Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males according to the latest report released by the International Agency for Research on Cancer (IARC) in 2008 [1]. It has been well established that PCa is one of the pronounced geographically and ethnically related human malignancies, with a much higher incidence observed in the Western world than in Asian countries [2]. Recently, accumulated evidence from genome-wide association studies (GWASs) suggests that more than 40 single nucleotide polymorphisms (SNPs) are associated with human PCa risk, some of which were also confirmed in Chinese male populations. However, almost all the candidate SNPs are reported to be in weak associations with PCa risk to date [3–6]. Therefore, it is still not fully understood to what extent genetic factors and their interactions with environmental attributes may play a role in the etiology of PCa.

The phosphoinositide-3 kinase-AKT-mammalian target of rapamycin pathway (PI3K/AKT/mTOR) is a major pathway controlling cell growth and tumorigenesis [7,8]. As a key downstream effector of PI3K/AKT/mTOR pathway, the mTOR has been confirmed to be a central regulator of vital cellular processes, such as cell growth, proliferation, metabolism, migration, and apoptosis, based on the in vivo and in vitro investigations [9–12]. Structurally, mTOR contains several important domains
across the whole protein, of these, the rapamycin-binding domain and the kinase domain was considered closely relevant to carcinogenesis [13]. Additionally, several studies have demonstrated that mTOR targeted therapies can be designed to block the induction of the proliferative, prosurvival, and oncogenic functions of mTOR [14]. Therefore, it was speculated that mTOR is a possible driver gene in carcinogenesis, and a promising target point and prognosis marker in cancer treatment as well.

Somatic aberrations of PI3K/AKT/mTOR pathway genes have been commonly observed in a variety of malignancies, including PCs [8]. And the mutations in the mTOR gene have been identified in a few human cancers [15]; however, the mechanism has not been well established to date. PCs harboring almost the same well-known mutations often presents with heterogeneous clinicopathologic characteristics. By the same token, almost the same well-known mutations often presents with heterogeneous clinicopathologic characteristics. Given that mTOR is one of the most important downstream components of the mTOR pathway, which can also receive signals from other pivotal pathways. Several studies have demonstrated that mTOR can serve as a promising therapeutic target in the future cancer treatment. And there have been few studies to date addressing the role of common, functional variants in the mTOR gene as PCa susceptibility factors, together with some variations of other pivotal genes in this pathway have been investigated as weak gene as PCa susceptibility factors, together with some variations of other pivotal genes in this pathway have been investigated as weak gene and their interactions with environmental factors.

Materials and Methods

Patients and controls

We recruited PCa patients and the matched cancer-free controls from genetically unrelated Chinese Han participants between January 2008 and January 2012. This analysis included 1004 patients who were inhabitants of the administrative regions of eastern China (including Shanghai city, Zhejiang province, Jiangsu province and the surrounding areas) and have been histopathologically confirmed primary prostate adenocarcinoma and 1051 cancer-free controls in an Eastern Chinese Han population. We tested the hypothesis that risk of PCs may be associated with SNPs in the mTOR gene and their interactions with environmental factors.

Multifactor Dimensionality Reduction (MDR) Analysis

Evidence indicated that gene–gene and gene–environment interactions are difficult to be fully characterized by using logistic regression model. And statistic power would decrease and type II errors would increase when detecting interactions by LR in case-control studies with relatively small sample sizes [18]. By contrast, the MDR analysis can overcome some of the limitations of logistic regression model for the interactions by collapsing high-dimensional data into a single dimensional variable with two levels. In the present study, we performed the MDR analysis, as described previously [19]. We used a model of 100-fold cross-validation and repeated the complete analysis for 10 times under different random seeds, and then the test was repeated 1000 times under the null hypothesis of no association. As a result, the model employing the minimized prediction error together with the maximized cross-validation consistency (CVC) was recommended. This analysis was performed by using the MDR V2.0 beta 8.2 software (http://www.multifactorialdimensionalityreduction.org/).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) for evaluation of genotype distributions of the controls was performed by a goodness-of fit $\chi^2$ test. Differences in the frequency distributions of the alleles, genotypes and the selected categorical variables between cases and controls were evaluated by Pearson’s $\chi^2$ test under various genetic models (including dominant model, recessive model, and additive model). Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated according to the significant genetic models by univariate and multivariate uncon-
ditional logistic regression models, respectively, to evaluate associations between the genotypes and risk of PCa with and without adjustment for by confounding factors. Given the present study was single ethnicity, and all of the SNPs loci were agree with HEW, the confounding factors which should be adjusted for was age, smoking status, and body mass index (BMI). Further stratification analyses were conducted to calculate the associations of SNP genotypes with PCa risk by demographic and clinic-pathologic variables, followed by the homogeneity Q-tests to detect any difference in the risk estimates between the strata. Based on the observed genotypes, haplotype frequencies and individual haplotypes were generated using Statistical Analysis Software PROC HAPLOTYPE, with a reference group of common haplotype, to calculate ORs for haplotypes associated with PCa risk in logistic regression analysis. For all the significant findings observed in our study, we calculated the false-positive report probability (FPRP) with prior probabilities of 0.0001, 0.001, 0.01, 0.1 and 0.25 to detect the possible false-positive associations [20]. Statistical power was estimated to detect an OR of 1.50/0.67 (for a risk/protective effect), with an α level equal to the observed P value. Only significant results with FPRP value less than 0.2 were considered a noteworthy association. All statistical analyses were performed with SAS 9.1 statistical software (SAS, Cary, NC, USA). All P values were two-sided with a significance level of P < 0.05.

