Associations of PI3KR1 and mTOR Polymorphisms with Esophageal Squamous Cell Carcinoma Risk and Gene-Environment Interactions in Eastern Chinese Populations

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Single nucleotide polymorphisms (SNPs) in the PI3K/PTEN/AKT/mTOR signaling pathway may contribute to carcinogenesis. We genotyped five potentially functional PIK3R1 and mTOR SNPs in 1116 esophageal squamous cell cancer (ESCC) patients and 1117 cancer-free controls to assess their associations with ESCC risk. We observed no association with ESCC risk for any of the selected SNPs. However, the combined analysis of these SNPs revealed that subjects with one-to-three risk genotypes had an increased ESCC risk. Stratified analysis by body mass index (BMI) found that ESCC risk was significantly associated with each of three mTOR SNPs among subjects with BMI ≤ 25.0. Specifically, we found that subjects carrying ≥ 1 risk genotypes had significantly increased ESCC risk, particularly for males, ever-smokers, ever-drinkers, and those with age ≥ 60, or BMI ≥ 25.0. Moreover, three mTOR haplotypes were associated with an increase in ESCC risk. Our meta-analysis of mTOR rs2295080 and cancer risk provided further evidence that mTOR SNPs might modulate cancer susceptibility. In this population, such risk effects might be modified by other risk factors, highlighting the importance of gene-environment interaction in esophageal carcinogenesis. Additional, larger studies are warranted to validate our findings.
Phosphatidylinositols (PtdIns) 3-kinases (PI3Ks), a family of lipid kinases, are divided into three different classes (I, II, and III), based on primary structure and biological features. Class I PI3Ks have been extensively studied because they are responsible for the production of PtdIns(3,4,5)P3 (also called PIP3) and PI3K catalytic subunit alpha (PIK3CA, alias: p110-α). PI3Ks debut in the cancer research field back in the mid-1980s. Since then, the dysregulation of the PI3K/PTEN/AKT/mTOR pathway has been observed in a variety of human cancers, including cancers of the endometrium, stomach, lung, and esophagus. Today, this pathway is well known to regulate important cellular events, including proliferation, adhesion, survival, and motility, which drive malignant transformation of cells and tumor progression. Growth factors and hormones, such as epidermal growth factor receptor (EGFR) and insulin growth factor-1 (IGF1), can stimulate class I PI3K by binding to the receptor tyrosine kinase (RTK). Activated class I PI3Ks convert PtdIns(4,5)P2 (called PIP2) to PIP3 by phosphorylating the hydroxyl group of the inositol ring of the former at the 3-position. The PIP3 then acts as a second messenger to trigger a downstream signaling cascade that is comprised of AKT, mTOR, and other proteins. Mammalian target of rapamycin/FK506 binding protein 12-rapamycin associated protein 1 (mTOR/FRAP1), a serine/threonine kinase, is a member of the PI3K-related kinase family and is known as a central effector of cell growth and proliferation through the regulation of protein synthesis.

Accumulating evidence has shown that mutations in some genes (PIK3CA, RAS, PTEN, and AKT) of the pathway could result in neoplastic transformation in both cellular and animal models, suggesting a critical role of the pathway in carcinogenesis. Aberrant activation of this pathway has been closely related to various cancers, including ESCC. For example, it was reported that 11.5% of the tumors from ESCC patients harbored PIK3CA mutations, and the aberrant activation of mTOR occurred in 69.5% and 25% of ESCC in Japanese patients and Caucasian patients in the Netherlands, respectively. The relatively high incidence of mutations in the PI3K pathway component provides strong evidence that dysregulation of this signaling pathway may contribute to the development of ESCC. Given the profound influence of aberrant activation of PI3K and mTOR on ESCC carcinogenesis, it is plausible that some potentially functional SNPs in genes encoding these proteins are likely to modulate ESCC susceptibility.

In contrast to extensive investigations regarding the mutations in these pathway genes, there are only a few studies exploring cancer risk associated with genetic variation in the same pathway genes. For example, Slattery et al. demonstrated that single nucleotide polymorphisms (SNPs) in PIK3CA and mTOR/FRAP1 genes were significantly associated with risk of colon and rectal cancers, respectively. Moreover, our group has previously reported associations of mTOR rs1883965 and rs2536 with risk of cancers of the esophagus, stomach, and prostate. Two independent studies indicated that the mTOR rs1883965 SNP was significantly associated with an increased risk of gastric cancer and ESCC.

In the present study, we expanded our previous studies by comprehensively analyzing additional potentially functional SNPs in the genes encoding class I PI3Ks and mTOR for their association with ESCC risk in an Eastern Chinese population.

### Results

#### Characteristics of the Study Population

Overall, demographic characteristics of the case and cancer-free controls were comparable (Table 1). No statistically significant difference was observed in the distributions of age and sex between the cases and controls. With respect to drinking and smoking habits, more cases tended to be smokers and drinkers in comparison with controls. Moreover, a significantly higher percentage of cases had BMI (weight in kilograms/height in meters) below 25.0, when compared with the controls. To minimize a possible confounding effect, these variables were then adjusted for in the subsequent multivariate logistic regression analyses.

