.57	0.06	1.00	(0.85,1.19)	0.94	(0.70,1.25)	1.07	(0.80,1.44)	0.64
12	rs11594179	<i>SUFU</i>	0.69	0.23	0.97	(0.88,1.07)	0.98	(0.84,1.15)	0.96	(0.81,1.14)	0.65
13	rs6953598	<i>SMO</i>	0.95	0.27	1.13	(1.03,1.23)	1.06	(0.92,1.24)	0.97	(0.82,1.14)	0.69
14	rs10786679	<i>SUFU</i>	0.13	0.26	1.00	(0.91,1.10)	0.93	(0.79,1.08)	1.03	(0.87,1.21)	0.73
15	rs6436100	<i>IHH</i>	0.34	0.17	0.93	(0.84,1.04)	1.04	(0.87,1.23)	0.97	(0.80,1.17)	0.73
16	rs7777470	<i>SHH</i>	1.00	0.42	1.04	(0.96,1.13)	1.03	(0.90,1.18)	0.98	(0.84,1.13)	0.74
17	rs2363923	<i>SHH</i>	0.28	0.34	1.00	(0.89,1.11)	1.10	(0.92,1.31)	1.03	(0.85,1.26)	0.75
18	rs7893954	<i>SUFU</i>	0.46	0.28	1.03	(0.94,1.12)	0.89	(0.77,1.04)	1.01	(0.87,1.19)	0.87
19	rs7776456	<i>SHH</i>	0.66	0.27	0.93	(0.85,1.02)	1.05	(0.91,1.22)	0.99	(0.84,1.16)	0.90
20	rs12755554	<i>PTCH2</i>	0.66	0.23	0.98	(0.89,1.08)	0.94	(0.80,1.10)	1.01	(0.85,1.20)	0.91

^aP-value for deviation from Hardy-Weinberg equilibrium;

^b minor allele frequency

Table 3

Odds ratios (ORs) and 95% confidence intervals (CI) for the association between n=22 VDR SNPs and risk of 1) Other Cancer Only; 2) BCC only; and 3) Both BCC plus another cancer. SNPs are ordered by p-value for the allelic trend test for the “BCC plus other cancer” versus “No cancer” comparison.

Rank	SNP	pHWE ^a	MAF ^b	Other Cancer Only		BCC Only		BCC plus Other Cancer	
				OR	95 % CL	OR	95% CL	OR	95% CL
1	rs11574085	0.14	0.06	1.08	(0.91,1.28)	1.20	(0.92,1.57)	1.38	(1.05,1.82)
2	rs4328262	0.06	0.43	1.06	(0.97,1.15)	0.95	(0.83,1.09)	0.87	(0.75,1.00)
3	rs3782905	0.28	0.32	0.97	(0.89,1.06)	1.08	(0.94,1.24)	0.87	(0.74,1.01)
4	rs4760655	0.27	0.37	1.02	(0.94,1.11)	0.96	(0.83,1.10)	0.88	(0.76,1.03)
5	rs4516035	0.24	0.42	0.90	(0.83,0.98)	0.95	(0.83,1.09)	1.12	(0.97,1.29)
6	rs11168287	0.74	0.49	0.91	(0.84,0.99)	0.92	(0.81,1.05)	1.10	(0.95,1.27)
7	rs757343	0.39	0.11	1.00	(0.88,1.14)	1.11	(0.90,1.37)	1.13	(0.90,1.41)
8	rs2238136	0.15	0.26	1.07	(0.98,1.17)	0.98	(0.84,1.14)	0.92	(0.78,1.08)
9	rs10875695	0.51	0.28	1.10	(1.01,1.21)	1.19	(1.03,1.38)	1.09	(0.92,1.27)
10	rs2239182	0.26	0.49	1.02	(0.94,1.11)	0.95	(0.83,1.09)	1.06	(0.92,1.23)
11	rs11168292	0.63	0.32	0.90	(0.82,0.98)	0.91	(0.79,1.05)	1.06	(0.91,1.24)
12	rs2107301	0.16	0.26	1.04	(0.95,1.14)	0.99	(0.85,1.15)	1.06	(0.90,1.25)
13	rs2853564	0.40	0.39	0.91	(0.84,0.99)	0.95	(0.83,1.09)	1.04	(0.90,1.21)
14	rs2189480	0.26	0.37	0.92	(0.85,1.01)	0.88	(0.76,1.01)	0.96	(0.83,1.12)
15	rs11568820	0.33	0.21	1.08	(0.98,1.19)	1.11	(0.94,1.30)	0.96	(0.81,1.15)
16	rs4760648	0.12	0.43	1.04	(0.96,1.13)	1.00	(0.87,1.14)	0.98	(0.85,1.13)
17	rs2239180	0.37	0.10	1.01	(0.89,1.15)	1.02	(0.82,1.26)	1.03	(0.81,1.29)
18	rs2254210	0.30	0.37	0.95	(0.87,1.03)	1.01	(0.88,1.16)	0.99	(0.85,1.15)
19	rs11574077	0.31	0.05	0.97	(0.81,1.16)	0.99	(0.74,1.33)	1.02	(0.74,1.39)
20	rs2239186	0.15	0.20	0.90	(0.81,0.99)	0.84	(0.71,0.99)	0.99	(0.83,1.18)
21	rs2248098	0.50	0.49	0.98	(0.91,1.07)	0.96	(0.84,1.10)	1.00	(0.87,1.16)
22	rs10735810	0.78	0.37	1.01	(0.92,1.09)	1.10	(0.96,1.26)	1.00	(0.86,1.16)

^aP-value for deviation from Hardy-Weinberg equilibrium;

^b minor allele frequency

Table 4

Odds ratios (ORs) and 95% confidence intervals (CI) for the association between n=8 FAS SNPs and risk of 1) Other Cancer Only; 2) BCC only; and 3) Both BCC plus another cancer. SNPs are ordered by p-value for the allelic trend test for the “BCC plus other cancer” versus “No cancer” comparison.

	SNP	pHWE ^a	MAF ^b	Other Cancer Only		BCC Only		BCC plus Other Cancer	
				OR	95 % CL	OR	95% CL	OR	P-value
1	rs9658761	0.07	0.11	1.01	(0.89,1.16)	0.93	(0.74,1.16)	1.14	(0.91,1.42)
2	rs1324551	0.11	0.49	0.98	(0.90,1.06)	0.90	(0.78,1.03)	0.93	(0.80,1.08)
3	rs2234768	0.81	0.13	1.05	(0.93,1.18)	0.90	(0.73,1.11)	0.90	(0.72,1.12)
4	rs1051070	0.26	0.12	1.11	(0.98,1.26)	1.09	(0.88,1.34)	1.10	(0.88,1.38)
5	rs983751	0.96	0.14	1.06	(0.94,1.19)	0.93	(0.76,1.13)	0.94	(0.76,1.16)
6	rs1468063	0.45	0.11	1.02	(0.90,1.17)	1.15	(0.93,1.42)	1.06	(0.84,1.33)
7	rs1977389	0.08	0.38	0.93	(0.85,1.01)	0.93	(0.81,1.07)	0.98	(0.84,1.13)
8	rs982764	0.18	0.30	1.01	(0.93,1.11)	1.04	(0.90,1.20)	0.99	(0.85,1.15)

^aP-value for deviation from Hardy-Weinberg equilibrium;

^bminor allele frequency