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ORIGINAL REPORT

Genetic Variations in the PI3K/PTEN/AKT/mTOR Pathway Are Associated With Clinical Outcomes in Esophageal Cancer Patients Treated With Chemoradiotherapy

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
The phosphoinositide-3-kinase (PI3K), phosphatase and tensin homolog (PTEN), v-akt murine thymoma viral oncogene homolog (AKT), and mammalian target of rapamycin (mTOR) signaling pathway has been implicated in resistance to several chemotherapeutic agents. In this retrospective study, we determined whether common genetic variations in this pathway are associated with clinical outcomes in esophageal cancer patients with adenocarcinoma or squamous cell carcinoma who have undergone chemoradiotherapy and surgery.

Patients and Methods
Sixteen tagging single nucleotide polymorphisms (SNPs) in *PIK3CA*, *PTEN*, *AKT1*, *AKT2*, and *FRAP1* (encoding mTOR) were genotyped in these patients and analyzed for associations with response to therapy, survival, and recurrence.

Results
We observed an increased recurrence risk with genetic variations in *AKT1* and *AKT2* (hazard ratio [HR], 2.21; 95% CI, 1.06 to 4.60; and HR, 3.30; 95% CI, 1.64 to 6.66, respectively). This effect was magnified with an increasing number of *AKT* adverse genotypes. In contrast, a predictable protective effect by *PTEN* genetic variants on recurrence was evident. Survival tree analysis identified higher-order interactions that resulted in variation in recurrence-free survival from 12 to 42 months, depending on the combination of SNPs. Genetic variations in *AKT1, AKT2,* and *FRAP1* were associated with survival. Patients homozygous for either of the *FRAP1* SNPs assayed had a more than three-fold increased risk of death. Two genes—*AKT2* and *FRAP1*—were associated with a poor treatment response, while a better response was associated with heterozygosity for AKT1:rs3803304 (odds ratio, 0.50; 95% CI, 0.25 to 0.99).

Conclusion
These results suggest that common genetic variations in this pathway modulate clinical outcomes in patients who undergo chemoradiotherapy. With further validation, these results may be used to build a model of individualized therapy for the selection of the optimal chemotherapeutic regimen.

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INTRODUCTION

An estimated 16,400 new cases of esophageal cancer (EC) will be diagnosed in 2008.[1] Surgery is one of the standard treatments for patients with resectable tumors but frequently preoperative chemoradiotherapy is used to treat both adenocarcinoma and squamous cell carcinoma ECs.[2-5] The most commonly utilized chemotherapy agents belong to fluoropyrimidines, taxanes, and platinum compounds. Unfortunately, even with the multimodal approach, current treatments result in a poor overall 5-year survival rate of 25% to 28%.[6-9]

Heterogeneity in response to chemoradiotherapy may be due to several factors, including age, sex, ethnicity, and drug-drug interactions. In addition, genetic variations in pharmacokinetic, pharmacodynamic, and drug action pathways have been shown to be important in determining sensitivity or resistance to treatment.[10] Therefore, one strategy to increase the effectiveness of chemoradiotherapy is to gain a better understanding of the influence a patient's genetic background has on response to treatment. Our group has previously reported that genetic variations in several drug action pathways were associated with variation in clinical outcomes in EC.[11] In this study, we expand those

Table 1. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Recurrence

SNP and Genotype	Any of the Three				Fluoropyrimidine + Any			
	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*
AKT1:rs3803304								
CC	60/26	1 (reference)			57/26	1 (reference)		
CG	54/25	1.44	0.75 to 2.78	.276	53/24	1.38	0.71 to 2.71	.341
GG	7/5	1.87	0.59 to 5.91	.289	7/5	1.72	0.54 to 5.54	.361
CG + GG		1.51	0.81 to 2.81	.200		1.43	0.76 to 2.72	.269
AKT1:rs2498804								
GG	50/20	1 (reference)			47/20	1 (reference)		
GT	63/30	2.04	0.95 to 4.37	.068	62/29	1.99	0.92 to 4.29	.080
TT	8/7	3.21	1.07 to 9.61	.037	8/7	3.06	1.01 to 9.28	.049
GT + TT		2.21	1.06 to 4.60	.034		2.14	1.02 to 4.50	.045
AKT1:rs2494738								
AA	105/49	1 (reference)			101/48	1 (reference)		
AG	18/7	0.94	0.36 to 2.44	.900	18/7	0.96	0.37 to 2.49	.936
AKT1:rs1130214								
GG	60/28	1 (reference)			57/27	1 (reference)		
GT	56/28	1.91	0.94 to 3.87	.073	55/28	1.91	0.94 to 3.87	.072
TT	7/2	1.83	0.34 to 9.38	.482	7/2	1.82	0.34 to 9.80	.488
GT + TT		1.90	0.94 to 3.85	.073		1.91	0.94 to 3.86	.072
AKT2:rs892119								
AA	95/33	1 (reference)			94/32	1 (reference)		
AG	25/23	3.48	1.66 to 7.28	.001	23/23	3.72	1.76 to 7.84	.001
GG	3/2	2.42	0.49 to 12.01	.280	2/2	2.57	0.51 to 12.88	.251
AG + GG		3.30	1.64 to 6.66	.001		3.52	1.73 to 7.17	.001
AKT2:rs8100018								
CC	61/24	1 (reference)			59/23	1 (reference)		
CG	47/30	1.26	0.64 to 2.46	.505	45/30	1.32	0.67 to 2.60	.430
GG	13/3	0.41	0.10 to 1.58	.193	13/3	0.44	0.11 to 1.74	.242
CG + GG		1.02	0.54 to 1.94	.944		1.09	0.57 to 2.09	.799
FRAP1:rs11121704								
CC	57/31	1 (reference)			54/30	1 (reference)		
CT	55/23	0.90	0.43 to 1.88	.783	54/23	0.94	0.48 to 1.96	.866
TT	11/3	1.79	0.45 to 7.09	.409	11/3	1.79	0.45 to 7.06	.404
CT + TT		0.97	0.48 to 1.99	.938		1.01	0.50 to 2.07	.974
FRAP1:rs2295080								
GG	47/21	1 (reference)			44/20	1 (reference)		
GT	56/29	1.81	0.81 to 4.05	.149	55/29	1.86	0.83 to 4.16	.132
TT	14/4	3.04	0.82 to 11.27	.096	14/4	3.00	0.82 to 11.04	.098
GT + TT		1.92	0.87 to 4.21	.105		2.00	0.61 to 6.48	.250
PIK3CA:rs7651265								
AA	95/40	1 (reference)			91/40	1 (reference)		
AG	23/15	1.54	0.74 to 3.19	.248	23/14	1.47	0.70 to 3.08	.312
GG	2/0				2/0			
AG + GG		1.43	0.69 to 2.95	.332		1.36	0.65 to 2.85	.409
PIK3CA:rs7640662								
CC	95/45	1 (reference)			93/44	1 (reference)		
CG	27/11	1.02	0.46 to 2.25	.970	25/11	1.06	0.48 to 2.34	.881
GG	2/1	1.26	0.14 to 11.76	.838	2/1	1.22	0.13 to 11.45	.864
CG + GG		1.04	0.48 to 2.21	.927		1.08	0.51 to 2.29	.849
PIK3CA:rs7621329								
CC	80/32	1 (reference)			77/35	1 (reference)		
CT	31/16	0.83	0.40 to 1.71	.620	30/15	0.84	0.41 to 1.74	.643
TT	6/3	1.76	0.42 to 7.38	.442	6/3	1.70	0.41 to 7.12	.467
CT + TT		0.92	0.47 to 1.83	.822		0.93	0.47 to 1.84	.838
PIK3CA:rs6443624								
AA	76/32	1 (reference)			73/32	1 (reference)		
AC	36/22	1.40	0.69 to 2.85	.348	35/21	1.39	0.68 to 2.82	.365
CC	10/4	0.95	0.28 to 3.16	.929	10/4	0.94	0.29 to 3.13	.926
AC + CC		1.30	0.66 to 2.57	.444		1.29	0.65 to 2.54	.461

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Table 1. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Recurrence (continued)