| Variables                        | Cases No. (%) | Controls No. (%) | P*  |
|----------------------------------|---------------|------------------|-----|
| All subjects                     | 1,116 (100.0) | 1,117 (100.0)    | 0.890 |
| Age, yr                          | 37–88         | 32–86            |     |
| Range                            | 60.4 ± 8.3    | 60.3 ± 10.2      |     |
| Meanb                            |               |                  |     |
| Age group ≤50                    | 143 (12.8)    | 151 (13.5)       | 0.410 |
| 51–60                            | 423 (37.9)    | 405 (36.3)       |     |
| 61–70                            | 411 (36.8)    | 405 (36.3)       |     |
| >70                              | 139 (12.5)    | 156 (13.9)       |     |
| Sex                              |               |                  |     |
| Males                            | 897 (80.4)    | 882 (79.0)       | <0.0001 |
| Females                          | 219 (19.6)    | 235 (21.0)       |     |
| Drinking status                  |               |                  |     |
| Ever                             | 498 (44.6)    | 369 (33.0)       | 0.0038 |
| Never                            | 618 (55.4)    | 748 (67.0)       |     |
| Smoking status                   |               |                  |     |
| Ever                             | 681 (61.0)    | 614 (55.0)       |     |
| Never                            | 435 (39.0)    | 503 (45.0)       | <0.0001 |
| Pack-years                       |               |                  |     |
| ≤16 (mean)                      | 435 (39.0)    | 503 (45.0)       |     |
| >16 (mean)                      | 150 (13.4)    | 246 (22.0)       |     |
| Body mass index                  |               |                  | <0.0001 |
| <25.0                            | 721 (64.6)    | 485 (43.4)       |     |
| ≥25.0                            | 395 (35.4)    | 632 (56.6)       |     |

*Two-sided z test for distributions between cases and controls.

*aData were presented as mean ± SD.
Association between selected SNPs and ESCC risk. Three genes (PIK3R1, PIK3CA, and mTOR) were initially searched for potentially functional SNPs. However, we only investigated SNPs in the PIK3R1 and mTOR genes in this study, because no potentially functional SNP in the PIK3CA gene met the SNP selection criteria. The genotype frequency distributions of all the selected SNPs in control subjects were in accordance with the Hardy Weinberg equilibrium (HWE). The minor allele frequencies (MAFs) of the SNP in these controls were similar to those reported in the CHB data from HapMap: 0.19 vs. 0.175 for rs3730089, 0.12 vs. 0.078 for rs1057079, and 0.078 vs. 0.109 for rs1064261, respectively. Odds ratios (ORs) were determined by logistic regression analyses with adjustment for the covariates, i.e., age, sex, drinking status, smoking status, and BMI. Results including genotype frequencies, crude OR and 95% confidence interval (CI), and adjusted OR (95% CI) are shown in Table 2. Risk estimates revealed that subjects with one, two, or three risk genotypes had significantly or borderline significantly increased risk of developing ESCC, compared with those without such risk genotypes. However, significant ESCC risk associated with three risk genotypes were categorized into the other group. Compared with the reference group, those with one or more risk genotypes had statistically, significantly increased ESCC risk (adjusted OR = 1.33, 95% CI = 1.10–1.62).

Stratified Analysis. In an attempt to further scrutinize potential associations between the selected SNPs and ESCC risk, the data were stratified by the dichotomized variables of age, sex, smoking status, drinking status, and BMI, individually, under the dominant genetic model. No significant ESCC risk associated with any PIK3R1 and mTOR SNPs was detected in the dichotomized subgroups by age, sex, smoking, and drinking status (Tables 3–5). However, significant ESCC risk associated with three mTOR SNPs, but not PIK3R1 SNPs, was each individually observed among subjects with BMI < 25.0 under the dominant genetic model (WV/VV vs. WW) (rs2295080: adjusted OR = 1.36, 95% CI = 1.07–1.73; rs1057079: OR = 1.31, 95% CI = 1.03–1.67, rs1014261: OR = 1.39, 95% CI = 1.01–1.92) (Tables 3–5).

Since the three mTOR SNPs were not in complete LD, their individual effects might be additive. We further explored the combined effects of mTOR risk genotypes using logistic regression analyses with adjustment for the covariates (age, sex, smoking status, drinking status, and BMI).

Table 2 | Logistic regression analysis of associations between the genotypes of PIK3R1 & mTOR and ESCC risk

