Abstract

Purpose: The development of second primary tumors (SPT) or recurrence alters prognosis for curatively treated head and neck squamous cell carcinoma (HNSCC) patients. The 13-cis-Retinoic acid (13-cRA) has been tested as a chemoprevention agent in clinical trials with mixed results. Therefore, we investigated whether genetic variants in the PI3K/PTEN/AKT/mTOR pathway could serve as biomarkers to identify which patients are at high risk of an SPT/recurrence, while also predicting response to 13-cRA chemoprevention.

Experimental Design: A total of 137 pathway single-nucleotide polymorphisms were genotyped in 440 patients from the Retinoid Head and Neck Second Primary Trial and assessed for SPT/recurrence risk and response to 13-cRA. Risk models were created based on epidemiology, clinical, and genetic data.

Results: Twenty-two genetic loci were associated with increased SPT/recurrence risk, with six also being associated with a significant benefit following chemoprevention. Combined analysis of these high-risk/high-benefit loci identified a significant (P = 1.54 × 10⁻⁴) dose–response relationship for SPT/recurrence risk, with patients carrying four to five high-risk genotypes having a 3.76-fold [95% Confidence Interval (CI), 1.87–7.57] increase in risk in the placebo group (n = 215). Patients carrying four to five high-risk loci showed the most benefit from 13-cRA chemoprevention, with a 73% reduction in SPT/recurrence (95% CI, 0.13–0.58) compared with those with the same number of high-risk genotypes who were randomized to receive placebo. Incorporation of these loci into a risk model significantly improved the discriminatory ability over models with epidemiology, clinical, and previously identified genetic variables.

Conclusions: These results show that loci within this important pathway could identify individuals with a high-risk/high-benefit profile and are a step toward personalized chemoprevention for HNSCC patients.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is estimated to be diagnosed in more than 48,000 individuals in the United States alone in 2010 (1). Worldwide, more than 600,000 diagnoses are made each year (2, 3). Treatment of early-stage (I and II) HNSCC with surgery with or without radiotherapy is successful in 60% to 80% of these patients, leading to a favorable long-term prognosis (4). The major challenge in these patients is the development of second primary tumors (SPT) and recurrences of the primary tumor, which develop in up to 20% of the patients within 5 years of curative treatment (5, 6). Because of this high risk in HNSCC patients, there has been a focus on retinoid-based chemoprevention to reduce or eliminate these events.

In a clinical trial of 13-cis-retinoic acid (13-cRA, also known as isotretinoin) for the prevention of SPT in 103 HNSCC patients who were disease free at time of enrollment, a significant reduction in SPTs was observed following treatment with 13-cRA (7, 8). This finding led to a larger phase III clinical trial using low-dose 13-cRA in 1,190 early-stage HNSCC patients. The results of this study showed no significant difference between incidence of SPT/recurrence in the placebo and chemoprevention arms (9). In follow-up analysis, our group identified common genotypes within
Patients diagnosed with early-stage head and neck cancer are at high risk of developing a second primary tumor or recurrence following curative-intent treatment. Toward this, retinoid-based chemoprevention has been explored as an approach to minimize these events and improve long-term prognosis. However, there are no clear biomarkers that can predict which patients are at high risk of developing a second primary tumor or recurrence, and more importantly, also predict favorable responses to 13-cis retinoic acid chemoprevention. This high-risk/high-benefit profile would go a long way toward identification of suitable candidates for chemoprevention interventions. In this study, we identified genetic predictors within a key signaling pathway, PI3K/PTEN/AKT/mTOR, that identified high-risk/high-benefit head and neck cancer patients. A risk prediction model generated from these data had a discriminatory ability of nearly 80%, suggesting that the application of genetic information to identify patients that would be candidates for chemoprevention way were included in this analysis of SPT/recurrence risk and 13-cRA response in 440 patients participating in the Retinoid Head and Neck Second Primary Trial.

