

Association of gene polymorphisms of *KLK3* and prostate cancer: A meta-analysis

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Conflict of interest

None declared

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Abstract

Previous studies have suggested that prostate-specific antigen (PSA) plays a role in the etiology of prostate cancer (PCa), and that polymorphisms of *KLK3* may be associated with PCa. However, these results were conflicting. Therefore, we performed a meta-analysis to illuminate this problem. We searched the PubMed and Web of Science databases. Ten single nucleotide polymorphisms (SNPs) were involved in this meta-analysis. The pooled results showed that the minor alleles of rs1058205, rs2735839, rs174776, rs17632542, rs266849, rs266878, and rs2569735 were significantly associated with PCa. Compared to genotypes of the common homozygotes, the heterozygous genotypes of rs1058205, rs2735839, rs174776, rs17632542, rs266849, and rs266878 were significantly associated with PCa, as well as the homozygous genotypes of rs1058205, rs2735839, rs17632542, rs266878, and rs2569735. Only rs2735839 was involved in the Gleason score (GS). The pooled results showed that when compared with GS ≥ 8 PCa, the A-allele was the protective factor for GS < 7 PCa. It was also a protective factor for GS $\geq 4+3$ when compared to GS $\leq 3+4$ PCa. A strong association was observed between PCa and rs1058205, rs2735839, rs266882, rs174776, rs17632542, rs266849, rs266878, rs1058274, and rs2569735. The G-allele of rs2735839 was a risk factor for GS < 7 PCa when compared with the GS ≥ 8 PCa, as well as for the GS $\geq 4+3$ when compared to the GS $\leq 3+4$ PCa. Therefore, these SNPs may be valuable as biomarkers for PCa in the future.

Key words: *KLK3*, prostate-specific antigen, polymorphisms, prostate cancer, meta-analysis

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Prostate cancer (PCa) is the 2nd most frequently diagnosed cancer in men around the world, and one of the leading causes of cancer death among men of all races.¹ With the aging of the population and the improvement of living conditions in recent years, the incidence of PCa has been increasing every year.² Serum levels of prostate-specific antigen (PSA) are widely used for screening for PCa. The PSA levels are known to be influenced by genetic components: Around 40–45% of the variance in PSA is thought to be explained by genetic components.^{3,4} Previous studies have revealed that kallikrein 3 (*KLK3*) is the strongest genetic factor to influence levels of PSA, and its single nucleotide polymorphism (SNP) loci have been shown to be associated with PCa.^{5–7}

The *KLK3* is located on chromosome 19q13.33, which encodes PSA and is a member of the serine protease kallikrein family. We searched the PubMed and Web of Science databases without language restrictions up to January 8, 2018, for relevant studies about the association of the SNPs of *KLK3* and PCa. We found that nearly 59 SNP loci were mentioned in studies in these databases, and among them, 21 SNP loci were involved in more than 2 studies (Fig. 1). However, these results were conflicting and there was still a lack of any relevant comprehensive analysis to clarify the confusion.

Therefore, in this study, we performed a literature review and a meta-analysis to explore the association between the risk of PCa and the 21 SNP loci of *KLK3* that were mentioned in more than 2 studies.

Material and methods

Search strategy

We searched the PubMed and Web of Science databases through September, 2018, without language restrictions, for relevant studies about associations of the SNPs of *KLK3* and PCa. The search term was (((*KLK3*) AND ((single nucleotide polymorphism) OR SNP))) AND ((prostate cancer) OR PSA).

Inclusion/exclusion criteria

The title, abstract and full text of the candidate studies were independently screened by 2 reviewers. A study was included when all of the following criteria were met: 1) Non-familial studies that examined the association between SNPs of *KLK3* and PCa were included; 2) studies that had complete data or data that could be used to calculate an odds ratio (OR) and a 95% confidence interval (95% CI) were included; 3) studies that had incomplete data were excluded.

Data extraction

Information was carefully extracted from all the eligible publications by 2 independent reviewers (Li and Fei), based on the aforementioned inclusion criteria. Any

disagreements were arbitrated by discussion with a 3rd reviewer (Shen). The following data were collected from each study: the 1st author's surname, the year of publication, the country, the laboratory methods used to detect *KLK3* polymorphisms, and the number of cases and controls.

Quality assessment

We used the Newcastle-Ottawa scale (NOS) to assess the quality of each eligible study. The NOS contains 8 items: 1) The cases were independently validated; 2) Cases were representative of a population; 3) There were community controls; 4) The controls had no history of PCa; 5A) The study was controlled for age; 5B) The study was controlled for additional factors; 6) Exposure was ascertained by blinded interview or record; 7) The same method of ascertainment was used for both the cases and the controls; 8) The non-response rate was the same for the cases and the controls. When a study fulfilled 1 criterion, it got 1 score. The NOS is arranged from 0 up to 9 scores, and a study is considered high quality if it gets more than 4 scores.