| Variants | Genotypes | Cases (N=1,116) | Controls (N=1,117) | P* | Crude OR (95% CI) | P | Adjusted OR (95% CI) | P** |
|----------|-----------|----------------|-------------------|----|------------------|---|---------------------|-----|
| PIK3R1 rs3730089 | GG | 736 (66.0) | 729 (65.4) | 0.880 | 1.00 | 1.00 | 0.763 |
| | AG | 331 (29.7) | 345 (30.9) | 0.95 (0.79–1.14) | 0.594 | 0.98 (0.81–1.19) | 0.857 |
| | AA | 48 (4.3) | 41 (3.7) | 1.16 (0.76–1.78) | 0.496 | 1.19 (0.76–1.85) | 0.452 |
| | AG/AA | 379 (34.0) | 386 (34.6) | 0.97 (0.82–1.16) | 0.755 | 1.00 (0.84–1.20) | 0.972 |
| PIK3R1 rs3730090 | CC | 837 (75.3) | 849 (76.4) | 0.463 | 1.00 | 1.00 | 0.475 |
| | CT | 255 (23.0) | 249 (22.3) | 1.04 (0.86–1.27) | 0.708 | 1.04 (0.85–1.28) | 0.690 |
| | TT | 19 (1.7) | 14 (1.2) | 1.38 (0.69–2.76) | 0.369 | 1.34 (0.65–2.75) | 0.434 |
| | CT/TT | 274 (24.7) | 263 (23.6) | 1.06 (0.87–1.28) | 0.578 | 1.06 (0.87–1.30) | 0.570 |
| mTOR rs2295080 | TT | 674 (60.6) | 702 (63.1) | 0.304 | 1.00 | 1.00 | 0.202 |
| | GT | 390 (35) | 362 (32.5) | 1.12 (0.94–1.34) | 0.199 | 1.13 (0.94–1.36) | 0.185 |
| | GG | 49 (4.4) | 49 (4.4) | 1.04 (0.69–1.57) | 0.841 | 1.12 (0.73–1.71) | 0.614 |
| | CT/GG | 439 (39.4) | 411 (36.9) | 1.11 (0.94–1.32) | 0.222 | 1.13 (0.95–1.36) | 0.167 |
| mTOR rs1057079 | TT | 702 (63.0) | 725 (65.0) | 0.321 | 1.00 | 1.00 | 0.248 |
| | CT | 367 (32.9) | 349 (31.3) | 1.09 (0.91–1.30) | 0.368 | 1.10 (0.91–1.32) | 0.322 |
| | CC | 45 (4.1) | 41 (3.7) | 1.13 (0.73–1.75) | 0.573 | 1.18 (0.75–1.85) | 0.484 |
| | CT/CC | 412 (37.0) | 390 (35.0) | 1.09 (0.92–1.30) | 0.324 | 1.11 (0.93–1.33) | 0.261 |
| mTOR rs1064261 | AA | 916 (82.2) | 945 (84.8) | 0.153 | 1.00 | 1.00 | 0.134 |
| | AG | 194 (17.4) | 164 (14.7) | 1.22 (0.97–1.53) | 0.085 | 1.22 (0.96–1.55) | 0.098 |
| | GG | 4 (0.4) | 6 (0.5) | 0.69 (0.19–2.45) | 0.563 | 0.87 (0.24–3.22) | 0.840 |
| | AG/GG | 198 (17.8) | 170 (15.2) | 1.20 (0.96–1.50) | 0.109 | 1.21 (0.96–1.53) | 0.108 |
| Combined effect of risk genotypes | 0 | 273 (24.6) | 322 (29.1) | 1.00 | 1.00 | 1.00 | 0.107 |
| | 1 | 362 (32.6) | 338 (30.6) | 1.27 (1.03–1.58) | 0.029 | 1.34 (1.07–1.68) | 0.012 |
| | 2 | 177 (16.0) | 157 (14.2) | 1.34 (1.03–1.75) | 0.032 | 1.42 (1.07–1.87) | 0.014 |
| | 3 | 209 (18.9) | 200 (18.1) | 1.24 (0.97–1.60) | 0.089 | 1.29 (0.99–1.67) | 0.058 |
| | 4 | 83 (7.48) | 83 (7.50) | 1.19 (0.84–1.68) | 0.322 | 1.21 (0.85–1.73) | 0.293 |
| | 5 | 5 (0.45) | 6 (0.54) | 0.99 (0.30–3.28) | 0.988 | 1.27 (0.35–4.54) | 0.717 |

*P<0.05.
**P<0.05.
†For additive genetic models. The results were in bold, if the 95% CI excluded 1 or P<0.05.
‡Adjusted for age, sex, smoking and drinking status in logistic regress models.
§Risk genotypes used for the calculation were PIK3R1 rs3730089 AG/AA + PIK3R1 rs3730090 CT/TT + mTOR rs2295080 GT/GG + mTOR rs1057079 CT/CC + mTOR rs1064261 AG/GG.
### Table 3 | Stratification analysis for associations between variant genotypes of PIK3R1 and ESCC risk

| Variables         | PIK3R1 3730089 (cases/controls) | PIK3R1 3730090 (cases/controls) |
|-------------------|---------------------------------|---------------------------------|
|                   | GG          | AG/AA    | Crude OR (95% CI) | P  | Adjusted OR (95% CI) | P  | P<sub>hom</sub> | CC          | CT/TT        | Crude OR (95% CI) | P  | Adjusted OR (95% CI) | P  | P<sub>hom</sub> |
| Age               |             |          |                  |    |                    |    |                  |             |              |                  |    |                    |    |                  |
| ≤60               | 373/365     | 192/190  | 0.99 (0.77–1.23) | 0.929 | 0.97 (0.74–1.25) | 0.790 | 0.853            | 415/420     | 150/132       | 1.13 (0.87–1.49) | 0.363 | 1.20 (0.90–1.59) | 0.222 | 0.455          |
| >60               | 363/364     | 187/196  | 0.96 (0.75–1.23) | 0.726 | 1.03 (0.79–1.34) | 0.844 | 0.97 (0.74–1.29) | 422/429     | 128/131       | 0.98 (0.74–1.29) | 0.873 | 0.96 (0.72–1.28) | 0.770 | 0.337          |
| Sex               |             |          |                  |    |                    |    |                  |             |              |                  |    |                    |    |                  |
| Females           | 153/152     | 65/82    | 0.79 (0.53–1.17) | 0.236 | 0.78 (0.51–1.18) | 0.248 | 0.174            | 158/181     | 60/53         | 1.27 (0.83–1.95) | 0.266 | 1.40 (0.88–2.22) | 0.153 | 0.337          |
| Males             | 582/576     | 314/305  | 1.02 (0.84–1.24) | 0.825 | 1.08 (0.88–1.33) | 0.450 |                 | 679/668     | 217/213       | 1.01 (0.81–1.25) | 0.948 | 0.98 (0.78–1.23) | 0.871 | 0.337          |
| Smoking status    |             |          |                  |    |                    |    |                  |             |              |                  |    |                    |    |                  |
| Never             | 286/321     | 149/182  | 0.92 (0.70–1.20) | 0.538 | 0.92 (0.70–1.21) | 0.542 | 0.556            | 318/386     | 115/115       | 1.21 (0.90–1.64) | 0.203 | 1.25 (0.92–1.71) | 0.157 | 0.239          |
| Ever              | 450/408     | 230/204  | 1.02 (0.81–1.29) | 0.852 | 1.06 (0.83–1.36) | 0.620 |                 | 519/463     | 159/148       | 0.96 (0.74–1.24) | 0.745 | 0.99 (0.76–1.30) | 0.957 | 0.337          |
| Drinking status   |             |          |                  |    |                    |    |                  |             |              |                  |    |                    |    |                  |
| Never             | 416/494     | 202/252  | 0.95 (0.76–1.19) | 0.670 | 0.97 (0.76–1.22) | 0.776 | 0.257            | 450/565     | 166/178       | 1.17 (0.92–1.50) | 0.207 | 1.16 (0.90–1.50) | 0.256 | 0.271          |
| Ever              | 298/235     | 199/134  | 0.97 (0.73–1.28) | 0.832 | 1.09 (0.81–1.47) | 0.574 |                 | 387/284     | 108/85        | 0.93 (0.68–1.29) | 0.670 | 0.90 (0.63–1.25) | 0.507 | 0.271          |
| BMI < 25.0        | 485/320     | 235/164  | 0.95 (0.74–1.21) | 0.677 | 0.95 (0.74–1.22) | 0.696 | 0.583            | 541/365     | 179/119       | 1.02 (0.78–1.33) | 0.903 | 1.02 (0.78–1.33) | 0.897 | 0.784          |
| BMI ≥25.0         | 251/409     | 143/222  | 1.05 (0.81–1.37) | 0.717 | 1.03 (0.79–1.35) | 0.824 |                 | 297/484     | 97/147        | 1.08 (0.80–1.45) | 0.632 | 1.10 (0.82–1.49) | 0.530 | 0.337          |