SNP and Genotype	Platinum Compound + Any				Taxane + Any			
	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*
AKT1:rs3803304								
CC	43/20	1 (reference)			28/12	1 (reference)		
CG	37/21	2.21	0.95 to 5.12	.065	31/13	2.24	0.75 to 6.72	.149
GG	5/3	1.62	0.37 to 7.15	.526	4/3	4.75	0.54 to 42.06	.162
CG + GG		2.08	0.94 to 4.60	.070		2.43	0.83 to 7.14	.107
AKT1:rs2498804								
GG	32/16	1 (reference)			26/7	1 (reference)		
GT	48/23	2.60	1.00 to 6.75	.049	31/17	11.95	1.89 to 75.41	.008
TT	5/5	3.55	0.97 to 13.02	.056	5/5	21.96	2.68 to 179.88	.004
GT + TT		2.79	1.13 to 6.89	.026		14.10	2.40 to 83.02	.003
AKT1:rs2494738								
AA	71/39	1 (reference)			57/24	1 (reference)		
AG	15/5	0.95	0.31 to 2.86	.921	8/4	1.33	0.23 to 7.63	.751
AKT1:rs1130214								
GG	45/23	1 (reference)			31/15	1 (reference)		
GT	37/21	2.08	0.87 to 4.98	.099	30/12	2.39	0.84 to 6.79	.102
TT	4/1	1.34	0.11 to 15.84	.817	4/2	10.94	1.55 to 76.98	.016
GT + TT		2.06	0.86 to 4.94	.104		2.72	0.97 to 7.62	.056
AKT2:rs892119								
AA	68/25	1 (reference)			49/16	1 (reference)		
AG	16/18	7.36	2.79 to 19.42	.000056	14/12	5.77	1.70 to 19.52	.005
GG	2/2	2.98	0.54 to 16.51	.211	2/1	2.56	0.16 to 40.00	.503
AG + GG		6.20	2.45 to 15.69	.00012		5.23	1.62 to 16.88	.006
AKT2:rs8100018								
CC	41/18	1 (reference)			35/14	1 (reference)		
CG	36/27	1.27	0.58 to 2.80	.554	23/12	1.35	0.44 to 4.17	.603
GG	7/0				7/3	0.33	0.05 to 2.29	.262
CG + GG		0.91	0.42 to 1.99	.822		0.97	0.34 to 2.81	.956
FRAP1:rs11121704								
CC	43/26	1 (reference)			30/15	1 (reference)		
CT	36/17	1.17	0.50 to 2.72	.718	30/12	0.47	0.12 to 1.90	.289
TT	7/2	2.56	0.40 to 16.30	.320	5/2	2.33	0.26 to 20.89	.450
CT + TT		1.29	0.58 to 2.86	.526		0.52	0.13 to 2.04	.346
FRAP1:rs2295080								
GG	35/18	1 (reference)			23/10	1 (reference)		
GT	35/22	2.35	0.88 to 6.23	.087	36/15	1.31	0.28 to 6.19	.730
TT	10/2	2.35	0.37 to 15.06	.367	5/3	12.35	1.19 to 128.38	.035
GT + TT		2.35	0.91 to 6.07	.079		1.43	0.30 to 6.82	.650
PIK3CA:rs7651265								
AA	68/30	1 (reference)			51/20	1 (reference)		
AG	14/13	2.13	0.81 to 5.58	.123	12/7	3.13	0.79 to 12.35	.103
GG	2/0				0/0			
AG + GG		1.75	0.70 to 4.38	.232				
PIK3CA:rs7640662								
CC	65/34	1 (reference)			51/24	1 (reference)		
CG	20/10	1.24	0.50 to 3.08	.639	13/4	1.15	0.29 to 4.51	.844
GG	2/1	1.38	0.14 to 13.53	.784	1/1	1.96	0.17 to 22.84	.589
CG + GG		1.26	0.53 to 2.99	.604		1.27	0.37 to 4.41	.705
PIK3CA:rs7621329								
CC	54/25	1 (reference)			45/17	1 (reference)		
CT	22/14	0.90	0.35 to 2.31	.822	16/8	0.50	0.15 to 1.71	.270
TT	5/2	1.08	0.18 to 6.66	.930	3/2	11.38	1.60 to 80.88	.015
CT + TT		0.92	0.37 to 2.28	.862		0.90	0.30 to 2.71	.846
PIK3CA:rs6443624								
AA	54/24	1 (reference)			40/17	1 (reference)		
AC	23/18	1.81	0.72 to 4.52	.205	20/9	0.77	0.24 to 2.46	.661
CC	8/3	1.26	0.30 to 5.37	.753	4/3	7.13	1.20 to 42.36	.031
AC + CC		1.69	0.69 to 4.13	.247		1.20	0.41 to 3.53	.737

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Table 1. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Recurrence (continued)

SNP and Genotype	Any of the Three				Fluoropyrimidine + Any			
	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*
PIK3CA:rs2699887								
AA	76/37	1 (reference)			74/36	1 (reference)		
AG	37/17	1.20	0.58 to 2.49	.618	35/17	1.29	0.62 to 2.71	.498
GG	6/3	0.73	0.18 to 2.93	.654	6/3	0.81	0.20 to 3.22	.762
AG + GG		1.09	0.55 to 2.17	.795		1.18	0.59 to 2.37	.639
PTEN:rs2299939								
AA	82/38	1 (reference)			79/37	1 (reference)		
AC	34/16	1.06	0.53 to 2.15	.864	34/16	1.05	0.52 to 2.13	.883
CC	5/3	0.45	0.09 to 2.21	.328	5/3	0.46	0.10 to 2.22	.335
AC + CC		0.91	0.47 to 1.76	.784		0.91	0.47 to 1.75	.776
PTEN:rs12569998								
GG	95/44	1 (reference)			93/44	1 (reference)		
GT	26/12	0.80	0.37 to 1.73	.570	24/11	0.81	0.37 to 1.76	.598
TT	1/1	1.40	0.12 to 15.73	.785	1/1	1.51	0.13 to 17.19	.742
GT + TT		0.83	0.39 to 1.75	.617		0.84	0.40 to 1.79	.650
PTEN:rs12357281								
CC	95/49	1 (reference)			92/48	1 (reference)		
CG	22/7	0.34	0.13 to 0.89	.027	21/7	0.33	0.13 to 0.87	.025
GG	1/0				1/0			
CG + GG		0.34	0.13 to 0.88	.027		0.33	0.13 to 0.87	.025
AKT1:rs2498804 and AKT2:rs892119 unfavorable genotype analysis								
No. of unfavorable genotypes								
0	36/13	1 (reference)			35/13	1 (reference)		
1	69/27	2.87	1.15 to 7.19	.024	67/26	2.80	1.12 to 7.03	.028
2	15/17	6.52	2.34 to 18.18	< .001	14/17	6.36	2.28 to 17.72	< .001

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results by analyzing an important signaling pathway comprised of phosphoinositide-3-kinase (PI3K), phosphatase and tensin homolog (PTEN), v-akt murine thymoma viral oncogene homolog (AKT), and mammalian target of rapamycin (mTOR).

Signaling through the PI3K/PTEN/AKT/mTOR pathway is responsible for balancing cell survival and apoptosis.[12,13] The signal is initiated by growth factors and hormones that bind receptor tyrosine kinases such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and platelet-derived growth factor receptor (PDGFR).[14] These receptors then activate PI3Ks resulting in a kinase cascade through AKT and mTOR, generating cell survival, growth, and angiogenesis signals.[15] PTEN negatively regulates this pathway by dephosphorylating phosphatidylinositol trisphosphate (PIP3) and negating the signal generated by PI3K.[16] This pathway has been shown to be commonly activated in cancer, including EC, and in the progression of Barrett's neoplasm to EC.[12,17,18] Furthermore, studies in several cancer types have demonstrated that this pathway has a key role in the development of resistance to platinum compounds, taxanes, and fluoropyrimidines.[19-24] To our knowledge, no studies have addressed how genetic variations in this pathway influence outcomes in patients with EC treated with these chemotherapeutic agents.

In this study, we determined whether common genetic variations in *AKT1*, *AKT2*, *PIK3CA* (catalytic subunit of PI3K), *PTEN*, and *FRAP1* (mTOR) were associated with clinical outcomes in patients who received chemoradiotherapy. Tagged single nucleotide polymorphisms (SNPs) were selected for each gene and genotyped in patients with EC. This pathway-based tagging approach allowed us to query genetic variations in the major effectors of this pathway and identify associations with clinical outcomes.

PATIENTS AND METHODS

Patient Population

This study included 210 patients with resectable adenocarcinoma (174 cases) or squamous cell carcinoma (36 cases) who were recruited between 1985 and 2003 at The University of Texas M. D. Anderson Cancer Center (Houston, TX).[11] All patients had undergone chemoradiotherapy followed by surgery, or induction chemotherapy followed by chemoradiotherapy and surgery.