Materials and Methods

Study population and epidemiologic data

Patients included in this study were stage I and II HNSCC cases enrolled in the Retinoid Head and Neck Second Primary Trial and randomized to receive either daily low dose (30 mg/d) of 13-cRA or placebo for a total of 3 years (5, 9). Patients must have remained cancer free for at least 16 weeks following surgery and/or radiation treatment to be enrolled in the trial. The primary endpoint was the development of SPT or recurrence within the 4-year follow-up. SPT was defined as a diagnosis of a new cancer with a different histologic type, cancer of identical histologic type appearing more than 3 years following treatment of the primary tumor, or discovery of a cancer separated from the primary tumor site by more than 2 cm of clinically normal epithelium. Recurrence was defined as any tumor of similar histology occurring within 2 cm or 3 years of the primary tumor. Written informed consent was obtained from all participants, and this study was approved by the University of Texas MD Anderson Cancer Center's Institutional Review Board.

Genotyping

Peripheral blood samples were collected from all participants and used for extraction of genomic DNA. Samples were stored at –80°C until use. A custom iSelect genotyping array (Illumina) was generated to query haplotype tagging and candidate functional single-nucleotide polymorphisms (SNP) from genes in 12 cancer-related pathways (22). A total of 9,465 SNPs were genotyped in 450 DNA samples blinded to patient outcome status and intervention arm following the standard Infinium II protocol (Illumina). Following quality control measures, data for 8,347 SNPs in 440 participants remained. Twenty genes within the PI3K/PTEN/AKT/mTOR signaling pathway were selected for further analysis: AKT1, AKT2, AKT3, MTOR, IRS1, IRS2, PDK1, PIK3CD, PTEN, RHEB, RPS6KA2, RPS6KA3, RPS6KA4, RPS6KA5, RPS6KA6, RPS6KB1, RPS6KB2, RPS6KC1, RPS6KL1, and TSC1. A total of 137 SNPs were genotyped from these candidate genes.

Statistical analysis

Hazard ratios (HRs) and 95% confidence intervals (95% CI) for each individual SNP and SPT/recurrence were estimated by fitting the Cox proportional hazards model while adjusting for age, gender, ethnicity, smoking status, tumor site, and tumor stage. We tested the proportional hazards assumptions for the 3 analytic approaches (placebo stratified by genotype, common genotype stratified by treatment, and variant genotype stratified by treatment). The proportionality assumptions were satisfied for with $P > 0.4$ for all 3 when all the covariates were included in the analyses. To compare
event-free durations, Kaplan–Meier curves and log-rank tests were constructed. High-risk genotypes were defined as those that significantly increased risk of SPT or recurrence for patients randomized to the placebo arm. Bootstrap resampling was used for internal validation of the 5 high-risk/high-benefit loci. Each bootstrap sample was drawn from the complete dataset with a corresponding P value for each of the 500 iterations. Combined effects of high-risk genotypes were assessed using a genetic risk score based on the main effect analysis within the placebo group and stratified by treatment group (placebo or 13-cRA). Receiver operating characteristic (ROC) curves and corresponding area under the curves (AUC) and P values were constructed for risk models, including combinations of epidemiology, clinical, and genetic factors. Bootstrap resampling was carried out 10,000 times to assess the differences between the AUCs for each of the models. Statistical analyses were done using STATA software (v10, STATA Corporation). All P values were 2-sided, and a P ≤ 0.05 was considered statistically significant.

Results

Patient population

The patient population for this study has been well described previously (5, 10, 22). Briefly, of the 440 HNSCC patients included in the analysis, 215 were randomized to the placebo arm, and 225 were selected to receive 13-cRA chemoprevention. There were no significant differences between patient characteristics of the treatment arms for age, gender, ethnicity, smoking status, years smoked, number of cigarettes per day, pack-year, alcohol consumption, tumor site, stage, and previous treatment regimens.

Genetic variation identifying high-risk HNSCC patients

To identify genetic variation that could serve as markers to identify patients at high risk for developing SPT or recurrence, we analyzed the effect of the 137 pathway SNPs in the 215 patients randomized to the placebo arm. A total of 22 SNPs were significantly associated with risk of development of SPT/recurrence. Of particular interest were the 15 genetic loci that were able to identify the majority of the population—those with the common genotype—that had an increased risk of SPT/recurrence ranging from 3.57- to 1.56-fold (Table 1). Interestingly, of these, 10 were located within TSC1. The 182 (84.7% of the population) HNSCC patients with the common genotype for the most significant genetic locus TSC1:rs7040593 had a greater than 3-fold increased risk of developing a SPT or recurrence (HR, 3.03; 95% CI, 1.37–6.67). Furthermore, these patients had a significant SPT/recurrence-free survival disadvantage compared with those with at least one variant allele (P = 0.038, Fig. 1).