CI, confidence interval; OR, odds ratio.

*Obtained in logistic regression models with adjustment for age, sex, smoking status and drinking status.

P<sub>hom</sub> derived from the homogeneity test.

The results were in bold, if the 95% CI excluded 1 or P < 0.05. 
Table 4 | Stratification analysis for associations between variant genotypes of mTOR and ESCC risk

| Variables          | mTOR rs1057079   | mTOR rs1014261   |
|--------------------|------------------|------------------|
|                    | (cases/controls) | (cases/controls) |
|                    | TT               | CT/CC            | Crude OR (95% CI) | P      | Adjusted OR (95% CI) | P      | P_{hom} | AA       | AG/GG   | Crude OR (95% CI) | P      | Adjusted OR (95% CI) | P      | P_{hom} |
| Age                |                  |                  |                   |        |                    |        |        |          |         |                    |        |                    |        |        |
| ≤60                | 364/362          | 201/193          | 1.04 (0.81–1.32)  | 0.780  | 1.05 (0.81–1.37)   | 0.692  | 0.550  | 464/468  | 101/87  | 1.16 (0.84–1.58)  | 0.368  | 1.20 (0.86–1.67)   | 0.276  | 0.727   |
| >60                | 338/363          | 212/197          | 1.15 (0.90–1.47)  | 0.261  | 1.15 (0.89–1.48)   | 0.284  | 451/477 | 99/83    | 1.25 (0.91–1.72) | 0.172  | 1.27 (0.91–1.77) | 0.167  |         |
| Sex                |                  |                  |                   |        |                    |        |        |          |         |                    |        |                    |        |        |
| Females            | 142/150          | 76/84            | 0.96 (0.65–1.41)  | 0.818  | 1.01 (0.67–1.53)   | 0.956  | 0.453  | 179/201  | 39/33   | 1.33 (0.80–2.20)  | 0.273  | 1.24 (0.71–2.15)   | 0.451  | 0.665   |
| Males              | 560/575          | 336/306          | 1.13 (0.93–1.37)  | 0.225  | 1.13 (0.93–1.39)   | 0.226  | 737/744 | 159/137  | 1.17 (0.91–1.50) | 0.215  | 1.17 (0.99–1.52) | 0.243  |         |
| Smoking status     |                  |                  |                   |        |                    |        |        |          |         |                    |        |                    |        |        |
| Never              | 267/314          | 166/188          | 1.04 (0.80–1.35)  | 0.780  | 1.02 (0.77–1.34)   | 0.897  | 0.566  | 352/414  | 81/87   | 1.10 (0.79–1.53)  | 0.594  | 1.08 (0.77–1.52)   | 0.678  | 0.405   |
| Ever               | 435/411          | 246/202          | 1.15 (0.91–1.45)  | 0.231  | 1.21 (0.95–1.54)   | 0.132  | 564/531 | 117/83   | 1.33 (0.98–1.80) | 0.069  | 1.31 (0.95–1.80) | 0.104  |         |
| Drinking status    |                  |                  |                   |        |                    |        |        |          |         |                    |        |                    |        |        |
| Never              | 388/480          | 228/266          | 1.06 (0.85–1.32)  | 0.604  | 1.08 (0.86–1.36)   | 0.516  | 0.631  | 506/636  | 110/110 | 1.25 (0.94–1.68)  | 0.121  | 1.24 (0.92–1.66)   | 0.167  | 0.585   |
| Ever               | 314/245          | 184/124          | 1.16 (0.87–1.54)  | 0.309  | 1.16 (0.86–1.57)   | 0.322  | 410/309 | 88/60    | 1.11 (0.77–1.58) | 0.585  | 1.10 (0.75–1.61) | 0.632  |         |
| BMI                |                  |                  |                   |        |                    |        |        |          |         |                    |        |                    |        |        |
| <25.0              | 437/323          | 283/161          | 1.30 (1.02–1.65)  | 0.033  | 1.31 (1.03–1.67)   | 0.031  | 0.022  | 585/415  | 135/69  | 1.39 (1.01–1.91)  | 0.042  | 1.39 (1.01–1.92)   | 0.043  | 0.167   |
| ≥25.0              | 265/402          | 129/229          | 0.86 (0.66–1.12)  | 0.246  | 0.87 (0.67–1.14)   | 0.311  | 331/530 | 63/101   | 1.00 (0.71–1.41) | 0.994  | 0.99 (0.70–1.40) | 0.962  |         |

CI, confidence interval; OR, odds ratio.

