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MTR D919G variant is associated with prostate adenocarcinoma risk: evidence based on 51106 subjects

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Abstract. – OBJECTIVE: Several case-control studies have identified the association of the D919G polymorphism of the methionine synthase *(MTR)* gene with the risk of prostate adenocarcinoma (PRAD). However, the results were inconclusive.

MATERIALS AND METHODS: Odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs) were evaluated to assess the correlation between *MTR* D919G variant and PRAD risk. In addition, *in silico* tools were used to demonstrate the relationship between *MTR* expression and PRAD risk and survival time.

RESULTS: The overall results from 10,617 PRAD cases and 40,489 control participants indicated the association of the *MTR* D919G variant with an increased risk of PRAD (allelic contrast: OR = 1.06, 95% CI = 1.01 - 1.11; GA *vs.* AA: OR = 1.08, 95% CI = 1.02 - 1.14; GG+GA *vs.* AA: OR = 1.08, 95% CI = 1.02 - 1.14). The stratified analysis yielded similar results for hospital based studies and those with larger sample sizes. Finally, the *in silico* results revealed lower *MTR* expression in PRAD tissue than in normal tissue (transcripts per million = 2.68 *vs.* 3.34, $p<0.05$). Furthermore, patients with high *MTR* expression and Gleason score = 6 exhibited reduced survival time ($p<0.0001$).

CONCLUSIONS: Our study indicated that the *MTR* D919G variant is associated with elevated risk to PRAD, especially for Asian descendants and hospital based studies. Moreover, the *MTR* D919G variant might be related to PRAD prognosis.

Key Words:
MTR, Genetic variation, Prostate adenocarcinoma, Polymorphism, *In silico*.

Abbreviations

South America; HWE: Hardy-Weinberg equilibrium of controls, HB: Hospital-based; PB: Population-based; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; GWAS: Genome-wide association study; CGEMS: Cancer Genetic Markers of Susceptibility; FHS: Framingham Heart Study; UKGPCS: UK Genetic Prostate Cancer Study; TCGA: The Cancer Genome Atlas; HB: hospital based; PB: population based; MTR: Methionine synthase; PRAD: prostate adenocarcinoma.

Introduction

Prostate adenocarcinoma (PRAD) is the most common non-cutaneous solid tumor; it is the leading cause of cancer associated male mortality in Western countries[1]. The incidence of PRAD in individuals of Asian descent is lower than that in their Western counterparts[2]. In recent years, the incidence of PRAD and the associated mortality rates have been increasing, particularly in Asian countries[3]. In China, the overall PRAD incidence is growing and has become the most frequent solid malignancy in urban males, thus highlighting the need for improved PRAD prevention and control strategies[4,5]. To date, several factors have been shown to be associated with PRAD development, including age, alcohol use, hormone exposure, positive family history, and gene mutations[6,7]. Genetic factors, in particular, might play a prominent role in the pathogenesis of PRAD and the susceptibility to malignant prostate tumors could be nearly 2-5 fold higher in individuals suffering from Lynch syndrome, which is caused by germline mutations in genes such as *MLH1* (Mutl homolog-1), *MSH6* (mutS homolog-6), or *PMS2* (postmeiotic segregation increased-2)[8-10].

Folate-metabolizing genes play a prominent role in carcinogenesis *via* involvement in both DNA repair and methylation[11,12]. Methionine synthase (*MTR*), located on chromosome-1 (1q43),

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has 34 exons and encodes a key enzyme containing 1,265 amino acids in folate pathway[13]. A common variant of the *MTR* gene harboring an A-to-G substitution at base-pair 2756 occurs in the activation domain; it alters the aspartic acid (D) at position 919 of the polypeptide chain to glycine (G) and is referred to as the D919G variant[14-16]. Sharp and Little[17] have shown the involvement of the D919G variant in DNA methylation and the elevation of homocysteine levels; thus, it regulates the enzymatic activity of *MTR*. Several studies have examined the association of the *MTR* D919G variant with PRAD susceptibility; however, the results were inconsistent. In 2009, a meta-analysis conducted by Collin et al[18] investigated the association between the *MTR* D919G variant and PRAD risk, but no statistically significant effects of this single nucleotide polymorphism (SNP) on PRAD susceptibility were reported. In 2016, Qu et al[19] evaluated the correlation between the *MTR* D919G variant and PRAD risk in the Han Chinese population and demonstrated an independent association between the MTR D919G polymorphism and prostatic carcinogenesis by decreasing the methylation potential. Our research aimed to study the correlation between the *MTR* D919G variant and susceptibility to PRAD in larger sample sizes using pooled analysis to reach a definite conclusion[18-31].

Materials and Methods

Identification and Selection of Relevant Literature

A comprehensive literature search on EMbase, PubMed, Web of Science, Wanfang and CNKI databases was conducted to include every eligible study using the following keywords: (*MTR* OR methionine synthase) AND (variant OR SNP OR polymorphism) AND (prostate carcinoma OR prostate carcinoma) (search was carried out till Dec 01, 2019). Furthermore, reference lists of reviews or supplementary data of source literature were also retrieved for additional studies.