Results

Characteristics of the subjects

The distributions of demographic characteristics of the subjects are presented in Table 1. Briefly, there were no statistical differences in the distributions of age and smoking status between 1004 cases and 1051 controls. The body mass index (BMI) for overweight (> 24.0 kg/m²) was more evident in controls than in cases (P < 0.0001), which was further adjusted for in subsequent multivariate logistic regression analyses. Among the case subjects, 178 (17.7%) cases were PSA≤10 ng/ml, 312 (31.1%) cases were Gleason score≤7 (3+4), and 601 (59.9%) cases were Gleason score=7 (4+3). For tumor staging, five (0.5%) cases had stage I disease, 431 (42.9%) had stage II disease, 140 (13.9%) had stage III disease, and 351 (35.0%) had stage IV disease. However, some cases had missing data because of the insufficient documented records, including 87 (8.7%) lacking serum PSA values, 91 (9.1%) lacking Gleason scores, and 77 (7.7%) lacking clinical staging status.

The mTOR allele and genotype distributions and associations with PCa risk

The genotype and allele distributions of the six selected SNPs among cases and controls are summarized in Table 2. The observed genotype frequencies of the six SNPs in controls agreed with the Hardy-Weinberg equilibrium. Furthermore, significant differences in genotype distributions were observed between cases and controls for rs2536 T>C (P=0.007), rs1034528 G>C (P=0.022), and rs2295080 T>G (P=0.012). Interestingly, the heterozygote genotypes of the above three SNPs were more likely to be significantly associated with PCa risk with adjusted OR (95% CI) and P value of 1.45 (1.15–1.84) and 0.002 for rs2536 TC, 1.31 (1.08–1.59) and 0.005 for rs1034528 GC, and 0.77 (0.64–0.93) and 0.006 for rs2295080 TG, respectively, compared with their respective wild-type genotypes, respectively. Additionally, we also found significant associations with PCa risk for SNPs in special genetic models, including rs2536 T>C [additive: adjusted OR = 1.34 (1.08–1.66), P = 0.006; dominant: adjusted OR = 1.42 (1.13–1.78), P = 0.003]; rs1034528 G>C [additive: adjusted OR = 1.21 (1.03–1.42), P = 0.019; dominant: adjusted OR = 1.29 (1.07–1.55), P = 0.007]; and rs2295080 T>G [additive: adjusted OR = 0.80 (0.69–0.94), P = 0.005; dominant: adjusted OR = 0.76 (0.64–0.92), P = 0.003]. Further analyses of the combined genotypes of these six SNPs revealed a significant increase in PCa risk with increasing numbers of putative high-risk alleles (P trend = 0.0005) (Table 3).

| Variables     | Cases No. (%) | Controls No. (%) | P    |
|---------------|---------------|------------------|------|
| All subjects  | 1004 (100)    | 1051 (100)       |      |
| Age, yr (Mean±SD) | 69.0±8.16    | 69.0±8.96        | 0.141|
| ≤69          | 510 (50.8)    | 494 (49.2)       |      |
| >69          | 568 (54.0)    | 483 (46.0)       |      |
| BMI (kg/m²)  |               |                  | <0.0001|
| ≤24          | 754 (75.1)    | 250 (24.9)       |      |
| >24          | 637 (60.6)    | 414 (39.4)       |      |
| Smoking status |              |                  | 0.572|
| Never        | 402 (40.0)    | 602 (60.0)       |      |
| Ever         | 408 (38.8)    | 643 (61.2)       |      |
| PSA value (ng/ml) |            |                  |      |
| ≤10          | 178 (17.7)    | 24 (2.3)         |      |
| 10–20        | 431 (42.9)    | 754 (71.7)       |      |
| >20          | 351 (35.0)    | 250 (24.9)       |      |
| Missing      | 77 (7.7)      |                  |      |
| Gleason score |              |                  |      |
| ≤7(3+4)      | 312 (31.1)    |                  |      |
| ≥7(4+3)      | 601 (59.9)    |                  |      |
| Missing      | 91 (9.1)      |                  |      |
| Stage of disease |            |                  |      |
| I            | 5 (0.5)       |                  |      |
| II           | 431 (42.9)    |                  |      |
| III          | 140 (13.9)    |                  |      |
| IV           | 351 (35.0)    |                  |      |
| Missing      | 77 (7.7)      |                  |      |

SD, standard deviation. BMI, body mass index.

Table 1. Distribution of demographic and clinical-pathologic characteristics of prostate cancer patients and cancer-free controls from Eastern Chinese men.

Stratification analysis of PCa risk associated with mTOR SNPs

In stratification analyses, as shown in Tables 4 and 5, the multivariate logistic regression analyses indicated, by assuming a dominant genetic model, that both mTOR rs2536 CT/CG and rs1034528 CG/CC genotypes were associated with an increased risk of PCa, particularly in subgroups of age≤69, BMI≤24 kg/m², ever smokers, Gleason score≤7 (3+4), and stage III/IV disease, compared with their homozygous wild-type genotypes, respectively. The rs17036508 CT/CC genotypes were also associated with an increased risk of PCa among subgroups of BMI≤24 kg/m², Gleason score≤7 (3+4), and stage III/IV disease.
Table 2. Logistic regression analysis of associations between mTOR genotypes and prostate cancer risk in Eastern Chinese men.