Statistical analysis

The strength of the association between *KLK3* polymorphism and the risk of PCa was shown using an OR with a 95% CI. If a study just provided the frequency (assumption: the frequency of allele 1 or genotype 1 in the case group was A; the frequency of allele 2 or genotype 2 in the case group was B; the frequency of allele 1 or genotype 1 in the control group was C; the frequency of allele 2 or genotype 2 in the control group was D), we used the formulas “ $OR = (A/B)/(C/D)$ ” and “ $95\% \text{ CI of } \ln OR = \ln(OR) \pm 1.96(1/A + 1/B + 1/C + 1/D)^{0.5}$ ” to calculate the OR and its 95% CI.

The statistical significance of the pooled OR was assessed with a Z-test, and a p-value of 0.05 was considered significant. A χ^2 -based Q-test was conducted to measure the heterogeneity of the eligible studies, and the heterogeneity was considered significant if the p-value for the heterogeneity test was 0.05. A sensitivity analysis in which 1 study was excluded at a time was conducted to evaluate the influence of an individual study on the results. Begg's funnel plot and Egger's regression test were used to evaluate the publication bias (no publication bias was indicated by a two-sided p-value ≥ 0.05). All the analyses were conducted using Stata v. 11.0 software (StataCorp LLC, College Station, USA), and a two-sided p-value ≥ 0.05 indicated no significance.

Results

Literature search

The study selection process is shown in Fig. 2. The primary literature search identified 45 studies. After the titles and abstracts were screened, 13 studies were excluded:

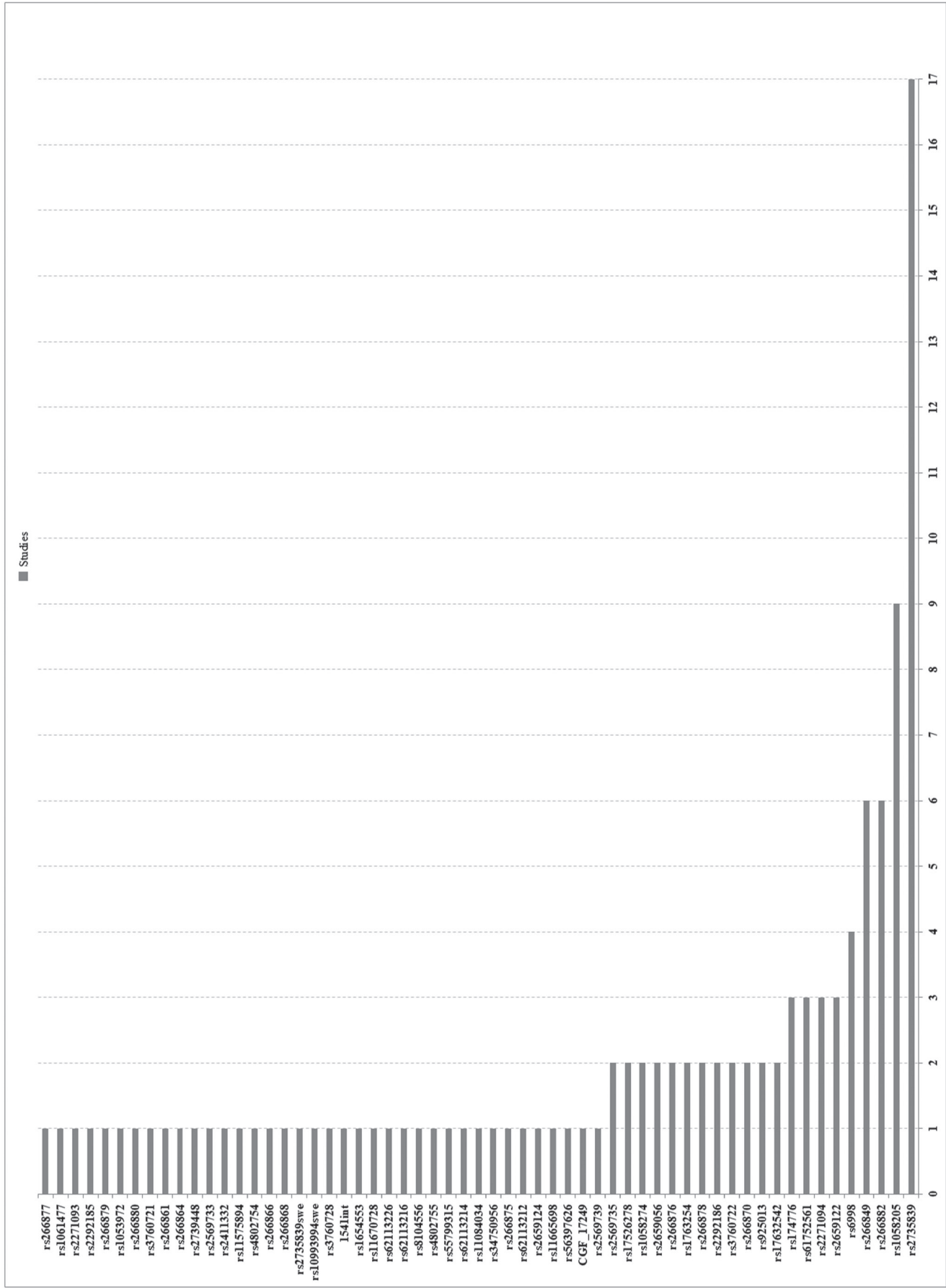


Fig. 1. 59 SNP loci of KLK3 that were mentioned in studies in the databases

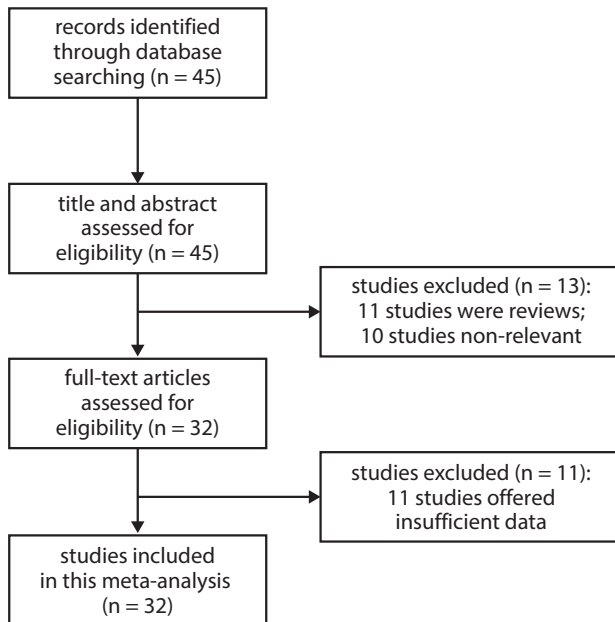


Fig. 2. The study selection process

3 were reviews and 10 were irrelevant studies. The full texts of the remaining 32 studies were then evaluated. As a result, 12 studies were excluded because of useless data and 21 studies were included in the meta-analysis.^{8–28} The 21 eligible studies were assessed with the NOS (Table 1). Each had a score more than 4, which means that all the studies were of high quality.

Meta-analysis of associations between SNPs and PCa risk

We found that 10 SNP loci were available to perform a meta-analysis to illuminate associations between the SNPs of *KLK3* and PCa risk. They were rs1058205, rs2735839, rs266882, rs174776, rs17632542, rs266849, rs266878, rs266876, rs1058274, and rs2569735.^{8–10,12,14,16–22,25–28} Their genetic information is presented in Table 2. The pooled results are shown in Table 3.

For the alleles, we found that except rs266882, rs266876 and rs1058274, the remaining 7 SNP loci were significantly

Table 1. Characteristics and quality assessment of eligible studies in the meta-analysis