In contrast, the remaining 7 genetic loci had the opposite effect on SPT/recurrence risk. For these SNPs, the variant-containing genotypes were the risk genotypes representing a minority of the population at increased risk of SPT/recurrence (Table 1). The largest increase in risk for this type of genetic loci was rs4972842, located within an intron of the gene encoding pyruvate dehydrogenase kinase 1 (PDK1) and carried by 69 of the 215 (33.7%) HNSCC patients. Patients with variant genotypes had a 5.16-fold increase in risk (95% CI, 2.21–12.03). Other significant increases in risk were observed for genetic loci in AKT2, PIK3CD, PTEN, and TSC1.

Effect of 13-cRA chemoprevention on patients at high risk

We next determined the effect of 13-cRA treatment compared with placebo for those carrying the high-risk genotypes (Table 1). Of these 22 genetic loci, 6 were also associated with a significant benefit for patients who received 13-cRA chemoprevention. Two TSC1 loci, rs4962225 and rs7035940, are in high linkage disequilibrium and resulted in a 43% reduction in SPT/recurrence following 13-cRA treatment in those with the common genotype (HR, 0.57; 95% CI, 0.37–0.88) compared with patients with the common genotype who did not receive chemoprevention. This significant decrease in SPT/recurrence incidence resulted in a greater than 17.9 month increase in event-free survival time from 75.1 months to more than 93 months (Fig. 2). Similar significant reductions in SPT/recurrence risk were associated with the common genotypes for TSC1:rs739442 and TSC1:rs7874234 (HR, 0.49; 95% CI, 0.27–0.87 and HR, 0.59; 95% CI, 0.38–0.92, respectively) following 13-cRA chemoprevention. These findings were similar to the reduction in risk when assessing the effect of the high-risk genotypes in the 13-cRA treatment arm, with the exception of TSC1:rs739442, which was not significantly associated with SPT/recurrence risk (Supplementary Table).

Carriers of the variant-containing genotypes for PIK3CD: rs4129341 and PTEN:rs1234221 had a significantly higher risk of developing SPT/recurrence compared with those with the common genotype (Table 1). These same variant genotypes were also associated with significant benefit following 13-cRA intervention. PIK3CD:rs4129341 was associated with a 52% reduction in SPT/recurrence (HR, 0.48; 95% CI, 0.26–0.87) and PTEN:rs1234221 associated with a 44% reduction (HR, 0.56; 95% CI, 0.34–0.94) in patients who received 13-cRA compared with those who received placebo.

The findings for these genetic loci with regard to response to 13-cRA were robust and remained significant (P < 0.05) for a majority of the 500 bootstrap resampling iterations for TSC1:rs739442 (80.8%), TSC1:rs4962225/rs7035940 (98.2%), TSC1:rs7874234 (87.0%), PIK3CD:rs4129341 (87.0%), and PTEN:rs1234221 (67.4%).

Combined analysis of PI3K/PTEN/AKT/mTOR pathway genetic variation

We carried out a combined analysis of the 6 genetic loci (TSC1:rs739442, TSC1:rs4962225, TSC1:rs7035940, TSC1: rs7874234, PIK3CD:rs4129341, and PTEN:rs1234221)
Table 1. Effect of PI3K/PTEN/AKT/mTOR genetic variation