*Obtained in logistic regression models with adjustment for age, sex, smoking status and drinking status.

P_{hom} derived from the homogeneity test.

The results were in bold, if the 95% CI excluded 1 or P < 0.05.
Table 5 | Stratification analysis for associations between variant genotypes of mTOR and ESCC risk

| Variables | mTOR rs2295080 | Combined risk genotypes |
|-----------|----------------|-------------------------|
|           | (cases/controls) | Crude OR (95% CI) | (cases/controls) | Crude OR (95% CI) | Adjusted OR* (95% CI) | Adjusted OR* (95% CI) |
| Age       |                |                       |                |                       |                        |                        |
| <60       | 342/350        | 223/205               | 1.12 (0.88–1.42) | 0.363                 | 1.14 (0.89–1.48)       | 0.303                   | 325/341               | 240/214               | 1.18 (0.93–1.49)       | 0.182                   | 1.20 (0.93–1.55)       | 0.157                   | 0.674                  |
| >60       | 332/345        | 218/205               | 1.11 (0.87–1.41) | 0.414                 | 1.12 (0.87–1.44)       | 0.381                   | 312/350               | 238/210               | 1.27 (1.00–1.61)       | 0.055                   | 1.28 (1.01–1.65)       | 0.049                   |                        |
| Sex       |                |                       |                |                       |                        |                        | 504/546               | 392/335               | 1.28 (1.01–1.65)       | 0.049                   | 1.28 (1.01–1.65)       | 0.049                   |                        |
| Females   | 136/146        | 92/88                 | 1.00 (0.81–1.21) | 0.529                 | 1.05 (0.87–1.26)       | 0.542                   | 134/146               | 84/88                 | 1.04 (0.71–1.52)       | 0.837                   | 1.11 (0.74–1.66)       | 0.631                   | 0.362                  |
| Males     | 538/556        | 358/325               | 1.14 (0.94–1.38) | 0.174                 | 1.14 (0.94–1.38)       | 0.181                   | 504/546               | 392/335               | 1.27 (1.05–1.53)       | 0.014                   | 1.28 (1.05–1.56)       | 0.014                   |                        |
| Smoking status |                |                       |                |                       |                        |                        | 392/312               | 264/256               | 1.27 (1.05–1.53)       | 0.014                   | 1.28 (1.05–1.56)       | 0.014                   |                        |
| Never     | 249/303        | 184/198               | 1.13 (0.87–1.47) | 0.357                 | 1.12 (0.85–1.47)       | 0.419                   | 237/299               | 198/204               | 1.22 (0.95–1.59)       | 0.126                   | 1.21 (0.92–1.58)       | 0.166                   | 0.937                  |
| Ever      | 425/399        | 255/213               | 1.12 (0.90–1.41) | 0.315                 | 1.18 (0.93–1.51)       | 0.404                   | 401/393               | 280/221               | 1.24 (0.99–1.55)       | 0.059                   | 1.31 (1.03–1.67)       | 0.028                   |                        |
| Drinking status |            |                       |                |                       |                        |                        | 261/235               | 198/190               | 1.31 (1.03–1.67)       | 0.028                   | 1.34 (1.00–1.79)       | 0.051                   |                        |
| Never     | 376/467        | 240/277               | 1.18 (0.86–1.64) | 0.513                 | 1.11 (0.88–1.39)       | 0.388                   | 357/460               | 261/288               | 1.17 (0.94–1.45)       | 0.167                   | 1.20 (0.95–1.50)       | 0.119                   | 0.527                  |
| Ever      | 298/235        | 199/134               | 1.17 (0.89–1.55) | 0.265                 | 1.20 (0.90–1.62)       | 0.216                   | 281/232               | 217/137               | 1.31 (0.99–1.72)       | 0.056                   | 1.34 (1.00–1.79)       | 0.051                   |                        |
| BMI       |                |                       |                |                       |                        |                        | 392/312               | 328/172               | 1.51 (1.19–1.91)       | 0.001                   | 1.52 (1.20–1.94)       | 0.001                   | 0.006                  |
| <25.0     | 420/315        | 300/169               | 1.34 (1.06–1.70) | 0.016                 | 1.36 (1.07–1.73)       | 0.014                   | 392/312               | 328/172               | 1.51 (1.19–1.91)       | 0.001                   | 1.52 (1.20–1.94)       | 0.001                   | 0.006                  |
| ≥25.0     | 256/386        | 138/245               | 0.87 (0.67–1.13) | 0.285                 | 0.88 (0.67–1.14)       | 0.323                   | 244/380               | 150/251               | 0.92 (0.71–1.29)       | 0.544                   | 0.95 (0.73–1.24)       | 0.711                   |                        |

CI, confidence interval; OR, odds ratio.
*Obtained in logistic regression models with adjustment for age, sex, smoking status and drinking status.
$P_{hom}$ derived from the homogeneity test.
The results were in bold, if the 95% CI excluded 1 or $P < 0.05$. 
effects of these three SNPs in stratified analyses by age, sex, smoking status, drinking status, and BMI and found that significantly increased ESCC risk was identified for subjects carrying at least one of the three putative risk genotypes (i.e., rs2295080 CT/GG, rs1057079 CT/CC, and rs1014261 AG/GG) among the following subgroups: >60 years of age (adjusted OR = 1.28, 95% CI = 1.01–1.65), males (adjusted OR = 1.28, 95% CI = 1.05–1.56), ever-smokers (adjusted OR = 1.31, 95% CI = 1.03–1.67), ever-drinkers (adjusted OR = 1.34, 95% CI = 1.00–1.79) or BMI < 25.0 (adjusted OR = 1.52, 95% CI = 1.20–1.94) (Table 5). Moreover, while evaluating the strength of associations between mTOR SNPs and ESCC risk among subgroups with BMI < 25.0, the OR (1.52, 95% CI = 1.20–1.94) of combined risk genotypes was larger than the ORs (1.31, 95% CI = 1.03–1.67 for rs1057079; 1.39, 95% CI = 1.01–1.92 for rs1014261; 1.36, 95% CI = 1.07–1.73 for rs2295080) of any individual risk genotype (Table 5), indicating that there was likely a combined effect of these three SNPs. These findings suggested that the effect of each SNP is likely necessary but not sufficient, depending on the presence of other genetic variants.