Table 1. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Recurrence (continued)

SNP and Genotype	Platinum Compound + Any				Taxane + Any			
	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*	No Recurrence/ Recurrence (No.)	HR*	95% CI	*P*
PIK3CA:rs2699887								
AA	53/27	1 (reference)			41/19	1 (reference)		
AG	25/15	1.35	0.58 to 3.10	.486	18/6	1.95	0.57 to 6.66	.286
GG	5/2	0.36	0.04 to 3.09	.353	4/3	1.15	0.25 to 5.36	.856
AG + GG		1.16	0.51 to 2.64	.726		1.59	0.56 to 4.49	.383
PTEN:rs2299939								
AA	59/28	1 (reference)			42/20	1 (reference)		
AC	21/14	0.94	0.39 to 2.26	.895	20/8	1.38	0.41 to 4.63	.605
CC	4/3	0.36	0.06 to 2.21	.267	1/1	0.37	0.01 to 11.18	.566
AC + CC		0.78	0.35 to 1.75	.544		1.17	0.37 to 3.68	.784
PTEN:rs12569998								
GG	67/34	1 (reference)			53/20	1 (reference)		
GT	17/10	1.09	0.40 to 2.93	.868	10/8	1.06	0.29 to 3.93	.927
TT	1/0				1/1	1.08	0.05 to 22.39	.962
GT + TT		1.06	0.39 to 2.82	.915		1.06	0.32 to 3.59	.920
PTEN:rs12357281								
CC	72/38	1 (reference)			44/26	1 (reference)		
CG	11/6	0.36	0.11 to 1.21	.098	17/2	0.05	0.006 to 0.461	.008
GG	0/0				1/0			
CG + GG						0.05	0.006 to 0.458	.008
AKT1:rs2498804 and AKT2:rs892119 unfavorable genotype analysis								
No. of unfavorable genotypes								
0	24/12	1 (reference)			18/4	1 (reference)		
1	49/17	3.54	1.10 to 11.46	.040	36/15	9.09	1.29 to 64.26	.027
2	11/15	10.73	3.21 to 35.82	< .001	8/10	83.37	9.56 to 726.78	< .001

Abbreviations: PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; AKT, v-akt murine thymoma viral oncogene homolog; mTOR, mammalian target of rapamycin; SNP, single nucleotide polymorphism; HR, hazard ratio.

*Adjusted for age, sex, smoking status, alcohol consumption, radiation dosage, chemoradiotherapy sequence, clinical stage, chemotherapy regimens, histologic tumor type, tumor location, pathologic stage, and histologic viability.

Clinical Data Collection

Patients with EC were staged as described previously.[11] After chemoradiotherapy, patients underwent restaging and surgery. Pathologic response to treatment was measured by previously described methodology.[25,26] Response was defined as no residual carcinoma in the primary tumor site. A poor response was any response less than a complete response. Study end points were pathologic response to therapy, recurrence, and survival. This study was approved by the M. D. Anderson Cancer Center institutional review board.

SNP Selection and Genotyping

Genomic DNA was extracted from paraffin slides using the PicoPure DNA extraction kit (Arcturus Bioscience, Mountain View, CA). Tagging SNPs were selected within and 5-kb flanking each gene using the tagger algorithm[28] with a cutoff of 0.80 for r^2 and a minor allele frequency between 0.10 and 0.35 based on data from Centre d'Etude du Polymorphisme Humain samples genotyped by the HapMap Project (www.hapmap.org).[27] A total of sixteen SNPs were selected to represent genetic variation of 95 SNPs in the pathway (Appendix Table A1, online only). TaqMan genotyping assays, including quality control measures, were performed as previously described[11] using the 7900HT sequence detection system (Applied Biosystems, Foster City, CA).

Statistical Analysis

Hazard ratios (HRs) for recurrence and survival end points were estimated by applying the Cox proportional hazards model while adjusting for age, sex, smoking status, alcohol consumption, radiation dosage, chemoradiotherapy sequence, clinical stage, chemotherapy regimens, histologic tumor type, tumor location, pathologic stage, and histologic viability. The Kaplan-Meier survival function and log-rank tests were used to assess differences in recurrence-free and overall survival times. For pathologic response to therapy, unconditional multivariate logistic regression analysis was done to estimate adjusted odds ratios (ORs) along with the corresponding 95% CIs for each SNP. We also evaluated the combined effects by the number of unfavorable genotypes identified from the main effects analysis of single SNPs. The statistical analyses described above were completed using the STATA software (version 8, STATA, College Station, TX). Survival tree analyses were used to identify higher-order gene-gene interactions. Survival tree analysis was performed using the STREE program (http://masal.med.yale.edu/stree/) which uses recursive-partitioning to identify subgroups of individuals at higher risk.

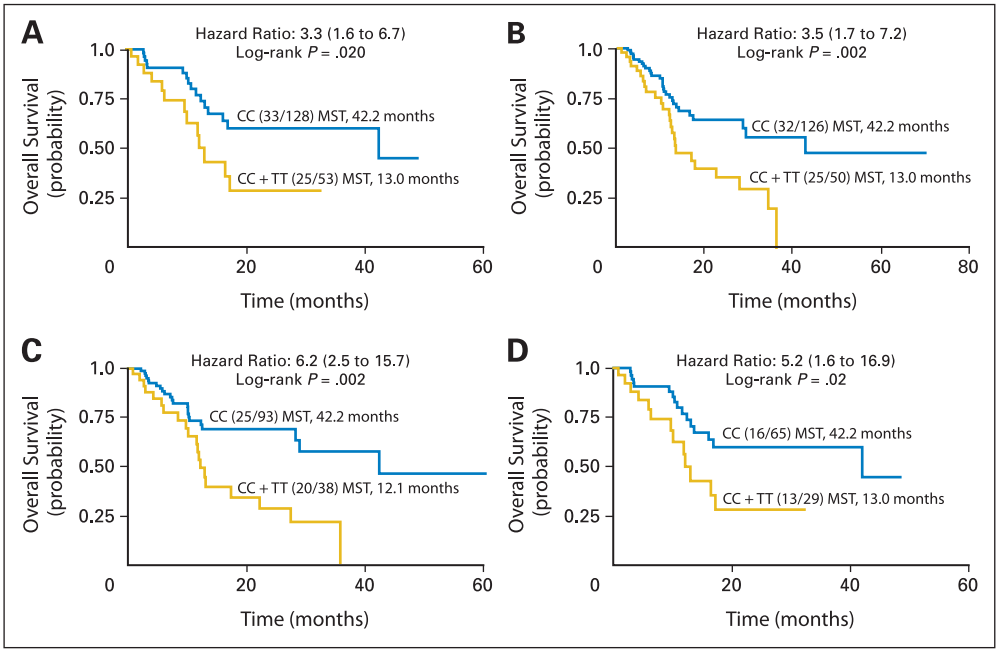

Fig 1. Kaplan-Meier curves of recurrence-free survival times in patients with esophageal cancer (EC) with AKT2:rs892119 treated with (A) any of the three drugs, (B) fluoropyrimidine, (C) platinum compound, and (D) taxane. The numbers in parentheses are the numbers of patients with EC with recurrence/total patients with the respective genotype. MST, median survival time in months.

All statistical analyses were two sided, and $P < .05$ was considered statistically significant.

RESULTS

Patient Characteristics

Of the 210 patients with EC enrolled in this study, DNA was available for 207 and of those, 186 were white (90%; Appendix Table A2, online only). Because of the small number of patients from other ethnic groups, we focused our study on white patients only. Fifty-nine percent of patients were ever smokers, and 33.1% used alcohol on a daily basis. Approximately half of patients (51%) presented with stage IIA disease. More than 97% of the patients (182) were treated with either a fluoropyrimidine, platinum agent, or taxane. Of these 182 patients, 177 received a fluoropyrimidine (95%), 132 received a platinum agent (71%), and 94 received a taxane (51%). The median follow-up time was 18.8 months, with 97 deaths and 59 recurrences. The overall median survival time was 34.5 months.

Associations Between SNPs and Recurrence Risk

Variant genotypes were analyzed for association with recurrence risk in patients after chemoradiotherapy. Three SNPs—AKT1:rs2498804, AKT2:rs892119, and PTEN:rs12357281—were associated with variation in recurrence risk (Table 1).