Inclusion Criteria and Exclusion Criteria

Published studies were included in our analysis based on the following criteria: (a) correlation assessment of PRAD and the *MTR* D919G polymorphism; (b) adequate sample sizes for all genotypes (or the potential to acquire them by calculation); and (c) case–control studies. In addition, studies were excluded if: (a) there was no control population; (b) the studies focused on other disorders instead of carcinoma; and (c) there was duplication of previous publications.

Data Extraction and Genetic Models

Two of the authors independently extracted all the data based on the selection criteria. The following details were obtained from the included case-control studies: name of the first author, year of publication, race and country of each case-control group, source of control (population based, PB; hospital based, HB), sample size of the test case and control, and genotype frequency and *p*-value for the Hardy-Weinberg equilibrium (HWE) in the cases and controls. Two alleles are involved in the *MTR* D919G polymorphism. Among these, the G-allele is a minor allele (mutated gene) and considered to be a high-risk allele. In contrast, the D-allele is the wild type and assumed to be a low-risk allele. Five genetic models were chosen for our study: allelic contrast (G-allele *vs.* D-allele), homozygous (GG *vs.* DD), heterozygous (GD *vs.* DD), dominant genetic (GG + GD *vs.* DD), and recessive genetic models (GG *vs.* GD + DD).

Statistical Analysis

The strength of association between *MTR* D919G variant and PRAD susceptibility was investigated *via* odds ratios (ORs) with 95% confidence intervals (CIs). *Z*-test was utilized to calculate statistical significance of ORs and heterogeneity assumption was investigated by Chi-square-based *Q*-test. Fixed-effect model (Mantel-Haenszel method) was applied to measure the pooled OR estimate if *p* value of *Q*-test was more than 0.01; on the other hand, Der Simonian and Laird method was used for random-effect model[32,33]. Stratified analyses were carried by ethnicity, sample size of case, and source of controls. We used web-based program (http://ihg2.helmholtz-muenchen.de/cgibin/hw/hwa1.pl) to calculate *p* values of Hardy-Weinberg equilibrium (HWE) for the control and case group[34]. *p*-value (for HWE) more than 0.05 indicated an HWE balance. Leave-one-out sensitivity analysis was conducted to measure stability of the pooled results[35]. I^2 was also evaluated to evaluate the heterogeneity. If I^2 value was less than 50%, it indicated no statistical heterogeneity of the studies. Begg's funnel plot and Egger's test were also performed to measure potential publication bias[36-37], with *p*-value more than 0.05 suggesting

no statistical significance. All statistical analyses were conducted by software STATA 11.0 (Stata Corporation, College Station, TX, USA).

In Silico Analysis of MTR Expression

A newly developed interactive online gene expression mini database was used to explore *MTR* expression in PRAD and paracancerous tissues (http://gemini.cancer-pku.cn/)[38]. This database includes the RNA expression profiles of 492 PRAD and 152 normal samples, extracted from the corresponding tissues. The Cancer Genome Atlas (TCGA) samples were examined to evaluate the gene-gene interaction of *MTR* among the PRAD subjects (http://ualcan.path.uab.edu/analysis.html). Protein Variation Effect Analyzer (PROVEAN) was used to investigate the *MTR* D919G mutation in *Homo sapiens* (http://provean.jcvi.org/seq_submit.php).

Results

Characteristics of Relevant Studies

In total, 98 articles were retrieved based on the inclusion and exclusion criteria from the PubMed, Embase, CNKI, Web of Science and Wanfang databases. The PRISMA checklist and study flow chart are presented in ***Supplementary Table I*** and ***Supplementary Figure 1***. 46 publications were rejected and 52 articles were selected for further evaluation. However, 38 of the 52 articles were excluded because they were test case-only study, duplicated publications, contained no useful information, or used ineligible samples. Thus, in total, 14 case-control study articles were selected, which included 10,617 PRAD cases and 40,489 control participants for the *MTR* variant (Table I). The enrolled studies were stratified based on the following independent criteria: (a) ethnicity: nine studies were carried out in Caucasian populations, three studies in Asian descendants, one study in Africans, and one study in South American descendants; (b) source of controls: six were HB studies, and the rest (eight) were PB studies; (c) size of case groups: eight studies involved a sample size less than 1,000 and the other six studies contained a sample size great than 1,000 samples each. Furthermore, we checked the minor allele frequencies (MAF) of *MTR* D919G variant in main worldwide populations: in Europeans, 0.1917; Americans, 0.1864; Asians, 0.2463; Africans, 0.2666; and Global, 0.2091 (Figure 1).