Association of High-Order Interactions with ESCC Risk by Multiple Dimension Reduction (MDR) Analysis. To further investigate the existence of possible gene-environmental interaction in association with ESCC risk, high-order interactions assessed by using the MDR analysis was performed with inclusion of the five selected SNPs (i.e., rs3730089, rs3730090, rs2295080, rs1057079, and rs1014261) and five known risk factors (i.e., age, sex, smoking status, drinking status, and BMI). In the MDR analysis, BMI was the best one-factor model with the highest cross-validation consistency (CVC) and the lowest prediction error among all ten factors, indicating that BMI was the strongest risk factor for ESCC. Moreover, the ten-factor model had a maximum CVC and a minimum prediction error, with the prediction error being statistically significant (Table 6). Taken together, the ten-factor model showed a better prediction than the one-factor model and represented the best model to predict ESCC risk for this study population.

mTOR Haplotypes and ESCC Risk. Since PIK3R1 and mTOR are located in different chromosomes, we only explored whether the haplotypes of three mTOR SNPs would influence ESCC risk. As presented in Table 7, seven mTOR haplotypes were identified. When the most frequent haplotype T-T-A was used as the reference group, three haplotypes, T-C-A, T-C-G, and G-T-A, were significantly associated with increased ESCC risk.

Gene-Gene and Gene-Environment Interactions. As presented in Table 8, logistic regression analyses identified significant gene-environment interactions of BMI with smoking status and drinking status were also noticeable (Table 8).

Meta-analysis for the Association between mTOR rs2295080 and Cancer Risk. To date, six published studies have explored the association of mTOR rs2295080 with the risk of various cancers but yielded conflicting results8–10,13,25,26, whereas fewer studies on other mTOR SNPs have been published. To better evaluate such an association, we performed a meta-analysis with all published studies and our new data, leading to a total of 4772 cases and 5264 controls. When all the data were combined, the mTOR rs2295080 SNP appeared to be modestly protective and significantly associated with a decreased cancer risk under most of the genetic models

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**Table 6** | MDR analysis for the prediction of ESCC risk with and without PIK3R1 & mTOR genotypes

| Best interaction models | Cross-validation | Average prediction error | P a |
|-------------------------|-----------------|--------------------------|-----|
| 1                       | 100/100         | 0.394                    | < 0.0001 |
| 1, 2                    | 100/100         | 0.394                    | < 0.0001 |
| 1, 2, 3                 | 96/100          | 0.386                    | < 0.0001 |
| 1, 2, 3, 4              | 93/100          | 0.379                    | < 0.0001 |
| 1, 2, 3, 4, 5           | 100/100         | 0.370                    | < 0.0001 |
| 1, 2, 3, 4, 5, 6        | 100/100         | 0.367                    | < 0.0001 |
| 1, 2, 3, 4, 5, 6, 7     | 92/100          | 0.358                    | < 0.0001 |
| 1, 2, 3, 4, 5, 6, 7, 8  | 94/100          | 0.350                    | < 0.0001 |
| 1, 2, 3, 4, 5, 6, 7, 8, 9  | 100/100 | 0.341                    | < 0.0001 |
| 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | 100/100 | 0.340                    | < 0.0001 |

MDR, multifactor dimensionality reduction.

a Obtained in logistic regression models with adjustment for age, sex, smoking status and drinking status.

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**Table 7** | Haplotype analysis for genotypes of mTOR and ESCC risk

| Haplotype frequencies | Cases (N=2232) | Controls (N=2234) | Crude OR (95% CI) | P | Adjusted OR (95% CI) | P a |
|-----------------------|---------------|-------------------|------------------|---|----------------------|---|
| T-T-A                 | 1700          | 76.23             | 1746             | 78.72 | 1.00                | 0.009 | 1.00 |
| T-CA                  | 23            | 1.03              | 9                | 0.41 | 2.63 (1.21–5.69)    | 0.015 | 2.96 (1.32–6.67)    | 0.023 |
| T-CG                  | 19            | 0.85              | 6                | 0.27 | 3.25 (1.30–8.16)    | 0.012 | 2.97 (1.16–7.60)    | 0.024 |
| G-TA                  | 40            | 1.79              | 18               | 0.81 | 2.28 (1.30–4.00)    | 0.004 | 2.41 (1.34–4.37)    | 0.003 |
| G-TG                  | 33            | 1.48              | 27               | 1.22 | 1.26 (0.75–2.10)    | 0.385 | 1.38 (0.81–2.35)    | 0.234 |
| T-CG                  | 265           | 11.88             | 271              | 12.22 | 1.01 (0.84–1.21)    | 0.963 | 1.03 (0.85–1.24)    | 0.795 |
| G-TA                  | 150           | 6.73              | 141              | 6.36 | 1.09 (0.86–1.39)    | 0.468 | 1.12 (0.87–1.43)    | 0.380 |

a Obtained in logistic regression models with adjustment for age, sex, smoking status and drinking status.
tested without obvious among-study heterogeneity (homozygous: OR = 0.79, 95% CI = 0.66–0.95; heterozygous: OR = 0.88, 95% CI = 0.78–1.02, dominant: OR = 0.87, 95% CI = 0.80–0.94, recessive: OR = 0.82, 95% CI = 0.69–0.90) (Figure 1). Moreover, the shape of funnel plots seemed symmetrical and Egger’s test showed no significance (data not shown), suggesting no publication bias.