| Variables (HWE)* | Cases (N = 1004) | Controls (N = 1051) | P | Crude OR (95% CI) | P | Adjusted OR (95% CI)b | P |
|------------------|------------------|---------------------|---|------------------|---|----------------------|---|
| rs2536 (HWE: P = 0.156) | | | | | | | |
| TT               | 804 (80.1)       | 894 (85.1)          | 0.007 | 1.00            | 1.00 | | |
| CT               | 192 (19.1)       | 147 (14.0)          | | 1.45 (1.15–1.84) | 0.002 | 1.45 (1.15–1.84) | 0.002 |
| CC               | 8 (0.8)          | 10 (0.9)            | | | | | |
| Additive model   | | | | 1.34 (1.08–1.66) | 0.008 | 1.34 (1.08–1.66) | 0.008 |
| Dominant model   | 0.003 | 1.42 (1.13–1.78) | 0.003 | 1.42 (1.13–1.78) | 0.003 |
| Recessive model  | 0.707 | 0.84 (0.33–2.13) | 0.709 | 0.83 (0.33–2.12) | 0.698 |
| rs1883965 (HWE: P = 0.904) | | | | | | | |
| GG               | 843 (84.0)       | 890 (84.7)          | 0.874 | 1.00            | 1.00 | | |
| AG               | 153 (15.2)       | 154 (14.7)          | | 1.21 (0.44–3.34) | 0.718 | 1.33 (0.48–3.70) | 0.588 |
| AA               | 8 (0.8)          | 7 (0.7)             | | | | | |
| Additive model   | 1.06 (0.85–1.32) | 0.622 | 1.08 (0.86–1.34) | 0.522 |
| Dominant model   | 0.655 | 1.06 (0.83–1.34) | 0.655 | 1.07 (0.84–1.36) | 0.574 |
| Recessive model  | 0.728 | 1.20 (0.43–3.32) | 0.728 | 1.32 (0.47–3.66) | 0.600 |
| rs1034528 (HWE: P = 0.443) | | | | | | | |
| GG               | 639 (63.7)       | 727 (69.2)          | 0.022 | 1.00            | 1.00 | | |
| CG               | 333 (33.2)       | 290 (27.6)          | | 1.31 (1.08–1.58) | 0.006 | 1.31 (1.08–1.59) | 0.005 |
| CC               | 32 (3.2)         | 34 (3.2)            | | | | | |
| Additive model   | 1.07 (0.65–1.76) | 0.787 | 1.09 (0.66–1.79) | 0.739 |
| Dominant model   | 0.008 | 1.28 (1.07–1.54) | 0.008 | 1.29 (1.07–1.55) | 0.007 |
| Recessive model  | 0.951 | 0.99 (0.60–1.61) | 0.951 | 1.00 (0.61–1.64) | 0.994 |
| rs17036508 (HWE: P = 0.085) | | | | | | | |
| TT               | 749 (74.6)       | 820 (78.0)          | 0.135 | 1.00            | 1.00 | | |
| CT               | 237 (23.6)       | 210 (20.0)          | | 1.24 (1.00–1.53) | 0.049 | 1.23 (0.99–1.52) | 0.055 |
| CC               | 18 (1.8)         | 21 (2.0)            | | | | | |
| Additive model   | 1.15 (0.96–1.38) | 0.128 | 1.15 (0.96–1.38) | 0.139 |
| Dominant model   | 0.068 | 1.21 (0.99–1.48) | 0.069 | 1.20 (0.98–1.48) | 0.076 |
| Recessive model  | 0.733 | 0.90 (0.47–1.69) | 0.734 | 0.89 (0.47–1.69) | 0.731 |
| rs3806317 (HWE: P = 0.746) | | | | | | | |
| AA               | 772 (76.9)       | 790 (75.2)          | 0.351 | 1.00            | 1.00 | | |
| AG               | 220 (21.9)       | 241 (22.9)          | | 0.93 (0.76–1.15) | 0.521 | 0.93 (0.76–1.15) | 0.500 |
| GG               | 12 (1.2)         | 20 (1.9)            | | 0.61 (0.30–1.27) | 0.186 | 0.61 (0.29–1.25) | 0.174 |
| Additive model   | 0.91 (0.74–1.11) | 0.360 | 0.91 (0.74–1.11) | 0.340 |
| Dominant model   | 0.195 | 0.62 (0.30–1.28) | 0.199 | 0.62 (0.30–1.27) | 0.188 |
| Recessive model  | 0.733 | 0.90 (0.47–1.69) | 0.734 | 0.89 (0.47–1.69) | 0.731 |
| rs2295080 (HWE: P = 0.334) | | | | | | | |
| TT               | 653 (65.0)       | 617 (58.7)          | 0.012 | 1.00            | 1.00 | | |
| GT               | 311 (31.0)       | 382 (36.4)          | | 0.77 (0.64–0.93) | 0.006 | 0.77 (0.64–0.93) | 0.006 |
| GG               | 40 (4.0)         | 52 (5.0)            | | 0.73 (0.47–1.11) | 0.143 | 0.73 (0.48–1.12) | 0.147 |
| Additive model   | 0.80 (0.69–0.93) | 0.004 | 0.80 (0.69–0.94) | 0.005 |
| Dominant model   | 0.003 | 0.76 (0.64–0.91) | 0.003 | 0.76 (0.64–0.92) | 0.003 |
| Recessive model  | 0.291 | 0.80 (0.52–1.22) | 0.292 | 0.8 (0.52–1.22) | 0.300 |

OR, odds ratio; CI, confidence interval.

*Hard-Wenborg equilibrium test for controls.

Two-sided Chi-square tests were used to calculate differences in the frequency distribution of genotypes between cases and controls.

Adjusted for age, smoking, and BMI status in logistic regress models.

The results were in bold, if the 95% CI excluded 1 or P < 0.05.

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III+IV diseases, compared with the TT homozygous variant genotype. In contrast, the rs2295080 GT/TT genotypes had a protective effect, particularly in subgroups of age>69, BMI, ever-smokers, Gleason score≥7 (3+4), and stage I+II diseases, compared with the GG homozygous variant genotype. However, further homogeneity tests indicated that there was no difference in risk estimates between subgroups for most of the strata with a few exceptions including BMI level by rs2536 CT/CC genotypes \([OR = 1.43 (1.13–1.80), P = 0.017]\) and by rs1034528 CC/CG genotypes \([OR = 1.28 (1.07–1.55), P = 0.039]\); disease stage by rs2536 CT/CC genotypes \([OR = 1.47 (1.20–1.90), P = 0.004]\) and by rs17036508 CT/CC genotypes \([OR = 1.24 (1.04–1.49), P = 0.024]\).