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>SNP location</th>
<th>MAF (%)</th>
<th>Genotype counts (cc/cc/cc/vv)</th>
<th>Risk genotype</th>
<th>Model</th>
<th>95% CI of HR</th>
<th>P</th>
<th>MST (risk genotype)</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>HR (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>rs9521509</td>
<td>IRS2</td>
<td>Intron</td>
<td>0.44</td>
<td>76/90/49</td>
<td>Common</td>
<td>Dominant</td>
<td>1.82 (1.14–2.86)</td>
<td>0.012</td>
<td>74.0</td>
<td>0.64 (0.37–1.12)</td>
<td>0.12</td>
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<tr>
<td>rs9515119</td>
<td>IRS2</td>
<td>Intron</td>
<td>0.31</td>
<td>105/86/24</td>
<td>Common</td>
<td>Dominant</td>
<td>1.67 (1.04–2.70)</td>
<td>0.035</td>
<td>75.1</td>
<td>0.70 (0.45–1.10)</td>
<td>0.12</td>
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<tr>
<td>rs7999797</td>
<td>IRS2</td>
<td>Intron</td>
<td>0.41</td>
<td>79/94/42</td>
<td>Common</td>
<td>Dominant</td>
<td>1.59 (1.00–2.66)</td>
<td>0.050</td>
<td>81.6</td>
<td>0.77 (0.46–1.30)</td>
<td>0.31</td>
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<tr>
<td>rs10274</td>
<td>RP56K18</td>
<td>3-UTR</td>
<td>0.37</td>
<td>85/98/30</td>
<td>Common</td>
<td>Dominant</td>
<td>1.75 (1.10–2.78)</td>
<td>0.020</td>
<td>64.7</td>
<td>0.62 (0.36–1.07)</td>
<td>0.088</td>
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<tr>
<td>rs7158047</td>
<td>RP56K18</td>
<td>5-UTR</td>
<td>0.40</td>
<td>77/102/36</td>
<td>Common</td>
<td>Dominant</td>
<td>2.17 (1.01–4.55)</td>
<td>0.048</td>
<td>&gt;93</td>
<td>0.91 (0.52–1.59)</td>
<td>0.73</td>
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<tr>
<td>rs7040593</td>
<td>TSC1</td>
<td>5-FR</td>
<td>0.08</td>
<td>182/33/0</td>
<td>Common</td>
<td>Dominant</td>
<td>3.03 (1.37–6.67)</td>
<td>0.0066</td>
<td>&gt;93</td>
<td>0.73 (0.31–1.04)</td>
<td>0.081</td>
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<tr>
<td>rs3827665</td>
<td>TSC1</td>
<td>Intron</td>
<td>0.06</td>
<td>188/27/0</td>
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<td>Dominant</td>
<td>3.57 (1.41–9.09)</td>
<td>0.0075</td>
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<td>0.73 (0.31–1.05)</td>
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<tr>
<td>rs739442</td>
<td>TSC1</td>
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<td>70/100/45</td>
<td>Common</td>
<td>Dominant</td>
<td>1.56 (1.12–2.17)</td>
<td>0.0089</td>
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<td>0.49 (0.28–0.87)</td>
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<td>rs2519760</td>
<td>TSC1</td>
<td>3-UTR</td>
<td>0.41</td>
<td>71/113/31</td>
<td>Common</td>
<td>Dominant</td>
<td>1.61 (1.12–2.33)</td>
<td>0.0095</td>
<td>75.1</td>
<td>0.69 (0.40–1.19)</td>
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<tr>
<td>rs2809243</td>
<td>TSC1</td>
<td>3-UTR</td>
<td>0.37</td>
<td>86/98/29</td>
<td>Common</td>
<td>Dominant</td>
<td>1.64 (1.23–2.38)</td>
<td>0.010</td>
<td>75.1</td>
<td>0.70 (0.43–1.15)</td>
<td>0.16</td>
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<tr>
<td>rs4962225</td>