AKT1 and AKT2 genetic variations. The *AKT1* and *AKT2* SNPs resulted in increased risk, with adjusted HRs of 2.21 (95% CI, 1.06 to 4.60) and 3.30 (95% CI, 1.64 to 6.66), respectively. These two SNPs were also associated with recurrence when the results were stratified by treatment (Table 1). Furthermore, AKT2:rs892119 resulted in dramatically different median recurrence-free survival times of 42 months for patients with wild-type genotype compared with 12 months for those with one or two variant alleles (Fig 1).

Because *AKT1* and *AKT2* SNPs were consistently associated with recurrence risk, we performed an unfavorable genotype analysis to determine the effect of having one or both of these SNPs. One unfavorable genotype resulted in a nearly three-fold increased recurrence risk (95% CI, 1.15 to 7.19; Table 1). This risk increased to more than six-fold (HR, 6.52; 95% CI, 2.34 to 18.18) in patients with two unfavorable genotypes. The same result was also observed when results were stratified by treatment, with HRs for two unfavorable genotypes of 6.36 (95% CI, 2.28 to 17.72), 10.73 (95% CI, 3.21 to 35.82), and 83.4 (95% CI, 9.56 to 726.78) for fluoropyrimidine, platinum compound, and taxane groups, respectively. These results demonstrate that *AKT1* and *AKT2* genetic variation has an additive effect on recurrence risk and recurrence-free survival rates.

PTEN genetic variation. In contrast, and as would be predicted by PTEN's negative regulation of signaling through the pathway, PTEN:rs12357281 was associated with a decreased recurrence risk. Patients carrying at least one variant allele had a significant reduction in risk (HR, 0.34; 95% CI, 0.13 to 0.88). This same pattern was also observed for fluoropyrimidine and taxane groups, with HRs of 0.33 (95% CI, 0.13 to 0.87) and 0.05 (95% CI, 0.0006 to 0.458), respectively (Table 1).

Higher-order gene-gene interactions. Overall, seven SNPs were found to be associated with recurrence risk in patients treated with a taxane as part of their overall treatment regimen. Survival tree analysis was used to identify interactions within these SNPs. AKT2:rs892119, PIK3CA:rs6443624, and PTEN:rs12357281 demonstrated gene-gene interactions, resulting in four terminal nodes with different recurrence-free survival times (Fig 2A). The initial split on the survival tree was due to AKT2:rs892119 (node 4), indicating that this SNP is the primary factor contributing to variation in recurrence risk in this population. The reference group for the analysis (node 1) was composed of individuals with wild-type AKT2:rs892119 and wild-type PIK3CA:rs6443624 genotypes. Patients in this node had the longest

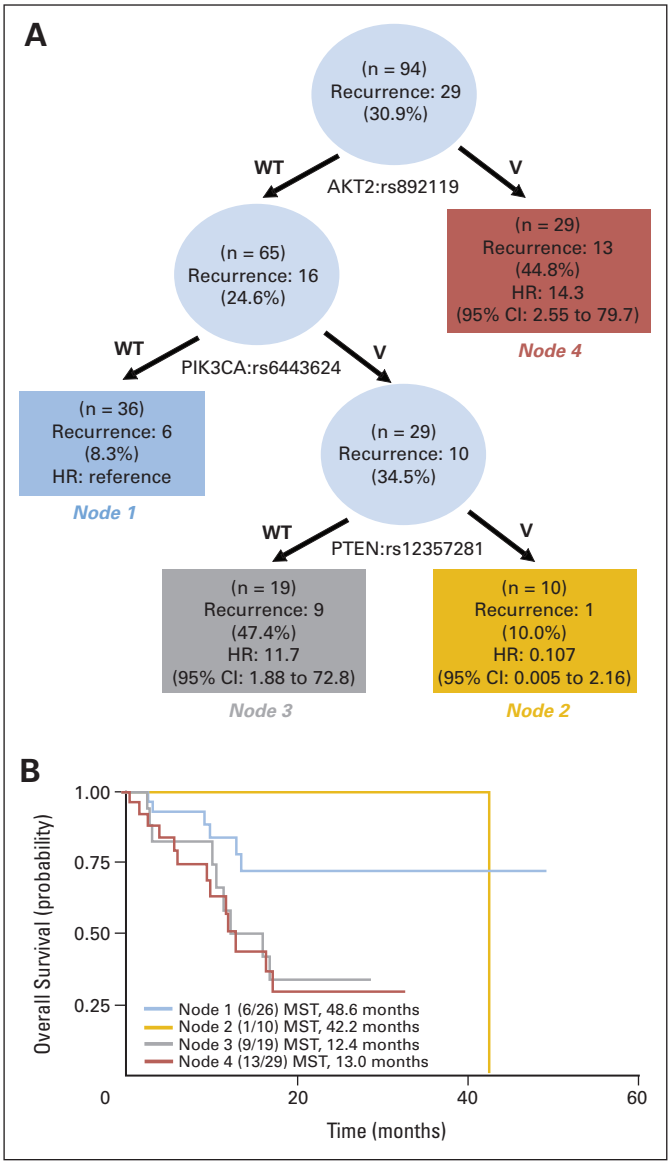

Fig 2. Gene-gene interactions in the phosphoinositide-3-kinase (PI3K), phosphatase and tensin homolog (PTEN), v-akt murine thymoma viral oncogene homolog (AKT), and mammalian target of rapamycin (mTOR) pathway that modified recurrence risk and recurrence-free survival in patients with esophageal cancer (EC) treated with a taxane. (A) Survival tree analysis showing the interactions between three SNPs. (B) Kaplan-Meier curves of recurrence-free survival times in patients in the four terminal nodes, as identified by a survival tree analysis. MST, median survival time; HR, hazard ratio.

recurrence-free survival time of 48 months. This duration was comparable to patients in node 2 who carried the wild-type alleles for AKT2:892119, but variants of both PIK3CA:rs6443624 and PTEN:rs12357281. These results suggest that the protective effect conferred by *PTEN* is able to counteract the negative consequences of *PIK3CA* genetic variation and shift the recurrence-free survival time from 12 to 42 months (Fig 2B).

Associations Between SNPs and Survival

The PI3K/PTEN/AKT/mTOR pathway did not appear to be a large contributor to variation in survival times. Of the 16 SNPs assayed, only four were found to be significantly associated with survival in any of the treatment groups: AKT1:rs1130214, AKT2:rs892119, FRAP1:rs11121704, and FRAP1:rs2295080 (Table 2).

FRAP1 genetic variations. Both of the *FRAP1* SNPs genotyped were significant with HRs of 3.53 (95% CI, 1.48 to 8.39) and 4.19 (95% CI, 1.83 to 9.61) for homozygous variants of FRAP1:rs11121704 and FRAP1:rs2295080, respectively (Table 2). These same SNPs were also associated with increased risk of death in the fluoropyrimidine and taxane treatment groups.

AKT1 and AKT2 genetic variations. *AKT1* and *AKT2* SNPs were associated with survival in taxane-treated patients only. AKT1:rs1130214 resulted in a nearly nine-fold (HR, 8.92; 95% CI, 1.56 to 51.17) increased risk of death in these patients, while AKT2:rs892119 was associated with a 3.5-fold increase (95% CI, 1.43 to 8.78). The relationships between AKT1:rs1130214 and AKT2:rs892119 with survival were not observed in any of the other treatment groups, suggesting that the effect of these genetic variants may be restricted to taxane-based therapy. However, the small sample size of the taxane group may be a factor contributing to these results.

Associations Between SNPs and Response to Therapy

Response to therapy was analyzed for associations with genetic variations in the pathway with three SNPs showing significance: AKT2:rs892119, AKT1:rs3803304, and FRAP1:rs1121704 (Table 3).

AKT1 genetic variation. Patients heterozygous for AKT1:rs3803304 experienced a better response to chemoradiotherapy than those with a wild-type genotype (OR, 0.50; 95% CI, 0.25 to 0.99).

AKT2 genetic variation. Interestingly, the same *AKT2* SNP (rs892119) that had been significantly associated with increased risk of both recurrence and death was also found to be associated with a poorer response (OR, 2.81; 95% CI, 1.27 to 6.21). This effect was also observed in the fluoropyrimidine (OR, 2.98; 95% CI, 1.33 to 6.68), and taxane (OR, 4.12; 95% CI, 1.18 to14.37) treatment groups.

FRAP1 genetic variation. The FRAP1:rs11121704 SNP, which was associated with poor survival, also contributed to a poor pathologic response (OR, 2.76; 95% CI, 1.04 to 7.37) in patients treated with a taxane with at least one variant allele.