Main Results

The pooled analysis of all the included studies revealed a higher risk of PRAD associated with the *MTR* D919G variant (Table II) with the three genetic models as described below: for the allelic contrast model (G-allele *vs.* D-allele): OR = 1.06, 95% CI = 1.01 - 1.11, $p_{heterogeneity}$ = 0.091, p = 0.012, I^2 = 35.6 (Figure 2A); for the heterozygous model (GD *vs.* DD): OR = 1.08, 95% CI = 1.02 - 1.14, $p_{heterogeneity}$ = 0.286, p = 0.010, I^2 = 15.3; and for the dominant genetic model (GG + GD *vs.* DD): OR = 1.08, 95% CI = 1.02 - 1.14, $p_{heterogeneity}$ = 0.138, p = 0.007, I^2 = 29.9. In the subgroup analysis by race, similar results were obtained for the Asian population in all of the five genetic models as described below: for the allelic contrast model: OR = 1.22, 95% CI = 1.06 - 1.40, $p_{heterogeneity}$ = 0.050, p = 0.006, I^2 = 66.6; for the homozygous model (GG *vs.* DD): OR = 1.93, 95% CI = 1.14 – 3.26, $p_{heterogeneity}$ = 0.390, p = 0.014, I^2 = 0; for the heterozygous model (GD *vs.* DD): OR = 1.18, 95% CI = 1.01 - 1.38, $p_{heterogeneity}$ = 0.044, p = 0.042, I^2 = 68.0; for the dominant genetic model (GG + GD *vs.* DD): OR = 1.21, 95% CI = 1.04 - 1.41, $p_{heterogeneity}$ = 0.024, p = 0.016, I^2 = 73.2; and for recessive genetic model (GG *vs.* GD + DD): OR = 1.72, 95% CI = 1.02 – 2.89, $p_{heterogeneity}$ = 0.767, p = 0.041, I^2 = 0. In the stratified analysis based on control source, a positive correlation was observed between the *MTR* D919G variant and PRAD risk in the hospital based studies (allelic contrast model: OR = 1.15, 95% CI = 1.02 - 1.29, $p_{heterogeneity}$ = 0.137, p = 0.018, I^2 = 40.3), but not in the population-based studies (OR = 1.04, 95% CI = 0.99 – 1.10, $p_{heterogeneity}$ = 0.209, p = 0.093, I^2 = 27.5) (Figure 2B). Furthermore, in stratified analysis based on sample size, positive correlations were observed between *MTR* D919G variant and PRAD susceptibility in studies with larger sample size (more than 1,000 cases) in three genetic models (allelic contrast: OR = 1.05, 95% CI = 1.00 - 1.11, $p_{heterogeneity}$ = 0.226, p = 0.039, I^2 = 27.8; heterozygote comparison: OR = 1.07, 95% CI = 1.00 - 1.13, $p_{heterogeneity}$ = 0.309, p = 0.036, I^2 = 16.2; dominant genetic model: OR = 1.07, 95% CI = 1.01 - 1.13, $p_{heterogeneity}$ = 0.277, p = 0.029, I^2 = 20.8).

In Silico Analysis of MTR Expression

The results of the *in silico* analysis revealed lower *MTR* expression in PRAD tissue than in normal tissue (Transcripts Per Million = 2.68 *vs.* 3.34, $p < 0.05$, Figure 3A). Furthermore, we evaluated whether the *MTR* expression level and Glea-

Table I. Study characteristics of the MTR D919G polymorphism included in the present analysis.

First author	Year	Country	Ethnicity	Design	Method	Subjects size	Sample size of case				pHWE					pHWE
							GG	GD	DD	Total		GG	GD	DD	Total	
Ebrahimi	2017	Iran	Asian	HB	PCR-RFLP	<1000	13	53	34	100	0.276	6	37	57	100	0.999
Qu	2016	China	Asian	HB	real-time PCR	>1000	20	316	1481	1817	0.496	15	319	1692	2026	0.993
Jackson	2013	Jamaica	African	HB	Taqman	<1000	20	82	97	199	0.664	24	82	99	205	0.274
López-Cortés	2013	Ecuador	SA	PB	PCR-RFLP	<1000	3	9	92	104	<0.001	1	4	105	110	0.001
Weiner	2012	Russia	Caucasian	PB	real-time PCR	<1000	15	134	221	370	0.339	16	96	173	285	0.580
Cai	2010	China	Asian	HB	PCR-RFLP	<1000	5	27	185	217	0.003	3	29	188	220	0.139
ProtecT	2009	UK	Caucasian	PB	Taqman	>1000	52	515	1033	1600	0.207	84	637	1355	2076	0.402
Stevens	2008	USA	Caucasian	PB	Taqman	>1000	42	351	701	1094	0.814	53	324	728	1105	0.032
UKGPCS	2008	UK	Caucasian	PB	GWAS	>1000	84	590	1176	1850	0.364	71	547	1268	1886	0.213
Marchal	2008	Spain	Caucasian	HB	Taqman	<1000	9	54	118	181	0.391	11	55	138	204	0.088
FHS	2007	USA	Caucasian	PB	GWAS	<1000	7	55	110	172	0.970	9	69	153	231	0.728
CGEMS	2007	USA	Caucasian	PB	GWAS	>1000	48	376	738	1162	0.990	38	340	734	1112	0.858
deCODE	2006	Iceland	Caucasian	PB	GWAS	>1000	60	466	1093	1619	0.242	1044	9160	20575	30779	0.532
Kimura	2000	Germany	Caucasian	HB	PCR-RFLP	<1000	4	41	87	132	0.753	4	44	102	150	0.773

SA: South America; HWE: Hardy-Weinberg equilibrium of controls, HB: Hospital-based; PB: Population-based; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; GWAS: Genome-wide association study; CGEMS: Cancer Genetic Markers of Susceptibility; FHS: Framingham Heart Study; UKGPCS: UK Genetic Prostate Cancer Study.