**Table 3. Combined effects of risk genotypes of mTOR by dominant genetic models.**

| mTOR Variables | Cases          | Controls       | \(\beta\)   | Crude OR (95% CI) | \(P\) | Adjusted OR (95% CI) | \(\beta\) |
|---------------|---------------|----------------|-------------|-------------------|------|---------------------|---------|
| Genotypes \((N = 1004)\) | \(N = 1051)\) |                |             |                    |      |                     |         |
| 0–1           | 466 (46.4)    | 543 (51.7)     | 0.004       | 1.00              | 1.00 |                     |         |
| 2             | 295 (29.4)    | 322 (30.6)     | 1.07        | 0.87–1.31         | 0.523| 1.07 (0.87–1.31)    | 0.516   |
| 3             | 162 (16.1)    | 129 (12.3)     | 1.46        | 1.13–1.90         | 0.004| 1.48 (1.14–1.93)    | 0.004   |
| 4             | 79 (7.9)      | 53 (5.0)       | 1.74        | 1.20–2.51         | 0.003| 1.74 (1.20–2.52)    | 0.003   |
| 5             | 2 (0.2)       | 4 (0.4)        | 0.58        | 0.11–3.20         | 0.534| 0.52 (0.09–2.86)    | 0.450   |

\(\beta_{\text{adj}}=0.0005\)

\(\text{Chi-square test was used to calculate the genotype frequency distributions.}\)

\(\text{Obtained under dominant models in logistic regression analyses with adjustment for age, smoking status and BMI.}\)

\(\text{The results were in bold, if the 95\% CI excluded 1 or } P<0.05.\)

**Discussion**

In this large, ethnic specific single institutional case-control study, we investigated the associations between six potentially functional SNPs of the mTOR gene and PCa risk, and we found that the rs2536 C, rs1034528 C, and rs2295080 G variant genotypes were associated with PCa risk, and the effects were more evident in subgroups of age≥69, BMI≥24 kg/m² and stage III+IV (FPRP = 0.112 and 0.055, respectively), and the similar results can be observed in the association with rs1034528 (CG/CC vs. GG) in subgroups of≥24 kg/m² BMI and stage III+IV (FPRP = 0.132 and 0.045, respectively) as well as the association with subgroup of stage III+IV (FPRP = 0.165) by rs17036508 (CT/CC vs. TT). In contrast, some greater FPRP values for the other significant associations between mTOR variants and prostate cancer risk suggested some possible bias in the findings, which need further validation in larger studies.
Table 4. Stratification analysis for associations between mTOR variants and prostate cancer risk by dominant genetic models in all subjects of Eastern Chinese men.

| Variables | rs2536 Adjusted OR (95%CI)* | rs1883965 Adjusted OR (95%CI)* | rs1034528 Adjusted OR (95%CI)* |
|-----------|-----------------------------|---------------------------------|---------------------------------|
| Age, yr (median) ≤ 69 | 103/84 407/484 | 1.42 (1.03–1.95) 0.031 | 90/88 420/480 | 1.19 (0.86–1.65) 0.291 |
| ≥ 69 | 97/73 397/410 | 1.38 (0.99–1.93) 0.057 | 71/73 423/410 | 0.94 (0.66–1.34) 0.176 |
| BMI, kg/m² ≤ 24 | 159/85 595/552 | 1.74 (1.30–2.32) 0.0002 | 117/95 637/542 | 1.05 (0.79–1.42) 0.726 |
| > 24 | 41/72 209/342 | 0.93 (0.61–1.41) 0.736 | 44/66 206/348 | 1.13 (0.74–1.71) 0.578 |
| Smoking status Never | 82/63 320/345 | 1.40 (0.98–2.02) 0.068 | 60/66 342/342 | 0.93 (0.63–1.36) 0.701 |
| Ever | 118/94 484/549 | 1.42 (1.06–1.92) 0.021 | 101/95 501/548 | 1.18 (0.87–1.60) 0.298 |
| Stage of disease<sup>c</sup> II | 67/157 245/894 | 1.58 (1.15–2.18) 0.005 | 56/161 256/890 | 1.24 (0.89–1.74) 0.208 |
| III+IV | 121/157 480/894 | 1.44 (1.11–1.87) 0.007 | 97/161 504/890 | 1.07 (0.81–1.41) 0.635 |
| Smoking status Never | 68/157 368/894 | 1.07 (0.78–1.46) 0.672 | 79/161 357/890 | 1.25 (0.93–1.68) 0.142 |
| Ever | 123/157 368/894 | 1.91 (1.47–2.49) <0.0001 | 75/161 416/890 | 1.00 (0.75–1.35) 0.980 |

| Variables | rs17036508 Adjusted OR (95%CI)* | rs306317 Adjusted OR (95%CI)* | rs2295080 Adjusted OR (95%CI)* |
|-----------|-----------------------------|---------------------------------|---------------------------------|
| Age, yr (median) ≤ 69 | 129/120 381/448 | 1.23 (0.92–1.63) 0.161 | 113/140 397/428 | 0.86 (0.65–1.14) 0.296 |
| ≥ 69 | 126/111 368/372 | 1.16 (0.87–1.56) 0.320 | 119/121 375/362 | 0.94 (0.70–1.25) 0.659 |
| BMI, kg/m² ≤ 24 | 200/139 554/498 | 1.29 (1.01–1.66) 0.042 | 172/164 582/473 | 0.85 (0.67–1.09) 0.206 |
| > 24 | 55/92 195/322 | 0.98 (0.67–1.43) 0.918 | 60/97 190/317 | 1.03 (0.71–1.48) 0.896 |
| Smoking status Never | 108/96 294/312 | 1.19 (0.86–1.63) 0.288 | 98/103 304/305 | 0.95 (0.69–1.31) 0.747 |
| Ever | 147/135 455/508 | 1.21 (0.93–1.58) 0.165 | 134/158 468/485 | 0.86 (0.66–1.12) 0.262 |
| Stage of disease<sup>c</sup> II | 90/231 222/820 | 1.44 (1.08–1.92) 0.013 | 75/261 237/790 | 0.95 (0.71–1.27) 0.720 |
| III+IV | 148/231 453/820 | 1.16 (0.92–1.47) 0.219 | 130/261 471/790 | 0.84 (0.66–1.06) 0.140 |

BMI, body mass index.
<sup>*</sup>Obtained under dominant models in logistic regression analyses with adjustment for age, smoking status and BMI.
<sup>a</sup>According to the current WHO recommendations.
<sup>b</sup>P<sub>hom</sub> P value for homogeneity test.