**Discussion**

Knowledge on the genetics of cancer can help health care professionals in providing high-risk individuals with better decisions on prevention and intervention strategies, such as cancer screening, early detection, and targeted therapy. Numerous studies have indicated that potentially functional SNPs in the important genes may confer host genetic susceptibility to cancer. Aberrant activation of the PI3K/PTEN/AKT/mTOR signaling pathway is a common event in a wide range of tumor types, suggesting a role for this pathway in carcinogenesis. In the present study, however, none of the studied SNPs in the PIKRI and mTOR genes exhibited an association with ESCC risk. However, the combined analysis of these SNPs revealed significant risk associations with one, two, and three risk genotypes, compared with zero risk genotype. Actually, lack of the main effect of individual SNPs on cancer risk does not necessarily rule out these SNPs as etiologic factors, because these SNPs may have low penetrance in cancer susceptibility, compared with environmental and lifestyle factors contributing to the risk. It is likely, however, that the relevant exposure, such as smoking, alcohol intake, and hormonal disorder, may interact with genetic factors27. This was the case in the present study. For example, our stratified analysis by BMI suggested a significantly increased ESCC risk associated with mTOR rs2295080, rs1057079, and rs1014261, individually, among subjects with BMI<25.0. When risk genotypes of these three mTOR SNPs were combined, subjects with ≥1 risk genotype exhibited an increased ESCC risk in the older participants (age>60), males, smokers, drinkers, or those with BMI<25.0, supporting gene-environment interactions on ESCC susceptibility.

Although few studies have investigated cancer risk associated with the SNPs we studied, some reported findings that are in line with ours. For example, one study observed an association between mTOR rs2295080 and a reduced risk of renal cancer in 710 cases and 760 controls25. In addition, mTOR rs2295080 was also shown to protect against gastric cancer risk in a Chinese population10. Functional analysis demonstrated that the rs2295080 variant G allele reduced transcriptional activity in both normal gastric mucosa epithelial cell lines (GES-1) and three different gastric cancer cell lines, compared with the wide-type T allele10. Moreover, mTOR mRNA expression levels in gastric cancer tissues with GT/GG genotypes were significantly lower than those with the TT genotype10, indicating that mTOR rs2295080 may decrease gastric cancer risk by affecting mTOR transcription.

Similarly, our meta-analysis of seven studies with 4772 cases and 5264 controls found that rs2295080 was significantly associated with a reduced cancer risk under homogenous (GG vs.TT) and recessive (GG vs.TT/TG) genetic models. However, due to the relatively small sample size in the current meta-analysis, large single studies with different cancer types and ethnic groups are needed to validate our findings. Moreover, additional meta-analyses with stratified analyses by cancer type are warranted to further determine the effect of this SNP on the risk of each specific cancer. For example, in contrast with other cancers, our study indicated that mTOR rs2295080 variant

| Study ID | Country | Site       | Cases/Controls | OR (95% CI) | % weight |
|----------|---------|------------|----------------|-------------|----------|
| Cao (2012) | China   | Kidney     | 710/760        | 0.90 (0.58, 1.40) | 16.00    |
| Chen (2012) | China   | Prostate   | 666/708        | 0.82 (0.49, 1.36) | 13.00    |
| Huang (2012) | China  | Blood      | 417/554        | 1.48 (0.81, 2.72) | 6.63     |
| Xu (2012) | China   | Stomach    | 753/854        | 0.53 (0.33, 0.86) | 18.33    |
| Wang (2012) | USA     | Endometrium| 113/219        | 0.53 (0.26, 1.08) | 8.84     |
| Li (2013) | China   | Prostate   | 1004/1051      | 0.80 (0.52, 1.22) | 18.98    |
| Zhu (Present) | China | Esophagus  | 1116/1117      | 1.00 (0.67, 1.50) | 18.22    |
| Overall (I² = 35.2%, P = 0.159) |          |             |                | 0.82 (0.69, 0.99) | 100.00   |

Figure 1 | Forest plot of overall cancer risk associated with mTOR rs2295080 (a genetic recessive model). This was derived from a meta-analysis of seven relevant case-control studies. The OR and 95% CI of each study are plotted with a box and a horizontal line. Quadrangles represent pooled ORs and 95% CIs; Chi², chi-square; df, degrees of freedom; I², index of heterogeneity.
In the present study, significant mTOR SNP-related increases in ERCC risk were detected among subjects with BMI < 25.0, but not among those with BMI ≥ 25.0, suggesting that BMI was a significant effect modifier of ERCC risk associated with mTOR SNPs. To support this finding, further MDR testing of the high-order interaction analysis consistently recognized BMI as a main risk factor for ESCC, which is consistent with the fact that body weight is reversely associated with risk of ESCC as well as with smoking, drinking, and nutrition. For example, previous studies conducted in both Western countries and China found that both poor nutrition and low BMI were associated with an increased ESCC risk. In the present study, we found a significant interaction between BMI and either mTOR rs1057079 or rs2295080 SNPs. Our results also showed that mTOR rs2295080 significantly interacted with either mTOR rs1057079 or rs1064261, suggesting that these SNPs of interest might collectively confer and modulate ESCC susceptibility.

There are some limitations in the present study. First, although age, sex, smoking, drinking, and BMI were considered, there were also a number of uncollected factors contributing to ESCC risk, including nutrition status; intake of hot beverages, fruits, vegetables; other genetic variations; and socioeconomic status. Failure to adequately control for these factors limited our ability to analyze gene-gene and gene-environment interactions. Second, the number of SNPs genotyped in the manuscript was also very limited, and some potentially functional SNPs in these two genes might be missed. Third, we performed multiple comparisons (single SNPs, SNPs combined, haplotypes, stratified by age, sex, BMI, etc.) in the present study, which may have led to chance findings (e.g., false positive findings). Therefore, these results should be interpreted with caution. Larger, more stringently designed studies are needed to validate our findings. Moreover, PIK3CA is a well-known oncogene, the activation of which has been implicated in various cancers. Failure to investigate the association of PIK3CA polymorphisms with ESCC risk is also a potential limitation.