<td>TSC1</td>
<td>5-FR</td>
<td>0.20</td>
<td>137/71/17</td>
<td>Common</td>
<td>Dominant</td>
<td>1.92 (1.13–3.23)</td>
<td>0.013</td>
<td>75.1</td>
<td>0.57 (0.37–0.88)</td>
<td>0.011</td>
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<tr>
<td>rs7035940</td>
<td>TSC1</td>
<td>5-FR</td>
<td>0.20</td>
<td>137/71/17</td>
<td>Common</td>
<td>Dominant</td>
<td>1.92 (1.13–3.23)</td>
<td>0.013</td>
<td>75.1</td>
<td>0.57 (0.37–0.88)</td>
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<tr>
<td>rs10491534</td>
<td>TSC1</td>
<td>3-UTR</td>
<td>0.10</td>
<td>172/41/2</td>
<td>Common</td>
<td>Dominant</td>
<td>2.00 (1.08–3.70)</td>
<td>0.028</td>
<td>&gt;93</td>
<td>0.71 (0.49–1.03)</td>
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<tr>
<td>rs2073869</td>
<td>TSC1</td>
<td>3-UTR</td>
<td>0.13</td>
<td>159/54/2</td>
<td>Common</td>
<td>Dominant</td>
<td>1.89 (1.08–3.33)</td>
<td>0.028</td>
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<td>0.70 (0.47–1.03)</td>
<td>0.072</td>
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<tr>
<td>rs7847243</td>
<td>TSC1</td>
<td>Intron</td>
<td>0.20</td>
<td>135/73/7</td>
<td>Common</td>
<td>Dominant</td>
<td>1.72 (1.03–2.86)</td>
<td>0.036</td>
<td>76.3</td>
<td>0.59 (0.38–0.92)</td>
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<tr>
<td>rs8921119</td>
<td>AKT2</td>
<td>Intron</td>
<td>0.16</td>
<td>151/58/6</td>
<td>Variant</td>
<td>Additive</td>
<td>3.06 (1.04–8.99)</td>
<td>0.042</td>
<td>76.3</td>
<td>0.78 (0.43–1.43)</td>
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<td>rs4972842</td>
<td>PKD1</td>
<td>Intron</td>
<td>0.18</td>
<td>146/60/9</td>
<td>Variant</td>
<td>Additive</td>
<td>5.16 (2.21–12.03)</td>
<td>0.00015</td>
<td>&gt;93</td>
<td>0.74 (0.42–1.33)</td>
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<td>rs4972839</td>
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<td>5-FR</td>
<td>0.21</td>
<td>132/71/10</td>
<td>Common</td>
<td>Dominant</td>
<td>1.56 (1.08–2.26)</td>
<td>0.017</td>
<td>67.7</td>
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<td>rs4129341</td>
<td>PIK3CD</td>
<td>5-FR</td>
<td>0.18</td>
<td>145/61/7</td>
<td>Variant</td>
<td>Dominant</td>
<td>1.68 (1.04–2.71)</td>
<td>0.035</td>
<td>81.6</td>
<td>0.46 (0.26–0.87)</td>
<td>0.016</td>
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<td>rs1234221</td>
<td>PTPN1</td>
<td>5-FR</td>
<td>0.22</td>
<td>130/76/9</td>
<td>Variant</td>
<td>Dominant</td>
<td>1.55 (1.05–2.30)</td>
<td>0.029</td>
<td>75.1</td>
<td>0.56 (0.34–0.94)</td>
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<tr>
<td>rs869116</td>
<td>TSC1</td>
<td>5-FR</td>
<td>0.50</td>
<td>54/108/53</td>
<td>Variant</td>
<td>Dominant</td>
<td>1.52 (1.08–2.13)</td>
<td>0.016</td>
<td>81.6</td>
<td>0.76 (0.52–1.10)</td>
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<tr>
<td>rs4376688</td>
<td>TSC1</td>
<td>Intron</td>
<td>0.50</td>
<td>55/107/53</td>
<td>Variant</td>
<td>Dominant</td>
<td>1.45 (1.04–2.02)</td>
<td>0.030</td>
<td>81.6</td>
<td>0.71 (0.48–1.04)</td>
<td>0.076</td>
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</tbody>
</table>