The results were in bold, if P<0.05.
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Table 5. Stratification analysis for associations between combined risk genotypes of mTOR variants and prostate cancer risk.

| Variables | Combined effect of risk genotypes (cases/controls) | Crude OR(95%CI) | Adjusted OR(95%CI)* | P* | Pb | Interaction |
|-----------|---------------------------------------------------|----------------|---------------------|----|----|------------|
| 0–1 at-risk genotype 2–6 at-risk genotype | | | | | | |
| Age, yr | | | | | | |
| ≤69 (median) | 236/301 | 274/267 | 1.31 (1.03–1.66) | 0.028 | **1.29 (1.02–1.65)** | 0.036 | 0.473 | 0.872 |
| >69 (median) | 230/242 | 264/241 | 1.15 (0.90–1.48) | 0.268 | 1.15 (0.90–1.48) | 0.271 | 0.473 | 0.872 |
| BMI, kg/m² | | | | | | |
| ≤24 | 348/336 | 406/301 | 1.30 (1.05–1.61) | 0.014 | **1.31 (1.06–1.61)** | 0.013 | 0.431 | 0.431 |
| >24 | 118/207 | 132/207 | 1.12 (0.82–1.53) | 0.484 | 1.11 (0.81–1.52) | 0.527 | 0.484 | 0.527 |
| Smoking status | | | | | | |
| Never | 185/201 | 217/207 | 1.14 (0.86–1.50) | 0.356 | 1.15 (0.87–1.52) | 0.322 | 0.470 | 0.470 |
| Ever | 281/342 | 321/301 | 1.30 (1.04–1.62) | 0.022 | **1.29 (1.03–1.61)** | 0.027 | | |
| Gleason score | | | | | | |
| ≤7(3+4) | 133/543 | 179/508 | 1.44 (1.12–1.86) | 0.005 | **1.46 (1.13–1.89)** | 0.004 | 0.258 | 0.258 |
| ≥7(4+3) | 284/543 | 317/508 | 1.19 (0.98–1.46) | 0.085 | 1.19 (0.98–1.46) | 0.084 | | |
| Stage of disease | | | | | | |
| II | 208/543 | 228/508 | 1.17 (0.94–1.47) | 0.165 | 1.18 (0.95–1.48) | 0.140 | 0.400 | 0.400 |
| III–IV | 218/543 | 273/508 | 1.34 (1.08–1.66) | 0.008 | **1.34 (1.08–1.66)** | 0.008 | | |

*Obtained in logistic dominant models with adjustment for age, smoking status and BMI.
 **P** for homogeneity test using the χ²-based Q test.
 P for multiplicative interaction obtained from logistic regression models with adjustment for age, smoking status and BMI.
 CI, confidence interval; BMI, body mass index.
 The results were in bold, if P<0.05.
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Table 6. The frequency of common inferred haplotypes of the mTOR gene based on the observed genotypes.

| rs2536 | rs1034528 | rs17036508 | rs2295080 | Case (N = 2014) | Control (N = 2134) | Adjusted OR (95% CI) | P* |
|--------|-----------|------------|-----------|----------------|-------------------|---------------------|----|
| T      | G         | T          | T         | 1515          | 1572              | 1.00                |    |
| T      | G         | T          | G         | 41            | 109               | 0.39 (0.27–0.56)    | <0.000 |
| T      | G         | C          | T         | 12            | 7                 | 1.77 (0.70–4.51)    | 0.231 |
| T      | G         | C          | G         | 46            | 76                | 0.63 (0.43–0.91)    | 0.014 |
| T      | C         | T          | T         | 55            | 40                | 1.44 (0.95–2.17)    | 0.087 |
| T      | C         | T          | G         | 127           | 145               | 0.92 (0.71–1.17)    | 0.481 |
| T      | C         | C          | T         | 3             | 2                 | 1.48 (0.24–8.90)    | 0.667 |
| T      | C         | C          | G         | 6             | 14                | 0.43 (0.16–1.11)    | 0.081 |
| C      | G         | T          | T         | 0             | 2                 | -                   |    |
| C      | G         | T          | G         | 0             | 2                 | -                   |    |
| C      | G         | C          | T         | 0             | 1                 | -                   |    |
| C      | G         | C          | G         | 0             | 2                 | -                   |    |
| C      | C         | T          | T         | 1             | 2                 | 0.55 (0.05–6.03)    | 0.622 |
| C      | C         | T          | G         | 1             | 9                 | 0.11 (0.01–0.89)    | 0.039 |
| C      | C         | C          | T         | 36            | 15                | 2.47 (1.34–4.52)    | 0.004 |
| C      | C         | C          | G         | 171           | 136               | 1.31 (1.03–1.66)    | 0.026 |

*Obtained under dominant models in logistic regression analyses with adjustment for age, smoking status and BMI.
 The results were in bold, if the 95% CI excluded 1 or P<0.05.
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is biologically plausible that functional SNPs affecting the pivotal domains described above may contribute to cancer susceptibility. However, in addition to the published GWAS studies, only a few reported post-GWAS studies have investigated the associations between functional SNPs of the mTOR gene and risk of PCa. In a Chinese study with 666 PCa and 708 cancer-free controls, Chen et al. [24] indicated that mTOR rs2295080 GT/GG genotypes had a protective effect on PCa risk, compared with the TT genotype, which was recently shown by Xu et al. in gastric cancer [25] and by Cao et al. in renal cell cancer [26]. These findings are consistent with those of the present study with a larger sample size. Additionally, Hildebrandt et al. found that individuals carrying the rs2295080 GG genotype had reversed clinical outcomes in Caucasian esophageal cancer patients treated with chemotherapy, compared with the TT homozygous wild-type genotype [27]. All studies that focused on Chinese populations indicated an association between rs2295080 GT/GG and cancer risk, suggesting an possibly ethnic-specific association. Nevertheless, the associations between mTOR rs2536 C variant or genotypes and cancer risk in Chinese populations were various in the literatures; for example, the mTOR rs2536 CT heterozygous genotype was found to be associated with decreased risk of Chinese childhood acute lymphoblastic leukemia [28]; however, this association was not observed in other tumor types, such as gastric cancer [29], prostate cancer [24], and esophageal squamous cell carcinoma [30]. On the contrary, in the present study, we found that the mTOR rs2536 CT/CC genotypes were associated with an increased PCa risk under a dominant genetic model, different from the findings of another previously published PCa study (666 cases and 708 controls), in which a null association was reported [24]. We speculated that the disagreement might be due to the different sample size or different inclusion criteria for the participaion, which needs large and better designed studies to confirm.