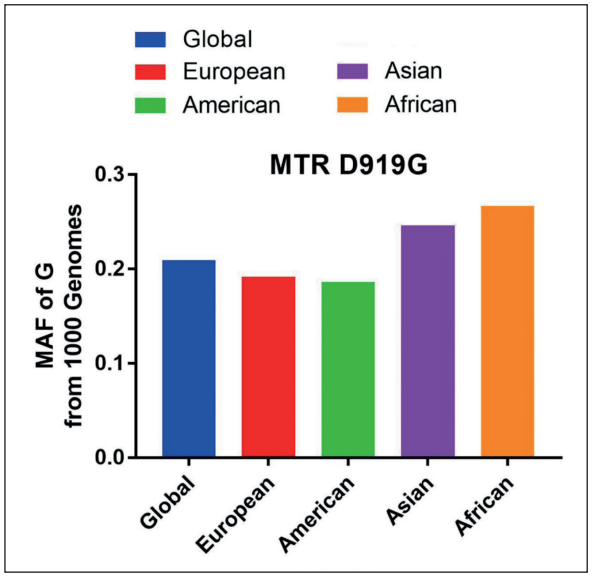

Figure 1. Minor allele frequency (MAF) of the *MTR* D919G variant in various races.

son score affected the survival time of patients with PRAD. As shown in Figure 3B, the patient group with high *MTR* expression and Gleason score = 6 had reduced survival time ($p < 0.0001$). The results from TCGA database[39] showed lower MTR expression in advanced-stage PRAD than in the normal prostate ($p < 0.05$, Figure 4A). In addition, no significant difference between normal and PRAD cases was observed in the Caucasian population ($p > 0.05$, Figure 4B). A similar result was also seen in the African-American population ($p > 0.05$, Figure 4B). We used PROVEAN tools to predict whether the *MTR* D919G variant could influence its protein expression. The PROVEAN score distribution for deleterious and neutral human protein variations is shown in Figure 5A. The default threshold is -2.5 (Figure 5C). The *MTR* D919G variant PROVEAN score was found to be -3.871, which indicates that this mutation is deleterious (Figure 5B). As shown in Figure 6A, more than 24 genes were shown to be associated with *MTR* in PRAD. The *BOD1L* gene (biorientation of chromosomes in cell division 1-like) was the most related gene (Pearson CC: 0.88, Figure 6B). There was a positive correlation between the *BOD1L* gene and *MTR* in PRAD (Figure 6C).

Sensitivity Analysis and Publication Bias

We conducted sensitivity analysis to explore the effect of each study on the pooled OR by the sequential exclusion of individual studies. The results from this analysis confirmed that the pooled OR data were trustworthy (G-allele *vs.* D-allele, Figure 7) and that no single study would significantly affect the overall OR. Both Begg's and Egger's tests were used to evaluate the publication bias; we observed no indication of publication bias for any of the models. For allelic comparison model of G-allele *vs.* D-allele: $t = 1.80$, $p = 0.097$; for heterozygote comparison model: $t = 1.91$, $p = 0.080$; for heterozygote comparison: $t = 1.74$, $p = 0.107$; for dominant genetic model: $t = 1.79$, $p = 0.099$; and for recessive genetic model: $t = 1.89$, $p = 0.083$.

Discussion

The identification of SNPs that influence genetic variants and contribute to cancer susceptibility could be used to predict cancer risk at the population level and understand the pathogenesis of cancer[40,41]. Globally, PRAD is one of the most common solid tumors among males, especially in Western countries. Several risk factors, including family history, phosphorus intake, lifestyle, and others, were shown to be associated with PRAD risk[42-45]. Hubner et al[46] indicated that the folate-metabolizing gene *MTR* might participate in the development of prostate carcinogenesis. However, the correlation between the *MTR* D919G variant, one of the critical SNPs involved in DNA methylation, and PRAD risk remains unclear. For example, Ebrahimi et al[22] showed the influence of the *MTR* D919G variant on the stability and activity of methionine synthase, which is related to PRAD risk, in males in Iran; in contrast, some researchers[26] demonstrated no apparent association between this *MTR* variant and PRAD risk. A previous 2009 meta-analysis[18] showed no statistically significant results. Since then, many investigators[19,20,22,23,25,26] confirmed their findings in different population subsets and larger sample sizes. Therefore, in the current analysis, we included all eligible studies according to a few inclusion criteria to assemble genetic data and obtain accurate conclusions on the association between the *MTR* D919G variant and PRAD risk. In total, 10,617 PRAD cases and 40,489 control participants were evaluated in our study to assess the *MTR* polymorphism.

Results from TCGA database showed lower *MTR* expression in PRAD than in the normal prostate among the overall population. According to the TCGA database, no significant difference between normal and PRAD cases was observed in the Caucasian and African-American population.

Table II. Stratified analysis of the MTR D919G variant on PRAD susceptibility.