NOTE: Underlined SNPs are in linkage disequilibrium, bold values are significant at P < 0.05.

*Adjusted for age, gender, ethnicity, smoking status, tumor site, and tumor stage.
that identified HNSCC patients at high risk of SPT/recurrence while also predicting response to 13-cRA (high-risk/high-benefit loci) to determine whether the cumulative effect of these genotypes within the pathway could better identify those patients at high risk of SPT/recurrence (Table 2). Because the 2 TSC1 SNPs rs4962225 and rs7035940 are in high linkage disequilibrium, we only included one of these loci in the analysis. A significant ($P = 1.54 \times 10^{-4}$) dose–response relationship was evident for SPT/recurrence risk in the placebo group. Patients with 2 or 3 high-risk genotypes (46.9% of the population) were at a 2.20-fold increased risk of SPT/recurrence (95% CI, 1.16–4.15) compared with those with 0 or 1 high-risk genotypes. This increase in SPT/recurrence risk reached to 3.76-fold in patients carrying 4 or 5 high-risk genotypes (95% CI, 1.87–7.57). These patients comprised 23% of the population had a significantly shorter median time to event of 75.1 months as well ($P = 0.0046$; Fig. 3A).

When we assessed the effect of 13-cRA compared with placebo in patients with different number of high-risk loci, patients at lowest risk of developing a SPT/recurrence (0-1 high-risk loci) received no benefit from 13-cRA. In fact, these individuals were at a significant 2.53-fold increased risk (95% CI, 1.28–5.01) of SPT/recurrence when receiving 13-cRA compared with the placebo arm patients (Fig. 3B). Carriers of 2 to 3 high-risk loci did not seem to benefit from 13-cRA treatment, with no difference in event-free survival durations ($P = 0.28$; Fig. 3C). This group of patients would carry an equal number of low-risk/low-benefit genetic loci, essentially canceling out the effect of the high-risk/high-benefit loci. However, 13-cRA had a dramatic benefit for HNSCC patients with the highest number of high-risk genotypes. Individuals with 4 or 5 high-risk genotypes had a highly significant ($P = 0.0056$) 73% reduction in SPT/recurrence (95% CI, 0.11–0.68) when receiving 13-cRA chemoprevention compared with those with the same number of high-risk genotypes who were randomized to receive placebo, resulting in a significant event-free survival difference between the 2 groups ($P = 0.010$; Fig. 3D).

**Risk prediction model for SPT/recurrence risk**

To better assess the ability of the 5 high-risk/high-benefit genetic loci in identifying HNSCC patients at high risk of SPT/recurrence, we constructed 3 risk prediction models based on the data from the placebo group. The first model included clinical and epidemiology variables associated with risk and adjusted for in our analysis: age, gender, ethnicity, smoking status, tumor site, and tumor stage (Fig. 4). The corresponding AUC was 65.5% and significantly increased to 76.3% ($P < 0.001$) with the addition of the 3 previously identified genetic loci (RXRA:rs3118570, JAK2:rs1887427, and CDC25C:rs6596428; ref. 10). There

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**Table 2. Effect of number of high-risk genotypes on SPT/recurrence risk in the placebo group**

<table>
<thead>
<tr>
<th>Number of high-risk genotypes</th>
<th>No SPT/recurrence, $N$ (%)</th>
<th>SPT/recurrence, $N$ (%)</th>
<th>aHR (95% CI)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>51 (79.7)</td>
<td>13 (20.3)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>62 (62.0)</td>
<td>38 (38.0)</td>
<td>2.20 (1.16–4.15)</td>
<td>0.015</td>
</tr>
<tr>
<td>4-5</td>
<td>26 (53.1)</td>
<td>23 (46.9)</td>
<td>3.76 (1.87–7.57)</td>
<td>2.1 × 10^{-4}</td>
</tr>
<tr>
<td>$P$ for trend</td>
<td>$1.54 \times 10^{-4}$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aAdjusted for age, gender, ethnicity, smoking status, tumor site, and tumor stage.*

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was an additional significant increase of 3.2% to 79.5% (P = 0.002) with the inclusion of the 5 high-risk/high-benefit genetic loci. These increases in the AUC with the addition of genetic information remained significant following bootstrap resampling.

Discussion

In this study, we showed that genetic variation within the PI3K/PTEN/AKT/mTOR pathway is able to identify HNSCC patients at high risk of SPT/recurrence while also predicting favorable response to 13-cRA chemoprevention. A total of 22 genetic loci were identified as high-risk genotypes, with 15 of these significant associations for the common genotype representing a majority of the patient population. A clear beneficial association between receiving 13-cRA chemoprevention compared with placebo was evident for 4 common genotypes and 2 variant-containing genotypes. Furthermore, a combinatory effect among these 6 genetic loci was observed that better stratified the patient population into those with a high-risk/high-benefit genetic background from those with a low-risk/no-benefit profile. A risk model was developed based on these results and showed excellent discriminatory ability. These results suggest that genetic variation within this important cellular signaling pathway could be used as biomarkers to select HNSCC patients to receive 13-cRA chemoprevention.

TSC1 complexes with TSC2 to regulate mTOR activity with inactivation of TSC1/2, resulting in increased mTOR signaling causing unrestrained cell growth and metabolism (13). The highest number of significant associations was for genetic variation within TSC1. Our results suggest that genetic variation within TSC1 plays an important role in modulating risk of SPT/recurrence as well as response to 13-cRA. In particular, the 4 high-risk/high-benefit genetic loci (rs739442, rs4962225, rs7035940, and rs7874234) were for the common genotype, suggesting that a majority of the patient population would be classified into this group and recommended to receive 13-cRA. These results also suggest that the common genotype for these loci is not sufficient to maintain TSC1 activity during tumor development and recurrence and thus unable to properly regulate mTOR signaling resulting in increased cell growth and survival signals.