DISCUSSION

The PI3K/PTEN/AKT/mTOR pathway plays an important role in balancing cell growth and death. This pathway is often activated in several cancer types—including EC—and has been shown to be important in the development of resistance to several commonly used classes of chemotherapeutic agents. In this study, we determined whether genetic variations in the genes for PI3K, PTEN, AKT1, AKT2, and mTOR were associated with variation in recurrence, survival, and pathologic response. To our knowledge, ours is the first study to apply a tagging SNP approach to determine the role of this pathway in clinical outcomes for any cancer type.

Significant associations were observed between several SNPs and clinical outcomes. In individual SNP analyses, we identified seven SNPs associated with recurrence risk and recurrence-free survival rates. Patients treated with any of the three chemotherapeutic agents had a dramatic increase in their recurrence risk with

Table 2. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Survival

SNP and Genotype	Any of the Three				Fluoropyrimidine + Any			
	No. Alive/ Dead	HR*	95% CI	*P*	No. Alive/ Dead	HR*	95% CI	*P*
AKT1:rs3803304								
CC	45/41	1 (reference)			43/40	1 (reference)		
CG	38/41	1.12	0.70 to 1.80	.645	37/40	1.14	0.7 to 1.84	.602
GG	8/4	1.09	0.36 to 3.33	.876	8/4	1.18	0.39 to 3.59	.775
CG + GG		1.12	0.71 to 1.76	.641		1.14	0.72 to 1.82	.579
AKT1:rs2498804								
GG	34/36	1 (reference)			32/35	1 (reference)		
GT	47/46	1.14	0.69 to 1.90	.601	46/45	1.18	0.71 to 1.98	.518
TT	9/6	1.50	0.57 to 3.97	.409	9/6	1.64	0.62 to 4.34	.321
GT + TT		1.18	0.72 to 1.93	.513		1.23	0.74 to 2.03	.424
AKT1:rs2494738								
AA	75/79	1 (reference)			72/77	1 (reference)		
AG	17/8	0.84	0.38 to 1.85	.668	17/8	0.89	0.41 to 1.95	.772
AKT1:rs1130214								
GG	46/42	1 (reference)			43/41	1 (reference)		
GT	40/44	1.26	0.76 to 2.09	.372	40/43	1.23	0.74 to 2.04	.426
TT	6/3	1.98	0.52 to 7.51	.315	6/3	2.13	0.56 to 8.15	.270
GT + TT		1.28	0.77 to 2.11	.337		1.25	0.76 to 2.06	.386
AKT2:rs892119								
AA	69/59	1 (reference)			69/57	1 (reference)		
AG	22/26	1.31	0.74 to 2.31	.354	20/26	1.47	0.83 to 2.61	.189
GG	1/4	1.08	0.32 to 3.70	.898	0/4			
AG + GG		1.27	0.75 to 2.16	.378		1.40	0.81 to 2.4	.229
AKT2:rs8100018								
CC	46/39	1 (reference)			44/38	1 (reference)		
CG	35/42	1.06	0.63 to 1.77	.825	34/41	1.03	0.61 to 1.74	.900
GG	10/6	0.65	0.26 to 1.66	.370	10/6	0.63	0.25 to 1.61	.335
CG + GG		0.96	0.60 to 1.56	.884		0.94	0.58 to 1.53	.800
FRAP1:rs11121704								
CC	43/45	1 (reference)			41/43	1 (reference)		
CT	44/34	0.84	0.50 to 1.43	.529	43/34	0.87	0.51 to 1.48	.599
TT	5/9	3.53	1.48 to 8.39	.004	5/9	3.82	1.58 to 9.23	.003
CT + TT		1.04	0.63 to 1.71	.878		1.08	0.65 to 1.78	.777
FRAP1:rs2295080								
GG	35/33	1 (reference)			33/31	1 (reference)		
GT	47/38	0.96	0.53 to 1.75	.906	46/38	1.00	0.54 to 1.84	.999
TT	6/12	4.19	1.83 to 9.61	.001	6/12	4.66	1.99 to 10.94	.0004
GT + TT		1.25	0.72 to 2.17	.433		1.30	0.74 to 2.31	.354
PIK3CA:rs7651265								
AA	70/65	1 (reference)			67/64	1 (reference)		
AG	18/20	1.18	0.67 to 2.10	.567	18/19	1.17	0.65 to 2.09	.602
GG	2/0				2/0			
AG + GG		1.02	0.59 to 1.78	.939		1.04	0.59 to 1.82	.902
PIK3CA:rs7640662								
CC	73/67	1 (reference)			72/65	1 (reference)		
CG	18/20	0.89	0.50 to 1.58	.685	16/20	0.99	0.55 to 1.77	.969
GG	1/2	2.13	0.45 to 10.11	.341	1/2	2.17	0.46 to 10.25	.330
CG + GG		0.95	0.55 to 1.66	.869		1.06	0.61 to 1.84	.844
PIK3CA:rs7621329								
CC	59/56	1 (reference)			57/55	1 (reference)		
CT	23/24	0.95	0.55 to 1.65	.857	22/23	0.97	0.56 to 1.67	.911
TT	6/3	0.72	0.20 to 2.62	.617	6/3	0.85	0.23 to 3.1	.810
CT + TT		0.91	0.55 to 1.52	.725		0.95	0.57 to 1.59	.854
PIK3CA:rs6443624								
AA	56/52	1 (reference)			54/51	1 (reference)		
AC	26/32	1.47	0.85 to 2.54	.163	25/31	1.53	0.88 to 2.65	.128
CC	9/5	0.84	0.30 to 2.35	.738	9/5	0.92	0.33 to 2.57	.867
AC + CC		1.31	0.79 to 2.18	.290		1.39	0.83 to 2.32	.213

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Table 2. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Survival (continued)

SNP and Genotype	Platinum Compound + Any				Taxane + Any			
	No. Alive/ Dead	HR*	95% CI	*P*	No. Alive/ Dead	HR*	95% CI	*P*
AKT1:rs3803304								
CC	31/32	1 (reference)			25/15	1 (reference)		
CG	24/34	1.51	0.85 to 2.67	.159	24/20	1.88	0.81 to 4.37	.143
GG	6/2	0.70	0.15 to 3.25	.653	4/3	2.17	0.35 to 13.3	.403
CG + GG		1.40	0.80 to 2.43	.239		1.90	0.83 to 4.36	.129
AKT1:rs2498804								
GG	21/27	1 (reference)			21/12	1 (reference)		
GT	33/38	1.28	0.69 to 2.37	.434	26/22	1.42	0.51 to 3.92	.503
TT	6/4	1.21	0.37 to 3.99	.757	5/5	2.48	0.57 to 10.77	.224
GT + TT		1.27	0.69 to 2.33	.436		1.54	0.57 to 4.15	.395
AKT1:rs2494738								
AA	49/61	1 (reference)			45/36	1 (reference)		
AG	12/8	0.84	0.37 to 1.91	.683	9/3	0.68	0.17 to 2.67	.576
AKT1:rs1130214								
GG	33/35	1 (reference)			28/18	1 (reference)		
GT	25/33	1.19	0.66 to 2.15	.562	23/19	1.69	0.73 to 3.94	.222
TT	3/2	1.85	0.37 to 9.26	.455	3/3	8.92	1.56 to 51.17	.014
GT + TT		1.21	0.67 to 2.18	.521		1.82	0.79 to 4.19	.157
AKT2:rs892119								
AA	45/48	1 (reference)			41/24	1 (reference)		
AG	16/18	1.38	0.71 to 2.68	.343	12/14	3.27	1.27 to 8.40	.014
GG	0/4				1/2	6.25	0.89 to 43.88	.065
AG + GG		1.39	0.75 to 2.59	.292		3.54	1.43 to 8.78	.006
AKT2:rs8100018								
CC	30/29	1 (reference)			26/23	1 (reference)		
CG	24/39	1.16	0.65 to 2.09	.617	22/13	0.76	0.32 to 1.83	.547
GG	6/1	0.17	0.02 to 1.30	.088	6/4	0.23	0.05 to 1.04	.056
CG + GG		1.01	0.56 to 1.79	.985		0.55	0.25 to 1.21	.138
FRAP1:rs11121704								
CC	31/38	1 (reference)			25/20	1 (reference)		
CT	26/27	0.97	0.53 to 1.76	.920	28/14	0.58	0.23 to 1.45	.244
TT	4/5	2.77	0.85 to 9.00	.091	1/6	7.03	1.81 to 27.35	.005
CT + TT		1.12	0.64 to 1.97	.692		0.85	0.37 to 1.97	.710
FRAP1:rs2295080								
GG	25/28	1 (reference)			20/13	1 (reference)		
GT	27/30	0.99	0.51 to 1.91	.971	33/18	0.32	0.10 to 1.01	.053
TT	5/7	2.66	0.91 to 7.75	.073	1/7	8.28	2.02 to 33.92	.003
GT + TT		1.15	0.61 to 2.16	.663		0.73	0.26 to 2.05	.554
PIK3CA:rs7651265								
AA	48/50	1 (reference)			41/30	1 (reference)		
AG	10/17	1.73	0.85 to 3.49	.130	11/8	0.78	0.25 to 2.38	.661
GG	2/0							
AG + GG		1.43	0.72 to 2.82	.305				
PIK3CA:rs7640662								
CC	46/53	1 (reference)			43/32	1 (reference)		
CG	14/16	0.79	0.41 to 1.53	.483	10/7	1.03	0.37 to 2.86	.952
GG	1/2	2.18	0.45 to 10.64	.335	1/1	0.87	0.09 to 8.24	.905
CG + GG		0.87	0.46 to 1.63	.665		1.00	0.39 to 2.60	.992
PIK3CA:rs7621329								
CC	38/41	1 (reference)			35/27	1 (reference)		
CT	15/21	1.19	0.61 to 2.35	.612	15/9	0.59	0.21 to 1.69	.328
TT	5/2	0.74	0.16 to 3.57	.712	3/2	1.38	0.24 to 7.87	.716
CT + TT		1.11	0.59 to 2.07	.752		0.70	0.27 to 1.81	.459
PIK3CA:rs6443624								
AA	38/40	1 (reference)			32/25	1 (reference)		
AC	15/26	1.55	0.82 to 2.93	.173	17/12	1.58	0.62 to 4.01	.336
CC	7/4	1.28	0.41 to 4.02	.675	4/3	1.22	0.21 to 7.05	.828
AC + CC		1.50	0.82 to 2.75	.186		1.52	0.62 to 3.71	.360