Variables	N[a]	Cases/ Controls	Allelic contrast OR (95%CI) p^b p I^2	Homozygous model OR (95%CI) p^b p I^2	Heterozygous model OR (95%CI) p^b p I^2	Dominant genetic model OR (95%CI) p^b p I^2	Recessive genetic model OR (95%CI) p^b p I^2
Total	14	10617/40489	1.06 (1.01-1.11) 0.091 0.012 35.6	1.07 (0.93-1.23) 0.331 0.321 11.1	1.08 (1.02-1.14) 0.286 0.010 15.3	1.08 (1.02-1.14) 0.138 0.007 29.9	1.04 (0.91-1.19) 0.528 0.553 0
Ethnicity							
Caucasian	9	8180/37828	1.04 (0.99-1.10) 0.671 0.104 0	1.03 (0.89-1.19) 0.614 0.710 0	1.06 (1.00-1.13) 0.687 0.056 0	1.06 (1.00-1.12) 0.694 0.062 0	1.01 (0.87-1.16) 0.602 0.930 0
Asian	3	2134/2346	1.22 (1.06-1.40) 0.050 0.006 66.6	1.93 (1.14-3.26) 0.390 0.014 0	1.18 (1.01-1.38) 0.044 0.042 68.0	1.21 (1.04-1.41) 0.024 0.016 73.2	1.72 (1.02-2.89) 0.767 0.041 0
SA	1	104/110	2.77 (1.05-7.29) - 0.039 -	3.42 (0.35-33.49) - 0.290 -	2.57 (0.77-8.02) - 0.127 -	2.74 (0.93-8.07) - 0.067 -	3.24 (0.33-31.63) - 0.312 -
African	1	199/205	0.95 (0.71-1.28) - 0.746 -	0.85 (0.44-1.64) - 0.629 -	1.02 (0.67-1.55) - 0.923 -	0.98 (0.66-1.45) - 0.928 -	0.84 (0.45-1.58) - 0.593 -
Source							
PB	8	7971/37584	1.04 (0.99-1.10) 0.209 0.093 27.5	1.03 (0.89-1.20) 0.398 0.650 4.1	1.06 (1.00-1.13) 0.373 0.057 7.4	1.06 (1.00-1.12) 0.293 0.058 17.4	1.01 (0.87-1.17) 0.395 0.857 4.5
HB	6	2646/2905	1.15 (1.02-1.29) 0.137 0.018 40.3	1.31 (0.92-1.87) 0.303 0.138 17.2	1.15 (1.01-1.32) 0.245 0.041 25.3	1.16 (1.02-1.32) 0.133 0.023 40.9	1.22 (0.86-1.73) 0.568 0.273 0
Size of case							
>1000	6	9142/38984	1.05 (1.00-1.11) 0.226 0.039 27.8	1.06 (0.92-1.23) 0.258 0.424 23.4	1.07 (1.00-1.13) 0.309 0.036 16.2	1.07 (1.01-1.13) 0.277 0.029 20.8	1.04 (0.90-1.21) 0.258 0.588 23.4
<1000	8	1475/1505	1.11 (0.98-1.27) 0.078 0.108 45.1	1.12 (0.80-1.57) 0.326 0.520 13.3	1.17 (0.99-1.38) 0.294 0.069 17.2	1.16 (0.99-1.36) 0.123 0.068 38.5	1.04 (0.75-1.45) 0.602 0.810 0

[a]Number of comparisons.
[b]p value of Q-test for heterogeneity test(pheter).
SA: South America; HB: Hospital-based; PB: Population-based.

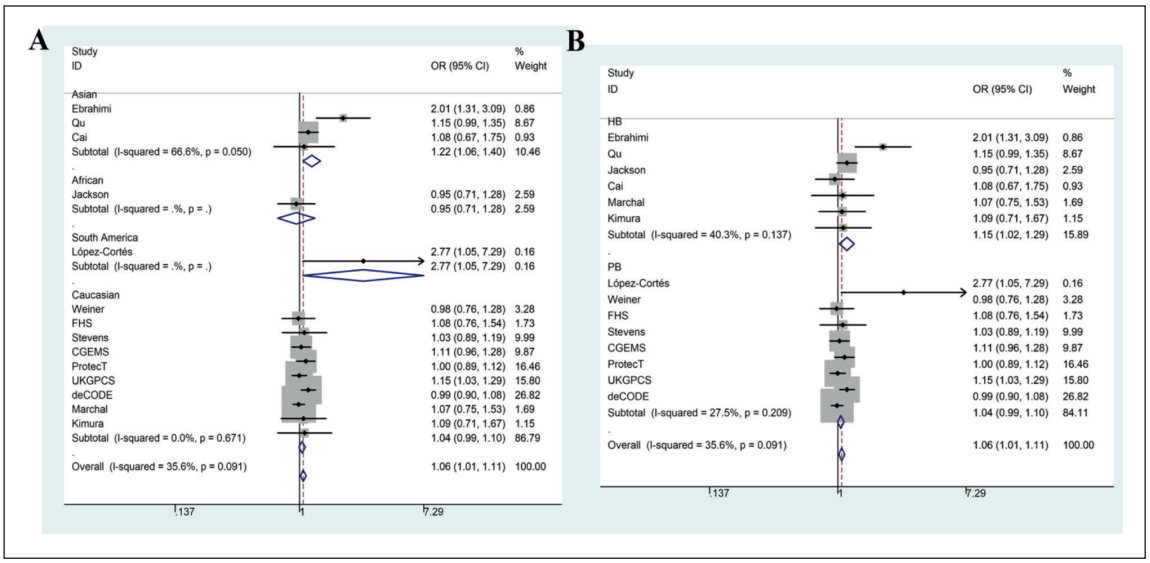

Figure 2. Forest plot shows odds ratio for the association between the *MTR* D919G polymorphism and PRAD risk in stratified analysis by ethnicity (**A**) and source of controls (**B**) (allelic contrast of G-allele *vs.* D-allele, fixed-effects).