In contrast to common genotypes for the 4 TSC1 loci, the variant genotypes of both PI3KCD:rs4129341 and PTEN: rs1234221 conferred high risk/high benefit. PIK3CD encodes for one of the catalytic domains of the phosphatidylinositol 3-kinase (PI3K) heterodimer, p110-delta. The PI3K-directed phosphorylation of PIP3 is the key initiation

Figure 3. Cumulative effect of high-risk/high-benefit genotypes (TSC1: rs739442, TSC1:rs4962225, TSC1:rs7035940, TSC1: rs7874234, PIK3CD:rs4129341, and PTEN:rs1234221) on (A) risk of SPT/recurrence in the placebo group and response to 13-cRA for individuals with (B) 0–1, (C) 2–3, or (D) 4 or 5 high-risk/high-benefit genotypes. N: number of events/total number of individuals; MST: median event-free survival time in months. Curves shown in dashed lines represent patients receiving 13-cRA.

Figure 4. Risk model for SPT/recurrence risk. ROC curves for 3 models with significantly different discrimination abilities.

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step that transmits signals from receptor tyrosine kinases and other cell surface receptors to downstream molecules such as AKT and mTOR. Overexpression of the wild-type p110-delta is oncogenic in cell culture and overexpression has been observed in several cancer sites, such as AML and glioblastoma. Although not as commonly mutated in solid tumors as p110-alpha (PIK3CA), somatic mutations have been reported in the COSMIC database (www.sanger.ac.uk/ genetics/CGP/cosmic/, v57) for head and neck cancer, as well as lung and skin tumors (23). Our results suggest that rs4129341 also behaves similar to oncogenic somatic mutations by increasing the risk of SPT/recurrence in HNSCC. PTEN is a well-known tumor suppressor and often down-regulated or inactivated in cancer, which results in unrestrained signaling through the pathway. Our results indicate that this may also be the function of the variant rs1234221 allele. Although both of the high-risk/high-benefit PI3KCD:rs4129341 and PTEN:rs1234221 genotypes were the variant-containing genotypes, they were still carried by approximately 30% and 40% of the population, respectively.

Other genes with loci shown to be significant markers for SPT/recurrence risk include IRS2, 2 isoforms of the S6 kinase (RPS6KB2 and RPS6KL1), AKT2, and PDK1. IRS2 (insulin receptor substrate 2) is an adaptor protein that links receptor tyrosine kinase signaling with downstream pathways, including the PI3K/PTEN/AKT/mTOR pathway (24, 25). Increased activity leads to increased signaling through this pathway, and previous studies have shown that IRS2 expression plays a role in metastasis (26). Our results suggest that the IRS2 common genotypes promote tumor progression and thus increase risk of SPT/recurrence in those carrying these genotypes. The S6 serine/threonine kinases are the most downstream effector of signaling through this pathway being activated by AKT and mTOR (27, 28). Increased pathway activity results in ribosomal protein S6 activation that subsequently increases protein translation. This biologic mechanism would suggest that the genetic loci significant for increased risk of SPT/recurrence would maintain signaling through this pathway or even enhance it. The 3 isoforms of the AKT kinase play an important role as a “hub” in transmitting the signals generated through this pathway. Interestingly, our group has previously shown that the variant AKT2:rs892119 was significantly associated with a poor response to chemotherapy, as well as increased risk of recurrence and dying, in a study of esophageal cancer patients (29). The results of this study are in line with these previous observations in which this variant is a marker for a poor outcome. PDK1 is a kinase that activates AKT following PI3K stimulation. The variant rs4972842 was associated with the most significant increase in SPT/recurrence risk, suggesting that this marker results in increased PDK1 activity and enhanced cell survival and proliferation signaling through increased pathway activation.