Studies have shown that the rs2295080 T allele could enhance the transcription activity of mTOR in HEK293, 786-O, HeLa, and GES-1 cell line in vitro [25,26]. Likewise, individuals carrying the TT genotype had higher levels of mTOR expression as well [25,26]. These suggest that the rs2295080 T allele could increase the affinity of special transcription factors to this region of the mTOR promoter and subsequently contribute to the increased mTOR activity in humans. Theoretically, miRNAs can bind to the 3’ UTR of target genes and inhibit gene expression translationally and/or by destabilizing the target mRNA. Based on a bioinformatics web server [http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo], the SNP rs2536 T>C was predicted to bind to miRNA-576 at the T variant allele or bind to miRNA-767 at the C allele. Therefore, we speculated that the expression of mTOR depended on the proportions of these two miRNAs or the affinity between miRNA and SNP rs2536 T>C, which has a growth advantage of immortalized cells and induces neoplastic transformation. It was indicated that disease-associated functional intronic variants may alter mRNA levels of the genes by affecting the transcriptional efficiency, RNA elongation, or splicing [31–33]. On the other hand, the SNP rs1034528 G>C located in the first intron region of the mTOR gene was also found to be associated with risk of PCa; however, both rs1034528 G and C alleles were predicted to bind to different transcription factors, respectively, in this region. Therefore, the exact mechanisms of the rs1034528 G>C underlying the observed PCa risk need additional functional studies.

There is evidence in the literature that each SNP may have a weak effect but the combination of multi-SNPs may present much stronger effects than any of the SNPs. This is particularly true in the present study, in which the haplotype and combined analyses confirmed the multi-SNPs effects in PCa. In the logistic regression model, a locus dose-response was found for the increased PCa risk with the increasing number of adverse genotypes of all studied SNPs. Additionally, we noticed the combined effects was more pronounced among subgroups of age ≤ 69 and BMI ≥ 24 kg/m². These findings agreed with the hypothesis that genetic susceptibility contributes to the risk of developing cancer in those who had an early age onset and minor exposures. Although the interaction between smoking and mTOR SNPs was not observed in the present study, we did find an obvious effect of the combined unfavorable genotypes on PCa risk, particularly among subgroups of ever smoker, suggesting that the effect of the tobacco smoke-related carcinogens may also depend on genetic factors.

In the present study, the number of positive findings from the stratified analyses was obviously decreased in the FPRP assessment. There are several possible explanations for the false positive findings. Firstly, some findings in the stratified analyses may be a chance finding due to the limited sample size in the subgroups. Secondly, some missing information and potential confounding factors might result in the false positive associations. Therefore, all positive results should be explained with caution. Extensive evidence from previous epidemiology studies has indicated that several genetic variant and environmental factors are involved in the initiation and development of cancer [34–37]. We also found the similar interactions by using logistic regression and MDR approaches (Table S1 in File S1). In the MDR analysis, BMI was found to be the most noteworthy factor in one-factor model;
however, the exact mechanisms for the association between BMI and PCa risk have not been established. Possible hypotheses include the effect of hormones, PSA, and adipose-related proteins [38]. In the present study, we found some evidence of the interactions between environmental factor (BMI) and genetic factors (rs17036508 T>C and rs2536 T>C), as shown in the best three-factor model, we speculated that those variations might alter the expression of mTOR and the subsequent synthesis of adipose-related proteins, but this finding needs to be validated in larger studies.

In summary, the present study investigated the associations between six selected potentially functional mTOR SNPs and PCa risk with a relative large sample size. However, several methodological issues and limitations of the present study should be