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Table 2. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Survival (continued)

SNP and Genotype	Any of the Three				Fluoropyrimidine + Any			
	No. Alive/ Dead	HR*	95% CI	*P*	No. Alive/ Dead	HR*	95% CI	*P*
PIK3CA:rs2699887								
AA	57/56	1 (reference)			56/54	1 (reference)		
AG	26/28	1.15	0.68 to 1.96	.601	24/28	1.28	0.75 to 2.2	.362
GG	5/4	0.48	0.15 to 1.50	.206	5/4	0.50	0.16 to 1.56	.230
AG + GG		0.99	0.60 to 1.65	.984		1.10	0.65 to 1.84	.728
PTEN:rs2299939								
AA	62/58	1 (reference)			60/56	1 (reference)		
AC	26/24	1.45	0.84 to 2.51	.186	26/24	1.46	0.84 to 2.52	.181
CC	3/5	1.35	0.44 to 4.09	.598	3/5	1.40	0.45 to 4.3	.559
AC + CC		1.43	0.86 to 2.40	.172		1.45	0.86 to 2.43	.163
PTEN:rs12569998								
GG	68/71	1 (reference)			66/71	1 (reference)		
GT	21/17	0.63	0.34 to 1.18	.148	20/15	0.62	0.33 to 1.16	.137
TT	1/1	3.52	0.40 to 30.89	.257	1/1	4.64	0.51 to 41.97	.172
GT + TT		0.66	0.36 to 1.22	.188		0.65	0.35 to 1.21	.178
PTEN:rs12357281								
CC	72/72	1 (reference)			70/70	1 (reference)		
CG	15/14	0.78	0.40 to 1.52	.468	14/14	0.77	0.39 to 1.52	.458
GG	1/0				0/0			
CG + GG		0.77	0.39 to 1.50	.444				

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two unfavorable *AKT1* or *AKT2* genotypes. This increased risk was also observed in fluoropyrimidine-, platinum-, and taxane-treated patients (6-, 10-, and > 80-fold increases, respectively). Although these results require further validation, they indicate that variations in these genes play a role in modulating EC recurrence. The importance of AKT2:rs892119 in determining recurrence risk was further supported in a survival tree analysis of patients treated with taxanes. This SNP was the basis of the initial split in the tree, suggesting that genetic variation tagged by this SNP is a major risk factor for developing a recurrence.

Interestingly, AKT2:rs892119 had consistent effect on variation in all three clinical outcomes studied and was not drug specific. AKT2:rs892119 is intronic and represents genetic variation across five SNPs genotyped in the Centre d'Etude du Polymorphisme Humain population. It is possible that AKT2:rs892119 is the functional SNP through alterations in normal splicing patterns or transcription of *AKT2*. However, it is likely that this SNP is not the functional variant but a surrogate marker for the underling genetic variation within that region on the genome. Additional studies will be required to identify the causative sequence variation and the mechanism(s) responsible for our observations. Nevertheless, our results suggest that the functional SNP tagged by AKT2:rs892119 results in activation of AKT2 and increased signaling through this pathway. This is of particularly intriguing because AKT plays a major role in regulating cell survival and growth.[13] AKT activation due to overexpression or gene amplification has been shown to be involved in resistance to several chemotherapeutic agents for cancers such as lung, uterine, and ovarian cancer.[19-22] However, to our knowledge, no studies have shown associations between common genetic variations in AKT and clinical outcomes in any cancer type.

We selected tagging SNPs for five genes in the PI3K/PTEN/AKT/mTOR pathway. These genes were chosen because they represent the core functional components of the pathway, but this pathway is complex, with several other genes warranting investigation on the basis of the results of this study. Two phosphoinositide-dependent kinases—PDK1 and PDK2—are responsible for phosphorylating AKT, resulting in AKT activation.[29] Directly downstream of AKT in the pathway are the tuberous sclerosis complex (TSC) tumor suppressor genes—*TSC1* and *TSC2*. AKT phosphorylation of TSC2 inhibits the function of this complex, allowing for the activation of mTOR.[30] Genetic variation in these four genes—*PDK1*, *PDK2*, *TSC1*, and *TSC2*—may contribute to additional variation in clinical outcome, especially in combination with genetically altered AKT.

PTEN acts as a negative regulator of PI3K/AKT/mTOR signaling by reversing PIP3 activation. Loss of PTEN function results in unrestrained signaling through this pathway and ultimately increased cell growth and proliferation.[31] This is a common feature of cancer and has been observed in several cancer types, including brain, breast, and prostate.[32-34] However, *PTEN* mutations occur infrequently in EC,[35] and decreased protein expression is found in approximately 40% of EC tumors.[36] Tachibana et al[37] reported that patients with positive PTEN expression in the nucleus had higher overall survival rates than did those without. In our study, we found that a SNP located in an intron of *PTEN* was associated with decreased recurrence risk—possibly due to increased expression of PTEN protein in esophageal tissue. Because PTEN:rs12357281 is a tagging SNP, it is likely not the functional SNP. Although, as with all tagging SNPs, there is a possibility that it is the functional variant. However,

Table 2. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Survival (continued)

SNP and Genotype	Platinum Compound + Any				Taxane + Any			
	No. Alive/ Dead	HR*	95% CI	*P*	No. Alive/ Dead	HR*	95% CI	*P*
PIK3CA:rs2699887								
AA	35/45	1 (reference)			36/24	1 (reference)		
AG	19/21	0.77	0.41 to 1.42	.397	12/12	2.32	0.84 to 6.38	.104
GG	4/3	0.30	0.07 to 1.27	.101	4/3	0.44	0.11 to 1.80	.253
AG + GG		0.68	0.37 to 1.23	.203		1.15	0.49 to 2.67	.749
PTEN:rs2299939								
AA	42/45	1 (reference)			36/26	1 (reference)		
AC	16/19	1.47	0.78 to 2.79	.234	16/12	1.43	0.58 to 3.52	.432
CC	2/5	1.99	0.56 to 7.08	.286	1/1	0.67	0.04 to 10.91	.778
AC + CC		1.55	0.85 to 2.80	.149		1.33	0.56 to 3.15	.515
PTEN:rs12569998								
GG	45/56	1 (reference)			42/31	1 (reference)		
GT	14/13	0.62	0.28 to 1.37	.234	10/8	0.35	0.12 to 1.02	.055
TT	0/1				1/1	3.11	0.21 to 46.74	.412
GT + TT		0.70	0.33 to 1.52	.373		0.42	0.15 to 1.18	.099
PTEN:rs12357281								
CC	50/60	1 (reference)			40/30	1 (reference)		
CG	9/8	0.73	0.30 to 1.76	.478	11/8	1.16	0.42 to 3.16	.777
GG	0/0				1/0			
CG + GG						1.11	0.41 to 3.02	.837

Abbreviations: PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; AKT, v-akt murine thymoma viral oncogene homolog; mTOR, mammalian target of rapamycin; SNP, single nucleotide polymorphism; HR, hazard ratio.