The PI3K/PTEN/AKT/mTOR pathway is complex and plays a role in regulating nearly all essential cellular processes. In this study, we genotyped variants from 20 genes that encode for the major effectors of this pathway. However, there are other proteins that function within this pathway that may also influence risk of SPT/recurrence as well as response to 13-cRA chemoprevention. Some candidate genes include TSC2, PDK2, RPTOR (Raptor), and EIF4EBP1 (4E-BP). A genome-wide approach would be necessary to identify all unknown factors but would not be possible within the current study design because of the small patient population.

In combined analysis of the 6 high-risk/high-benefit loci (TSC1:rs739442, TSC1:rs4962225, TSC1:rs7035940, TSC1:rs7874234, PIK3CD:rs4129341, and PTEN:rs1234221), a clear dose–response relationship was evident. Patients with the highest number of high-risk loci from the PI3K/PTEN/AKT/mTOR pathway received the greatest benefit from 13-cRA, providing support that a pathway-based approach to identifying HNSCC patients at high risk who would most benefit from 13-cRA chemoprevention would potentially reduce the incidence of SPT/recurrence in these HNSCC patients. Furthermore, these results suggest that potential combination therapy with a pathway inhibitor in addition to 13-cRA could be of benefit in reducing not only risk of SPT/recurrence but also response to 13-cRA as well. Several drugs have shown effectiveness in blocking signaling through this pathway, including mTOR inhibitor rapamycin (sirolimus) and its analogs, everolimus and temsirolimus, AKT inhibitor MK-2206, and the PI3K inhibitors LY-294002 and wortmannin and their derivatives (30). Unfortunately, the toxicity and side effect profiles for these agents may not be ideal for a chemopreventive agent that will be administered over many years. However, this pathway is of great interest for drug development, and targeted agents may be identified in the future that are suitable for chemoprevention. Additional studies will be necessary to test this hypothesis in vitro and to determine whether or not these agents work synergistically with 13-cRA and followed up with clinical trials to determine whether, indeed, they aid in preventing SPT/recurrence.

The usefulness of these genetic profiles within the clinical setting would be noteworthy, as a majority of HNSCC patients would carry high-risk/high-benefit genotypes. Selection of these individuals before 13-cRA chemoprevention would be able to avoid exposing patients to unnecessary and severe side effects if there is low risk of SPT or recurrence occurring and if the intervention will not provide a benefit to the patient. This is especially true for chemoprevention interventions that are taken by the patient over a long period of time. A strategy of using genetic information to inform whether or not a patient should receive chemotherapy, providing support that a pathway-based approach to identifying HNSCC patients at high risk who would most benefit from 13-cRA chemoprevention would potentially reduce the incidence of SPT/recurrence in these HNSCC patients.

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risk of SPT/recurrence. Further model validation and calibration are necessary to determine whether this model is appropriate for clinical use. However, the results are intriguing and may provide a tool to identify patients who would be candidates for 13-cRA chemoprevention.

The Retinoid Head and Neck Primary Trial was the largest randomized, placebo-controlled, double-blind phase III chemoprevention trial of its kind to investigate the effects of 13-cRA on SPT/recurrence in head and neck cancer patients. It has provided the platform to investigate the role of genetic variation in chemoprevention interventions, but it makes it difficult to assess the effects of the identified high-risk/high-benefit genotypes in an independent population. Potentially these variants could be integrated into a future prospective study. Furthermore, because of the retrospective nature of this secondary analysis, our sample size is limited and there may be slight differences in minor allele frequencies (MAF) between the 2 groups (Supplementary Table). However, the effect sizes for each genetic locus are robust (HR > 1.45 in the placebo analysis), suggesting that sample size may not be a major concern.

Long-term prognosis for early-stage HNSCC patients is highly dependent on the development of SPT or recurrence. Clinical trials of 13-cRA chemoprevention have given mixed results, but the use of molecular markers to select candidates for this intervention may have a significant clinical impact and help to prevent SPT/recurrences in a majority of HNSCC patients. Here we showed that genetic variation within the important PI3K/PTEN/AKT/mTOR pathway modulates the risk of an individual of SPT/recurrence as well as predicting response to 13-cRA chemoprevention. These results may help in design of future 13-cRA clinical trials as well as determining whether or not a HNSCC patient would be a suitable candidate for 13-cRA chemoprevention.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Genetic Variants in the PI3K/PTEN/AKT/mTOR Pathway Predict Head and Neck Cancer Patient Second Primary Tumor/Recurrence Risk and Response to Retinoid Chemoprevention

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