| mTOR SNP genotype | Crude OR (95%CI) | P | Statistical powerb | Prior probability |
|-------------------|-----------------|---|-------------------|-----------------|
|                   |                 | 0.25 | 0.1 | 0.01 | 0.001 | 0.0001 |
| All patients      |                 |     |     |     |     |     |
| rs2536, CT vs TT  | 1.45 (1.15–1.84)| 0.0018 | 0.614 | 0.009 | 0.026 | 0.225 | 0.746 | 0.967 |
| rs1034528, CG vs GG | 1.31 (1.08–1.58)| 0.0058 | 0.93 | 0.018 | 0.053 | 0.382 | 0.862 | 0.984 |
| rs2295080, GT vs TT | 1.24 (1.03–1.49)| 0.0203 | 0.982 | 0.058 | 0.157 | 0.672 | 0.954 | 0.995 |
| rs2295080, GG vs TT | 0.09 (0.02–0.30)| 0.0001 | 0.007 | 0.044 | 0.12 | 0.601 | 0.938 | 0.993 |
| rs2295080, GG vs GT/TT | 0.09 (0.03–0.28)| 0.0001 | 0.008 | 0.036 | 0.101 | 0.552 | 0.926 | 0.992 |
| rs2536, CT/CC vs TT |                 |     |     |     |     |     |
| All patients      | 1.42 (1.13–1.78)| 0.0029 | 0.699 | 0.012 | 0.036 | 0.291 | 0.806 | 0.976 |
| Age≤69 yrs        | 1.46 (1.06–2.00)| 0.0192 | 0.573 | 0.091 | 0.232 | 0.788 | 0.971 | 0.997 |
| BMI≥24 kg/m²      | 1.74 (1.30–231)| 0.0002 | 0.157 | 0.004 | 0.011 | 0.112 | 0.559 | 0.927 |
| Ever smoking      | 1.42 (1.06–1.92)| 0.0194 | 0.642 | 0.083 | 0.214 | 0.749 | 0.968 | 0.997 |
| Gleason score≥7(3+4) | 1.56 (1.13–2.14)| 0.0062 | 0.406 | 0.044 | 0.121 | 0.602 | 0.938 | 0.993 |
| Gleason score≥7(4+3) | 1.44 (1.11–1.87)| 0.0066 | 0.634 | 0.030 | 0.086 | 0.507 | 0.912 | 0.990 |
| Stage III+ IV     | 1.90 (1.46–2.48)| 0.0001 | 0.17 | 0.002 | 0.005 | 0.055 | 0.371 | 0.855 |
| rs1034528, CG/CC vs GG |                 |     |     |     |     |     |
| All patients      | 1.28 (1.07–1.54)| 0.0080 | 0.958 | 0.024 | 0.07 | 0.452 | 0.893 | 0.988 |
| Age≤69 yrs        | 1.41 (1.10–1.82)| 0.0076 | 0.681 | 0.032 | 0.091 | 0.525 | 0.918 | 0.991 |
| BMI≥24 kg/m²      | 1.47 (1.17–1.84)| 0.0009 | 0.586 | 0.005 | 0.014 | 0.132 | 0.605 | 0.939 |
| Ever smoking      | 1.31 (1.04–1.67)| 0.0237 | 0.870 | 0.076 | 0.197 | 0.730 | 0.965 | 0.996 |
| Gleason score≥7(3+4) | 1.48 (1.14–1.92)| 0.0032 | 0.539 | 0.017 | 0.051 | 0.370 | 0.856 | 0.983 |
| Gleason score≥7(4+3) | 1.30 (1.05–1.60)| 0.0162 | 0.917 | 0.050 | 0.137 | 0.636 | 0.946 | 0.994 |
| Stage III+ IV     | 1.53 (1.22–1.91)| 0.0002 | 0.443 | 0.001 | 0.004 | 0.043 | 0.311 | 0.819 |
| rs17036508, CT/CC vs TT |                 |     |     |     |     |     |
| BMI≥24 kg/m²      | 1.29 (1.01–1.66)| 0.0417 | 0.892 | 0.123 | 0.296 | 0.822 | 0.979 | 0.998 |
| Gleason score≥7(3+4) | 1.44 (1.08–1.91)| 0.0121 | 0.615 | 0.056 | 0.151 | 0.661 | 0.952 | 0.995 |
| Stage III+ IV     | 1.50 (1.18–1.91)| 0.0010 | 0.499 | 0.006 | 0.018 | 0.165 | 0.667 | 0.952 |
| rs2295080, GT/GG vs TT |                 |     |     |     |     |     |
| Age≤69 yrs        | 1.30 (1.01–1.66)| 0.0380 | 0.885 | 0.114 | 0.279 | 0.810 | 0.977 | 0.998 |
| Gleason score≥7(3+4) | 1.34 (1.03–1.73)| 0.0274 | 0.815 | 0.092 | 0.232 | 0.769 | 0.971 | 0.997 |
| Stage III+ IV     | 1.35 (1.08–1.68)| 0.0074 | 0.885 | 0.024 | 0.070 | 0.453 | 0.893 | 0.988 |
| Combined effect   |                 |     |     |     |     |     |
| 4 variable genotypes | 1.23 (1.04–1.47)| 0.017 | 0.988 | 0.052 | 0.141 | 0.643 | 0.948 | 0.995 |
| mTOR haplotypes (rs2536-rs1034528-rs17036508-rs2295080) | T-G-T-G | 0.39 (0.27–0.56) | <0.0001 | 0.088 | 0.003 | 0.010 | 0.101 | 0.531 | 0.919 |
| T-G-C-G        | 0.63 (0.43–0.91) | 0.0137 | 0.378 | 0.098 | 0.246 | 0.782 | 0.973 | 0.997 |
| C-C-C-G        | 1.31 (1.03–1.65) | 0.0269 | 0.895 | 0.083 | 0.213 | 0.748 | 0.968 | 0.997 |

OR, odds ratio; CI, confidence interval; BMI, body mass index.

bStatistical power was calculated using the number of observations in the subgroup and the OR and P values in this table.

The results in false-positive report probability analysis were in bold, if the prior probability < 0.2.

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discussed. Firstly, some participants might be misclassified due to the lack of PSA serum information; for example, some silent tumors (stage A1, usually asymptomatic) may have been included as normal controls, which could subsequently bias the results to the null. Secondly, although hormonal, occupational, dietary, inflammation and other factors have been suggested as etiological factors of PCa, we did not adequately documented these covariates for adjustment. Thirdly, only six potentially functional SNPs of mTOR were investigated in the present study, which did not cover all variants in the mTOR gene. Therefore, additional larger and well-designed studies are warranted to confirm our findings.