*Adjusted for age, sex, smoking status, alcohol consumption, radiation dosage, chemoradiotherapy sequence, clinical stage, chemotherapy regimens, histologic tumor type, tumor location, pathologic stage, and histologic viability.

even without the identification of the functional SNP, our results imply that common variation in *PTEN* is an important modulator of recurrence risk in patients with EC. Furthermore, the gene-gene interactions identified in our survival tree analysis highlight the complexity of the effects of genetic variation on recurrence risk and recurrence-free survival and support the importance of AKT2:rs892119 and PTEN:rs12357281 in modulating these outcomes.

In contrast to PTEN that acts as a brake for this pathway, increased mTOR (*FRAP1*) activity results in increased growth signals through phosphorylation of 4EBP and p70S6K.[38] mTOR is activated in many cancers, including EC.[18] In our study, patients who were homozygous for either of the *FRAP1* SNPs had an increased risk of death. In addition, FRAP1:rs11121704 homozygosity was associated with a poor response to taxane. These observations are consistent with those of increased mTOR signaling, resulting in poorer clinical outcomes for patients with genetically polymorphic *FRAP1*.

We observed that genetic variations in the PI3K/PTEN/AKT/mTOR pathway appeared to have more effect on clinical outcomes in patients treated with taxanes than in patients treated with either a fluoropyrimidine or platinum-containing agent. This may be partly due to the small sample size of the taxane treatment group, but this observation was particularly evident for associations with recurrence risk, with at least one SNP from every gene studied found to be significant in patients treated with taxane. The pathway's involvement in clinical outcomes in platinum-treated patients was limited to associations with recurrence risk for *AKT1* and *AKT2* SNPs. Similarly, few significant associations were found between clinical outcome and genetic variation in fluoropyrimidine-treated patients. The observations in the fluoropyrimidine and platinum agent groups were replicated in the larger group of patients treated with any of the three drugs. In contrast, several associations were observed only in the taxane treatment group. Although sample size may be an issue, these drugs have different mechanisms of action, and these differences may account for the differences in association between this pathway and clinical outcome.

In conclusion, we found significant associations between common genetic variants in the PI3K/PTEN/AKT/mTOR pathway and clinical outcomes in patients with EC. Although we limited our analyses to patients receiving chemoradiotherapy, we are not able to conclude that these markers are predictive of drug response since we are unable to exclude that they may be prognostic factors. It would be interesting to analyze these SNPs in a control group undergoing surgery alone to assess their prognostic impact, but we did not have enough patients in this category in this study. Nevertheless, if validated as predictive markers for chemotherapy, these results, with the integration of clinical, epidemiological, and genetic data, could become the basis for individualizing therapy For example, markers predictive of a good drug response could be useful for preselecting patients to a specific chemotherapeutic. In contrast, patients with a poor marker signature who are predicted to receive no benefits from chemotherapy may receive only surgery. The ultimate goal is to allow for the

Table 3. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Response to Therapy

SNP and Genotype	Any of the Three				Fluoropyrimidine + Any			
	Response/No Response	OR*	95% CI	*P*	Response/No Response	OR*	95% CI	*P*
AKT1:rs3803304								
CC	25/61	1 (reference)			25/58	1 (reference)		
CG	35/44	0.50	0.25 to 0.99	.047	34/43	0.54	0.27 to 1.08	.083
GG	4/8	0.93	0.25 to 3.53	.920	4/8	0.96	0.25 to 3.63	.953
CG + GG		0.54	0.28 to 1.05	.071		0.59	0.30 to 1.15	.118
AKT1:rs2498804								
GG	23/47	1 (reference)			23/44	1 (reference)		
GT	36/57	0.75	0.37 to 1.52	.419	35/56	0.83	0.40 to 1.70	.605
TT	5/10	1.15	0.33 to 4.01	.821	5/10	1.21	0.35 to 4.20	.767
GT + TT		0.79	0.40 to 1.58	.510		0.87	0.43 to 1.75	.703
AKT1:rs2494738								
AA	59/95	1 (reference)			58/91	1 (reference)		
AG	6/19	2.01	0.73 to 5.57	.177	6/19	2.07	0.75 to 5.75	.163
AKT1:rs1130214								
GG	31/57	1 (reference)			30/54	1 (reference)		
GT	31/53	1.12	0.56 to 2.25	.754	31/52	1.06	0.52 to 2.15	.879
TT	4/5	0.74	0.17 to 3.2	.693	4/5	0.71	0.16 to 3.11	.652
GT + TT		1.08	0.54 to 2.13	.833		1.02	0.51 to 2.04	.961
AKT2:rs892119								
AA	53/75	1 (reference)			53/73	1 (reference)		
AG	13/35	2.54	1.14 to 5.65	.023	12/34	2.68	1.18 to 6.06	.018
GG	0/5				0/4			
AG + GG		2.81	1.27 to 6.21	.010		2.98	1.33 to 6.68	.008
AKT2:rs8100018								
CC	33/52	1 (reference)			32/50	1 (reference)		
CG	26/51	1.30	0.66 to 2.58	.446	26/49	1.20	0.60 to 2.39	.612
GG	6/10	0.83	0.24 to 2.84	.769	6/10	0.83	0.24 to 2.84	.768
CG + GG		1.22	0.63 to 2.36	.549		1.13	0.58 to 2.20	.713
FRAP1:rs11121704								
CC	37/51	1 (reference)			36/48	1 (reference)		
CT	25/53	1.35	0.68 to 2.67	.395	25/52	1.32	0.66 to 2.63	.437
TT	4/10	1.92	0.53 to 6.87	.318	4/10	1.88	0.52 to 6.77	.336
CT + TT		1.43	0.74 to 2.74	.283		1.40	0.72 to 2.70	.320
FRAP1:rs2295080								
GG	29/39	1 (reference)			28/36	1 (reference)		
GT	26/59	1.60	0.77 to 3.32	.210	26/58	1.58	0.76 to 3.31	.224
TT	8/10	0.99	0.33 to 2.95	.983	8/10	0.99	0.33 to 2.97	.985
GT + TT		1.44	0.72 to 2.86	.301		1.43	0.71 to 2.86	.316
PIK3CA:rs7651265								
AA	53/82	1 (reference)			52/79	1 (reference)		
AG	11/27	1.45	0.61 to 3.42	.399	11/26	1.40	0.59 to 3.33	.443
GG	0/2				0/2			
AG + GG		1.61	0.69 to 3.75	.273		1.56	0.66 to 3.65	.307
PIK3CA:rs7640662								
CC	50/90	1 (reference)			50/87	1 (reference)		
CG	14/24	0.83	0.37 to 1.84	.639	13/23	0.85	0.37 to 1.95	.704
GG	2/1	0.34	0.03 to 4.00	.390	2/1	0.35	0.03 to 4.21	.410
CG + GG		0.76	0.35 to 1.65	.493		0.79	0.35 to 1.74	.552
PIK3CA:rs7621329								
CC	44/71	1 (reference)			43/69	1 (reference)		
CT	15/32	1.27	0.57 to 2.84	.562	15/30	1.16	0.51 to 2.61	.726
TT	3/6	1.64	0.36 to 7.37	.519	3/6	1.68	0.37 to 7.64	.501
CT + TT		1.34	0.64 to 2.79	.442		1.25	0.59 to 2.63	.560
PIK3CA:rs6443624								
AA	44/64	1 (reference)			43/62	1 (reference)		
AC	18/40	1.57	0.74 to 3.35	.239	18/38	1.46	0.68 to 3.13	.328
CC	4/10	2.06	0.57 to 7.48	.270	4/10	2.02	0.55 to 7.33	.288
AC + CC		1.67	0.82 to 3.36	.155		1.56	0.77 to 3.19	.217

(continued on following page)

Table 3. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Response to Therapy (continued)