Moreover, no relevant research in individuals of Asian descent can be acquired from the database. In our present analysis, we selected 14 case-control studies. The overall results from three genetic models indicated a positive correlation between the *MTR* D919G variant and PRAD susceptibility. In the subgroup analysis by race, we observed a higher risk of PRAD associated with the *MTR* D919G variant in the Asian descendants, but not other populations. This finding is consistent with the TCGA results. Furthermore, the conclusions obtained in our study were: an individual carrying the *MTR* G-allele may have an elevated PRAD risk. The *MTR* D919G variant may

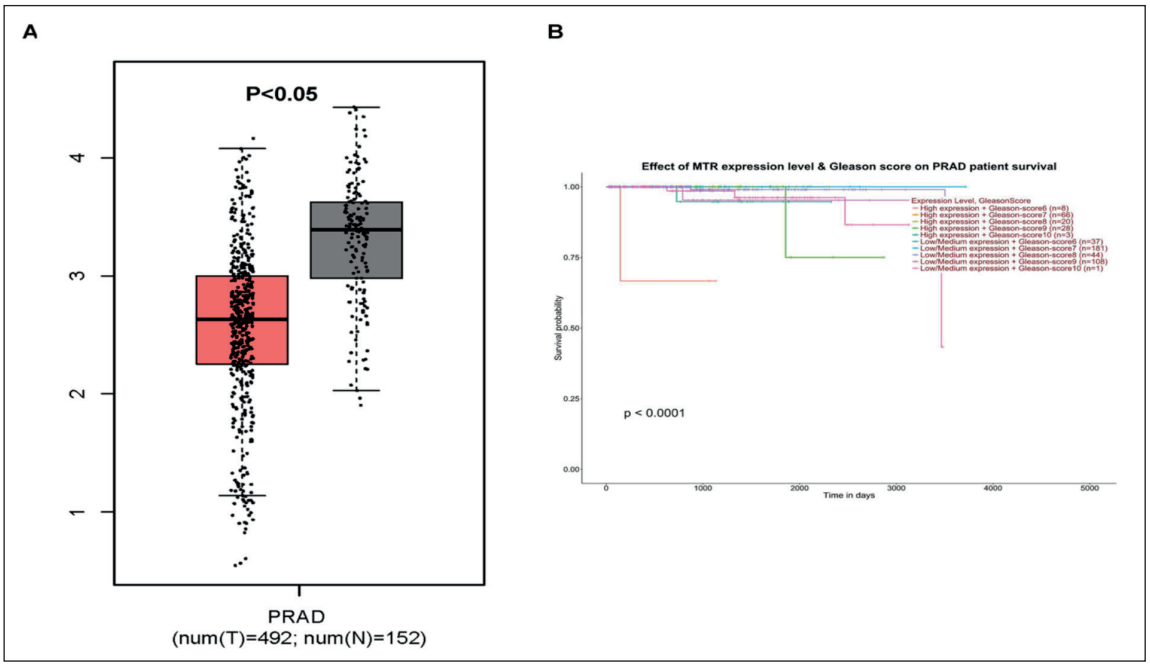

Figure 3. *In silico* analysis of *MTR* expression. (**A**) The relative expression of *MTR* in PRAD tissue and normal paracancerous tissue. (**B**) Effect of *MTR* expression level and Gleason score on prostate adenocarcinoma patients' survival time.