In summary, we found that rs2295080, rs1057079, and rs1064261 SNPs in the mTOR gene may modify the host’s genetic susceptibility to ESCC risk; however, these effects were largely dependent on other risk factors, i.e., BMI, age, sex, smoking and drinking status. Our results emphasize the importance of gene-environment interactions in determining the ESCC susceptibility, supporting the idea that the low-penetrant genetic effects of common SNPs on cancer predisposition may be fundamentally governed by the interplaying of SNPs and specific environmental exposures during the process of carcinogenesis.

**Methods**

**Study Population.** This case-control study was conducted at Fudan University, Shanghai Cancer Center. Briefly, the cases (n = 1116) were patients with newly-diagnosed and histopathologically confirmed ESCC from March 2009 to September 2011, who were all genetically unrelated Han Chinese and residents in Eastern China. The patients who had one or more of the following features were excluded: other types of cancer, primary tumors outside the esophagus, and cancers with unknown primary sites. Age and sex-matched, cancer-free controls (n = 1117) were selected from the Taizhou cohort following a procedure of matching with cases on age (± 5 years) and sex. A structured questionnaire was used to obtain the following information from each of the participants during personal interviews: demographic data and specific environmental exposures during the process of carcinogenesis.

**SNP Selection and Genotyping.** We searched the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) for
common, potentially functional SNPs in the PIK3CA, PIK3R1 and mTOR genes based on the following criteria: (1) located at exons, the 5'-near gene, 5'-untranslated regions (UTR), 3'-UTR, 3'-near gene and splice sites; (2) the minor allele frequency (MAF) ≥ 5% in Chinese Han population; (3) potentially functional SNPs as predicted by SNPinfo software (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); (4) having low linkage disequilibrium (LD) with each other using an r² threshold of <0.8; and (5) not investigated in the published genome-wide association studies (GWAS) of ESCC. No SNP in the PIK3CA gene met the criteria. Ultimately, five SNPs (PIK3CA: rs7330089 and rs7330090; mTOR: rs1057079, rs1064261, and rs2295080) for the study. The SNP selection process is indicated in Figure 2.

We isolated genomic DNA from blood samples by using the Qiagen Blood DNA Mini Kit (Qiagen Inc., Valencia, CA) and performed the TaqMan assay for genotyping as described previously. Briefly, we labeled allele-specific probes for SNPs of interest with the fluorescent dyes VIC and FAM. During extension, the 5'-exonuclease activity of the Taq polymerase cleaves the fluorophore from the non-fluorescent quencher. By using the ABI 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA), we used a post-amplification allelic discrimination run on the machine to determine genotypes based on the relative amount of fluorescence of VIC and FAM. Finally, we performed PCR reactions in a total reaction volume of 5 μl in 384-well plates. Individuals involved in genotyping were blind to patient status.

Statistical Methods. We used the χ² test to assess differences in the frequency distributions of the selected demographic variables, risk factors, and genotypes of the selected SNPs between the cases and controls. We tested the Hardy–Weinberg equilibrium (HWE) for genotype distribution in controls by a goodness-of-fit χ² test. Crude and adjusted ORs and their 95% CIs for the association of ESCC risk with selected SNPs were calculated using both univariate and multivariate logistic regression analyses with adjustment for co-variates including age, sex, smoking, drinking, and BMI, respectively. These co-variates were selected because of their importance in possible interaction with genetic factors, and were entered into the model at the same time as a group of categorical variables defined in Table 3. The P-value for multiplicative interaction between these selected SNPs and co-variates (age, sex, BMI, etc) was calculated by adding the product terms to the logistic regression model. A two-tailed P < 0.05 was used as the criterion of statistical significance. We also evaluated the associations in stratified analyses by age, sex, smoking, and drinking status. Four genetic models, 1) homozygous (WW vs. VV), 2) heterozygous (WW vs. WW, VV), 3) dominant (WW vs. WW/VV), and 4) recessive (WW/ WW vs. VV), were adopted for these analyses, with W and V representing wild and variant alleles of each SNP, respectively. In the present study, we defined the haplotype as a combination of rs2295080, rs1014261, and rs1057079 SNPs in the mTOR gene. The unphased genotype data were used to determine haplotype frequencies and individual haplotypes. Logistic regression analysis was performed to calculate ORs for the association of haplotypes with ESCC risk, while the haplotype of the highest frequency was considered as the reference group. Moreover, genotypes with one or two variant alleles of a SNP were referred to as risk genotype. Risk genotypes for the association with PIK3R1 rs7330089 AG/AA, PIK3R1 rs7330090 CT/TT, mTOR rs2295080 GT/GG, mTOR rs1057079 CT/CC, mTOR rs1064261 AG/GG. All tests were two-sided, and a P <0.05 was considered statistically significant. All statistical analyses were performed with SAS software (version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC). Furthermore, we used MDR software (V2.0 version 9.1; SAS Institute, Cary, NC).
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Author contributions
Conceived and designed the experiments: Q.Y.W., J.Q.X. Performed the experiments: J.H.Z., M.Y.W. Analyzed the data: J.H.Z., M.Y.W. Contributed reagents/materials/analysis tools: M.L.Z., J.H., J.C.W., J.L., X.F.W., Q.Y.W. Wrote the paper: J.H.Z., M.Y.W., J.Q.X., Q.Y.W.

Additional information
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