Supporting Information

File S1 File includes: Supplementary Table S1 for Stratification analysis of significant SNPs by age, smoking status, and BMI; S1-1 Stratification analysis of significant SNPs by age; S1-2 Stratification analysis of significant SNPs by smoking status; and S1-3 Stratification analysis of significant SNPs by BMI. (DOCX)

Author Contributions

Conceived and designed the experiments: DY QW. Performed the experiments: QL CG MW. Analyzed the data: JH MZ TS XZ. Contributed reagents/materials/analysis tools: YZ LJ YY JW. Wrote the paper: QL QW DY.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90.
2. Matsuda T, Saika K (2009) Comparison of time trends in prostate cancer incidence [1973-2002] in Asia, from cancer incidence in five continents, Vol IV-IX. Jpn J Clin Oncol 39: 468–469.
3. Godmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, et al. (2007) Genome-wide association study identifies a second prostate cancer susceptibility variant at 2q24. Nat Genet 39: 631–637.
4. Thomas G, Jacob J, Veager M, Kraft P, Wacholder S, et al. (2008) Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 40: 310–315.
5. Eyles RA, Kote-Jarai Z, Al OA, Giles GG, Guy M, et al. (2009) Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. Nat Genet 41: 1116–1121.
6. Stalder ZK, Vijai J, Thom P, Kirchhoff T, Hansen NA, et al. (2010) Genome-wide association studies of cancer predisposition. Humatol Oncol Clin North Am 24: 973–996.
7. Moore T, Beltran L, Carbajal S, Strom S, Traag J, et al. (2008) Dietary energy balance modulates signaling through the Akt/mammalian target of rapamycin pathways in multiple epithelial tissues. Cancer Prev Res (Phila) 1: 65–76.
8. Vivanco I, Sayers CL (2002) The phosphatidylinositol 3-kinase AKT pathway in human cancer. Nat Rev Cancer 2: 489–501.
9. Yang Q, Guan KL (2007) Expanding mTOR signaling. Cell Res 17: 666–681.
10. Fingar DC, Richardson CJ, Tao AR, Cheatham I, Tsou C, et al. (2004) mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation initiation factor e.
11. Edinger AL, Thompson CB (2002) Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. Mol Biol Cell 13: 2276–2288.
12. Patel PH, Chadalavada RS, Chaganti RS, Motzer RJ (2006) Targeting von Hippel-Lindau disease in renal cell carcinoma. Clin Cancer Res 12: 7215–7220.
13. Zhou H, Huang S (2010) The complexes of mammalian target of rapamycin. Cell 124: 471–484.
14. Hidalgo M, Rowinsky EK (2000) The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene 19: 6680–6686.
15. Zhou H, Huang S, Rowinsky EK, et al. (2003) Genetic variations in mTOR with risk of childhood acute lymphoblastic leukemia in a Chinese population. Leuk Lymphoma 53: 947–951.
16. He J, Wang MY, Qiu LX, Zhu ML, Shi TY, et al. (2013) A functional variant in mTORC1 genes modulate risk of gastric cancer in an Eastern Chinese population. Mol Carcinog.
17. Iida N, Arai Y, Shimada H, et al. (2005) A functional polymorphism (rs2295080) in mTOR promoter region and its association with gastric cancer in a Chinese population. PLoS One 7: e40817.
18. Hildebrandt MA, Yang H, Hung MC, Izzo JJ, Huang M, et al. (2009) Genetic variations in the PIK3/PTEK7/mTOR/pathway are associated with clinical outcomes in esophageal cancer patients treated with chemoradiotherapy. J Clin Oncol 27: 857–867.
19. Huang L, Huang J, Wu P, Li Q, Rong L, et al. (2012) Association of genetic variations in mTOR with risk of childhood acute lymphoblastic leukemia in a Chinese population. Leuk Lymphoma 53: 947–951.
20. Wacholder S, Chanock S, Garcia-Closas M, El GL, Rotman N (2004) Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 96: 434–442.
21. Guertin DA, Sabatini DM (2005) An expanding role for mTOR in cancer. Trends Mol Med 11: 355–361.
22. Sarbassov DD, Ali SM, Sabatini DM (2005) Growing roles for the mTOR pathway. Curr Opin Cell Biol 17: 596–603.
23. Wittlich-Siliger LE, Loveth R, Hall MN (2006) TOR signaling in growth and metabolism. Cell 124: 471–484.
24. Chen J, Shao P, Cao Q, Li P, Li, et al. (2012) Genetic variations in a PTEN/ AKT/mTOR axis and prostate cancer risk in a Chinese population. PLoS One 7: e40817.
25. Xu M, Tao G, Kang M, Gao Y, Zha H, et al. (2013) A polymorphism (rs2295080) in mTOR promoter region and its association with gastric cancer in a Chinese population. PLoS One 8: e60080.
26. Cao Q, Xu J, Li P, Meng X, Shao P, et al. (2012) A functional variant in the mTOR promoter modulates its expression and is associated with renal cell cancer risk. PLoS One 7: e50502.
27. Zhou J, Fan L, Pan X, Tao X, et al. (2013) Genetic variations of mTORC1 genes and risk of gastric cancer in an Eastern Chinese population. Leuk Lymphoma.
28. Zhou J, Fan L, Pan X, Tao X, et al. (2013) Polymorphisms in mTORC1 Genes Modulate Risk of Esophageal Squamous Cell Carcinoma in Eastern Chinese Populations. J Thorac Oncol 8: 788–795.
29. Tokudome S, Takeuchi M, Takino M, Nakamura Y, et al. (2003) Genetic variations with gastric cardia adenocarcinoma in Chinese population: a multiple interaction analysis. Carcinogenesis 34: 336–342.
30. Liu L, Liu C, Wang Y, Zheng R, Wang F, et al. (2011) Association of candidate genetic variations with gastric cardia adenocarcinoma in Chinese population: a multiple interaction analysis. Carcinogenesis 32: 336–342.
31. Zong R, Liu Z, Zou L, Sheng W, Zhu B, et al. (2013) Genetic variations in the TGFBeta signaling pathway, smoking and risk of colorectal cancer in a Chinese population. Carcinogenesis 34: 936–942.
32. Zhao XM, Zhang R, Liu L, Wang Y, Yuan JX, et al. (2011) Smoking and COX-2 functional polymorphisms interact to increase the risk of gastric cardia adenocarcinoma in Chinese population. PLoS One 6: e21894.
33. Zhang X, Zheng R, Zhang Z, Yuan J, Liu L, et al. (2011) Interaction of cyclooxygenase-2 polymorphisms with Helicobacter pylori infection and risk of gastric cancer. Mol Carcinog 50: 876–883.
34. Freeman MR, Solomon KR (2004) Cholesterol and prostate cancer. J Cell Biochem 91: 54–69.