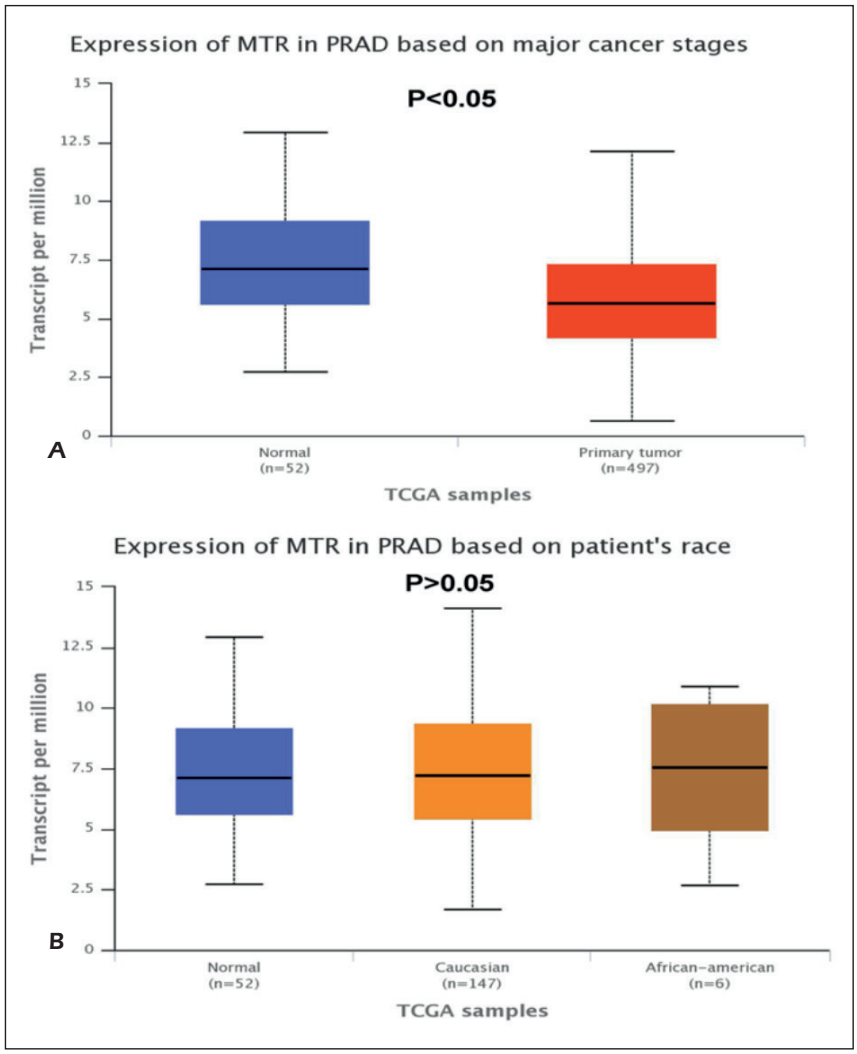

Figure 4. Expression of *MTR* in prostate adenocarcinoma based on major cancer stages (**A**) and patients' ethnicity (**B**).

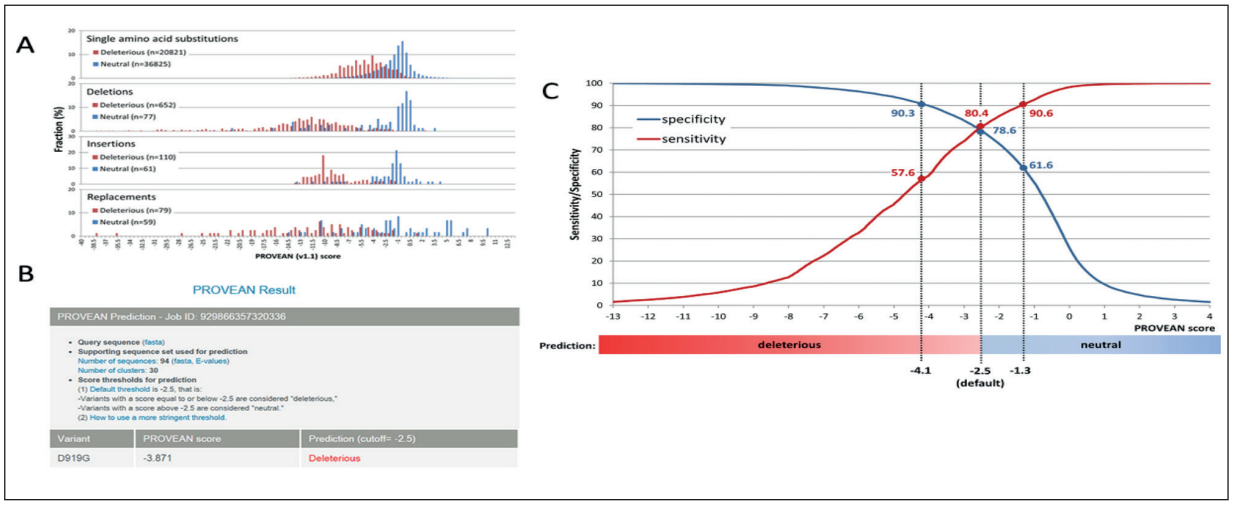

Figure 5. Evaluation of the *MTR* D919G variant by Protein Variation Effect Analyzer (PROVEAN, v1.1). PROVEAN (v1.1) score distribution for deleterious and neutral UniProt human protein variations (**A**). The Default threshold is -2.5 (**C**). The PROVEAN score of *MTR* D919G variant is -3.871, which indicate that this mutation is deleterious (**B**).