SNP and Genotype	Platinum Compound + Any				Taxane + Any			
	Response/No Response	OR*	95% CI	*P*	Response/No Response	OR*	95% CI	*P*
AKT1:rs3803304								
CC	18/45	1 (reference)			12/28	1 (reference)		
CG	26/32	0.45	0.19 to 1.04	.060	18/26	0.56	0.20 to 1.55	.265
GG	2/6	1.44	0.24 to 8.72	.694	3/4	0.67	0.11 to 4.05	.664
CG + GG		0.51	0.23 to 1.16	.108		0.58	0.22 to 1.54	.271
AKT1:rs2498804								
GG	15/33	1 (reference)			12/21	1 (reference)		
GT	27/44	0.67	0.28 to 1.61	.373	18/30	0.83	0.29 to 2.38	.736
TT	3/7	1.29	0.26 to 6.38	.752	4/6	0.90	0.18 to 4.49	.896
GT + TT		0.73	0.31 to 1.72	.470		0.85	0.31 to 2.34	.746
AKT1:rs2494738								
AA	41/69	1 (reference)			31/50	1 (reference)		
AG	5/15	1.79	0.56 to 5.72	.323	3/9	2.54	0.52 to 12.35	.248
AKT1:rs1130214								
GG	23/45	1 (reference)			19/27	1 (reference)		
GT	23/35	0.89	0.38 to 2.07	.790	14/28	1.39	0.51 to 3.85	.521
TT	1/4	2.07	0.20 to 21.71	.545	2/4	1.15	0.17 to 7.85	.886
GT + TT		0.94	0.41 to 2.17	.888		1.36	0.51 to 3.62	.540
AKT2:rs892119								
AA	37/56	1 (reference)			28/37	1 (reference)		
AG	10/24	2.18	0.85 to 5.60	.105	7/19	3.68	1.05 to 12.89	.042
GG	0/4				0/3			
AG + GG		2.46	0.97 to 6.23	.059		4.12	1.18 to 14.37	.026
AKT2:rs8100018								
CC	23/36	1 (reference)			20/29	1 (reference)		
CG	20/43	1.35	0.60 to 3.06	.471	11/24	1.22	0.43 to 3.44	.703
GG	3/4	0.85	0.15 to 4.67	.849	4/6	0.47	0.08 to 2.70	.396
CG + GG		1.29	0.58 to 2.85	.536		1.06	0.39 to 2.84	.914
FRAP1:rs11121704								
CC	27/42	1 (reference)			21/24	1 (reference)		
CT	18/35	1.12	0.49 to 2.56	.779	12/30	2.73	0.97 to 7.66	.057
TT	2/7	2.81	0.48 to 16.40	.250	2/5	2.94	0.47 to 18.52	.250
CT + TT		1.29	0.59 to 2.82	.521		2.76	1.04 to 7.37	.042
FRAP1:rs2295080								
GG	22/31	1 (reference)			15/18	1 (reference)		
GT	17/40	1.69	0.69 to 4.10	.248	16/35	2.11	0.74 to 6.03	.165
TT	5/7	1.15	0.29 to 4.49	.843	3/5	1.64	0.30 to 9.04	.569
GT + TT		1.55	0.68 to 3.58	.300		2.02	0.74 to 5.51	.172
PIK3CA:rs7651265								
AA	38/60	1 (reference)			29/42	1 (reference)		
AG	8/19	1.73	0.59 to 5.05	.317	4/15	2.10	0.55 to 8.02	.279
GG	0/2				0/0			
AG + GG		1.96	0.68 to 5.63	.211				
PIK3CA:rs7640662								
CC	32/67	1 (reference)			28/47	1 (reference)		
CG	13/17	0.53	0.21 to 1.35	.184	6/11	0.85	0.25 to 2.93	.794
GG	2/1	0.23	0.02 to 2.87	.255	1/1	0.97	0.04 to 24.89	.987
CG + GG		0.49	0.20 to 1.19	.116		0.86	0.27 to 2.79	.804
PIK3CA:rs7621329								
CC	29/50	1 (reference)			25/37	1 (reference)		
CT	12/24	1.19	0.45 to 3.15	.725	7/17	1.51	0.47 to 4.86	.490
TT	2/5	2.02	0.32 to 12.63	.452	2/3	0.94	0.14 to 6.45	.948
CT + TT		1.33	0.55 to 3.23	.530		1.35	0.47 to 3.85	.573
PIK3CA:rs6443624								
AA	30/48	1 (reference)			24/33	1 (reference)		
AC	14/27	1.28	0.51 to 3.21	.597	9/20	1.84	0.62 to 5.43	.271
CC	3/8	2.50	0.56 to 11.11	.227	2/5	1.36	0.22 to 8.58	.744
AC + CC		1.50	0.64 to 3.51	.353		1.72	0.64 to 4.61	.280

(continued on following page)

Table 3. PI3K/PTEN/AKT/mTOR Pathway Genotypes and Response to Therapy (continued)

SNP and Genotype	Any of the Three: Response/No Response	OR*	95% CI	P	Fluoropyrimidine + Any: Response/No Response	OR*	95% CI	P
PIK3CA:rs2699887								
AA	41/72	1 (reference)			41/69	1 (reference)		
AG	20/34	0.94	0.45 to 1.97	.880	19/33	0.98	0.47 to 2.08	.965
GG	3/6	1.06	0.22 to 5.06	.939	3/6	1.14	0.24 to 5.46	.874
AG + GG		0.96	0.48 to 1.93	.910		1.00	0.49 to 2.05	.991
PTEN:rs2299939								
AA	48/72	1 (reference)			48/68	1 (reference)		
AC	16/34	1.28	0.61 to 2.68	.507	16/34	1.33	0.64 to 2.79	.446
CC	1/7	4.63	0.51 to 42.07	.174	1/7	4.92	0.54 to 45.00	.158
AC + CC		1.47	0.72 to 2.98	.289		1.53	0.75 to 3.11	.243
PTEN:rs12569998								
GG	54/85	1 (reference)			53/84	1 (reference)		
GT	10/28	1.73	0.73 to 4.15	.216	10/25	1.50	0.62 to 3.64	.369
TT	1/1	0.64	0.04 to 11.45	.761	1/1	0.59	0.03 to 10.76	.725
GT + TT		1.63	0.70 to 3.79	.259		1.41	0.60 to 3.34	.430
PTEN:rs12357281								
CC	51/93	1 (reference)			51/89	1 (reference)		
CG	9/20	1.24	0.50 to 3.03	.642	8/20	1.44	0.57 to 3.66	.440
GG	1/0				1/0			
CG + GG		1.11	0.46 to 2.66	.813		1.26	0.51 to 3.10	.614

SNP and Genotype	Platinum Compound + Any: Response/No Response	OR*	95% CI	P	Taxane + Any: Response/No Response	OR*	95% CI	P
PIK3CA:rs2699887								
AA	26/54	1 (reference)			25/35	1 (reference)		
AG	17/23	0.54	0.22 to 1.31	.171	7/17	1.73	0.53 to 5.63	.362
GG	3/4	0.47	0.08 to 2.88	.417	2/5	1.94	0.28 to 13.46	.502
AG + GG		0.53	0.23 to 1.23	.139		1.78	0.61 to 5.22	.295
PTEN:rs2299939								
AA	36/51	1 (reference)			25/37	1 (reference)		
AC	9/26	1.84	0.73 to 4.65	.200	9/19	1.27	0.44 to 3.65	.663
CC	1/6	4.12	0.43 to 39.61	.220	0/2			
AC + CC		2.05	0.85 to 4.98	.112		1.50	0.53 to 4.22	.440
PTEN:rs12569998								
GG	39/62	1 (reference)			30/43	1 (reference)		
GT	6/21	2.22	0.74 to 6.67	.155	4/14	2.29	0.56 to 9.34	.246
TT	1/0				1/1	0.59	0.03 to 10.84	.723
GT + TT		1.89	0.66 to 5.39	.237		1.88	0.51 to 6.87	.342
PTEN:rs12357281								
CC	37/73	1 (reference)			26/44	1 (reference)		
CG	7/10	0.71	0.23 to 2.17	.549	5/14	1.82	0.53 to 6.20	.340
GG	0/0				1/0			
CG + GG						1.46	0.46 to 4.67	.522

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; AKT, v-akt murine thymoma viral oncogene homolog; mTOR, mammalian target of rapamycin.
*Adjusted for age, sex, smoking status, alcohol consumption, radiation dosage, chemoradiotherapy sequence, clinical stage, chemotherapy regimens, histologic tumor type, tumor location, pathologic stage, and histologic viability.

selection of the optimal therapy that would provide the most benefit and least toxicity for patients with EC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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