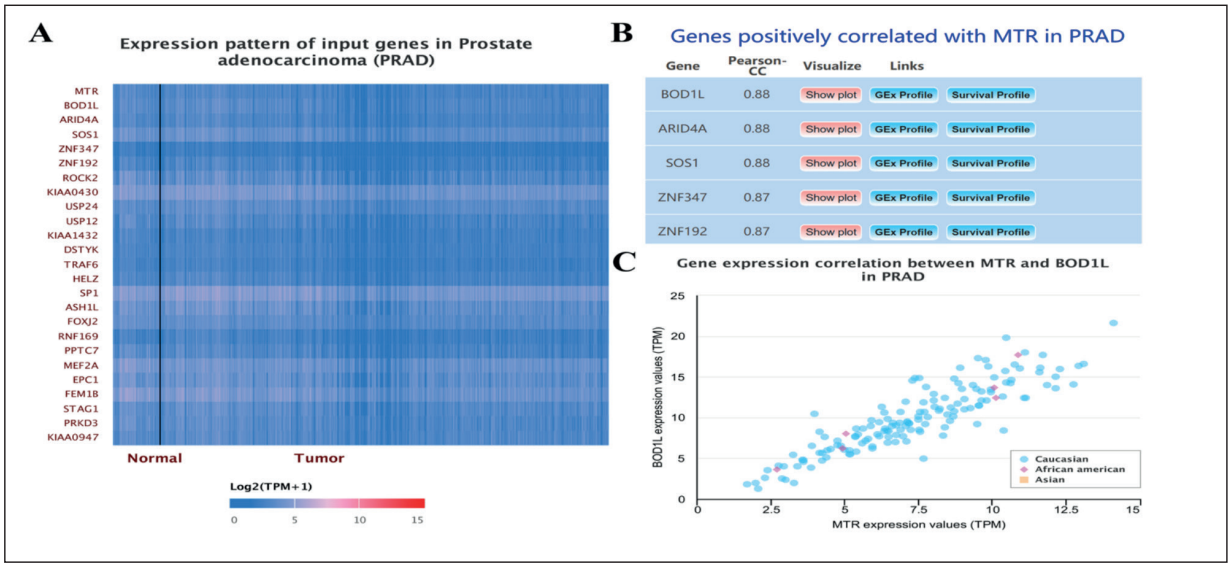

Figure 6. Gene-gene correlation of *MTR*. 24 genes have been predicted to participate in the interaction of *MTR* (**A**). *BOD1L* gene (biorientation of chromosomes in cell division 1-like) was the most related gene (Pearson CC is 0.88, **B**). There was a positive correlation between *BOD1L* gene and *MTR* in PRAD (**C**).

increase PRAD susceptibility, as seen in studies involving hospital based studies and studies with large sample sizes. We also used PROVEAN tools to predict whether the *MTR* D919G variant could influence its protein expression. The *MTR* D919G variant PROVEAN score was found to be -3.871, which indicates that this mutation is deleterious and this variant can affect the expression of *MTR*. We further evaluated whether the *MTR* expression level and Gleason score affect the survival time in patients with PRAD. We observed that PRAD patients with high expression of *MTR* and Gleason score = 6 exhibited reduced survival time.

Although the evaluation of the correlation between the *MTR* D919G variant and PRAD susceptibility required the use of considerable resources, certain limitations should be addressed. First, only three Asian studies involving the *MTR* D919G variant and PRAD risk were selected. Thus the total participant count for the Asian population was relatively low to allow a very comprehensive analysis. Second, all the included publications were ret-

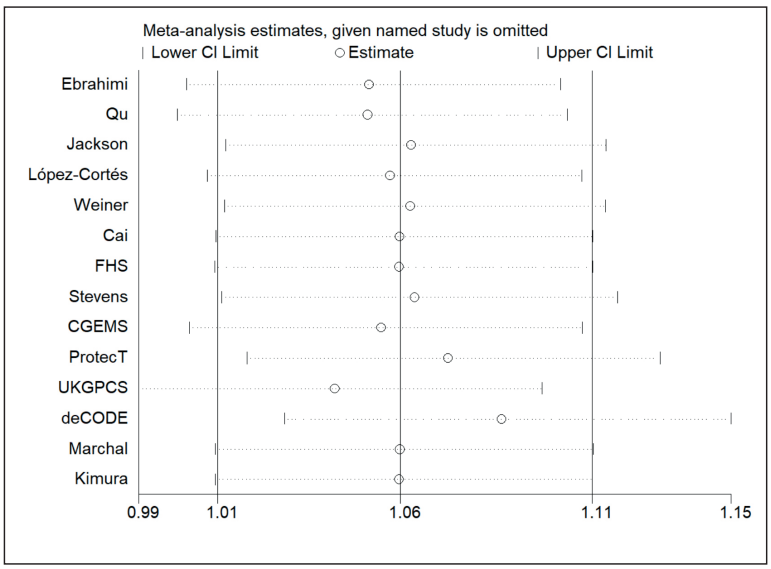

Figure 7. Sensitivity analysis about the *MTR* D919G variant and PRAD risk (G-allele *vs.* D-allele). Results were evaluated by removing each study in turn. Two ends of the dotted lines represent for 95% CI.

rospective case-control studies according to the inclusion criteria, which may have caused a selection bias in the above analysis. Third, the lack of specific data, including family history, body weight, lifestyle, tumor stage, and smoking exposure, limited the ability to further examine the adjusted OR or gene-environment interactions. Fourth, only articles published in English and Chinese were selected; therefore, some publications written in other languages were not included, which may have caused a further bias in the risk estimation processes. Despite these limitations, the present analysis has several advantages. First, the HWE is very important while studying SNPs. The *p*-value for the HWE in the control group is higher than 0.05 in the majority of the included articles, except for two[23,28], which indicates the superior quality of the selected studies. Second, a larger sample size significantly improved the statistical efficiency. Third, no heterogeneity was observed while evaluating the *MTR* D919G variant. Hence, the conclusions of the present analysis are more compelling than those of the earlier studies. Results from TCGA samples indicated the involvement of at least 24 genes in the interaction with *MTR* in PRAD. Among these, the *BOD1L* gene was the most related gene. Nevertheless, there is insufficient research on the involvement of this gene in PRAD. Further studies are required to investigate these interactions in detail.

Conclusions

In summary, our analysis of the studies on the Asian population as well as hospital based and large sample size studies indicated an association between the *MTR* D919G variant and PRAD susceptibility. Furthermore, the *MTR* D919G variant might be related to PRAD prognosis. Further studies with more information on lifestyle, tumor stage, and smoking exposure are warranted for an in-depth analysis of this association.

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Author Contribution

HJ and LZ designed this study, LY and HY analyzed and acquired the data, HJ and TL were involved in the collection of full-text papers, LY and TL drafted the manuscript. HY and LZ prepared all figures and tables. TL and LZ provided funding support. All the authors agreed to submission of this work.

Availability of Data and Materials

All the data generated in the current research is contained in this manuscript.

Conflict of Interests

The authors declare that they have no conflict of interests.

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