Fist author	Patients	Detection method	Year	Quality indicators from NOS										Score
				1	2	3	4	5A	5B	6	7	8		
Choe EK ⁸	Korean	genotyping arrays	2017	yes	yes	no	yes	no	no	yes	yes	yes	6	
Chen C ⁹	Chinese	PCR-HRM	2017	yes	yes	no	yes	yes	yes	yes	yes	yes	8	
Stegeman S ¹⁰	European	Illumina Infinium Array	2015	yes	yes	no	yes	no	no	yes	yes	yes	6	
He Y ¹¹	Caucasian men	Illumina BeadXpress Reader	2014	yes	yes	no	yes	no	no	yes	yes	yes	6	
Hu J ¹²	Chinese	TaqMan/MGB Assay	2014	yes	yes	yes	yes	no	no	yes	yes	yes	7	
Shui IM ¹³	European	TaqMan Assay	2014	yes	yes	no	yes	no	no	yes	yes	yes	6	
Wang NN ¹⁴	Chinese	PCR-HRM	2013	yes	yes	no	yes	no	no	yes	yes	yes	6	
Soni A ¹⁵	India	PCR-RFLP	2012	yes	yes	no	yes	no	no	yes	yes	yes	6	
Kwon EM ¹⁶	Caucasian and African American men	genotyping arrays	2012	yes	yes	yes	yes	yes	no	yes	yes	yes	8	
Kote-Jarai Z ¹⁷	UK/Australian	genotyping arrays	2011	yes	yes	no	yes	no	no	yes	yes	yes	6	
Penney KL ¹⁸	American	Sequenom technology	2011	yes	yes	no	yes	no	no	yes	yes	yes	6	
Lindstrom S ¹⁹	European	TaqMan Assay	2011	yes	yes	no	yes	no	no	yes	yes	yes	6	
Ciampa J ²⁰	European	Illumina Chips	2011	yes	yes	no	yes	no	no	yes	yes	yes	6	
Parikh H ²¹	European	TaqMan Assays	2011	yes	yes	no	yes	no	no	yes	yes	yes	6	
Gudmundsson J ²²	Icelandic	Illumina Chips	2010	yes	yes	no	yes	yes	no	yes	yes	yes	7	
Gallagher DJ ²³	Ashkenazi Jewish ancestry	Mass ARRAY QGE iPLEX System	2010	yes	yes	no	yes	no	no	yes	yes	yes	6	
Kader AK ²⁴	European	Mass ARRAY QGE iPLEX System	2009	yes	yes	no	yes	no	no	yes	yes	yes	6	
Xu J ²⁵	European	Mass ARRAY QGE iPLEX System	2008	yes	yes	no	yes	no	no	yes	yes	yes	6	
Eeles RA ²⁶	UK and Australia	sequencing	2008	yes	yes	no	yes	no	no	yes	yes	yes	6	
Lai J ²⁷	Caucasian men	PCR-RFLP	2007	yes	yes	no	yes	no	no	yes	yes	yes	6	
Cicek MS ²⁸	American	PCR-RFLP	2005	yes	yes	no	yes	no	no	yes	yes	yes	6	

PCR-HRM – high-resolution melting curve polymerase chain reaction method; PCR-RFLP – PCR-restriction fragment length polymorphism; NOS – Newcastle-Ottawa scale.

Table 2. Genetic information for 10 SNPs of *KLK3*

SNP	Chromosome ^a	Functional consequence ^a	Position (bp) ^a		Minor allele	Major allele
			GRCh38.p7	GRCh37.p13		
rs1058205	19:50860142	URT variant 3 prime	50860142	51363398	C allele	T allele
rs2735839	19:50861367	downstream	50861367	51364623	A allele	G allele
rs266882	19:50854757	upstream variant 2KB	50854757	51358013	A allele	G allele
rs174776	19:50856596	intron variant	50856596	51359852	T allele	C allele
rs17632542	19:50858501	missense	50858501	51361757	T allele	C allele
rs266849	19:50845834	intron variant	50845834	51349090	G allele	A allele
rs266878	19:50855858	intron variant	50855858	51359114	G allele	C allele
rs266876	19:50857562	intron variant	50857562	51360818	C allele	T allele
rs1058274	19:50860192	URT variant 3 prime	50860192	51363448	G allele	A allele
rs2569735	19:50861013	downstream variant 500B	50861013	51364269	A allele	G allele

^a The information was provided by the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>); URT – untranslated regions; SNP – single nucleotide polymorphism; *KLK3* – kallikrein 3.

Table 3. Meta-analysis of associations between SNPs and PCa risk

SNP	Number of studies	Test for overall effect			Test for heterogeneity		Test for publish bias	
		OR (95% CI)	Z-score	p-value	I ²	p-value	P _{egger's}	P _{begg's}
rs1058205								
C allele vs T allele	8 [9, 10, 16–18, 21] a7 [9, 10, 16–18, 21]	0.79 (0.73~0.87) 0.85 (0.82~0.88)	5.09 8.81	<0.001 <0.001	83.2% 10.8%	<0.001 0.347	– 0.085	– 0.133
TC vs TT	7 [9, 10, 17, 18, 21] a6 [9, 10, 17, 18, 21]	0.79 (0.72~0.86) 0.84 (0.80~0.88)	5.08 7.87	<0.001 <0.001	77.6% 15.8%	<0.001 0.312	– 0.303	– 0.707
CC vs TT	7 [9, 10, 17, 18, 21] a6 [9, 10, 17, 18, 21]	0.62 (0.49~0.77) 0.67 (0.61~0.73)	4.28 8.29	<0.001 <0.001	72.2% 0.0%	0.001 0.540	– 0.520	– 1.000
rs2735839								
A allele vs G allele	14 [8, 12, 14, 17, 19–22, 25, 26] b11 [8, 14, 17, 19–22, 25, 26]	0.78 (0.71~0.86) 0.86 (0.82~0.90)	4.96 6.35	<0.001 <0.001	87.5% 36.4%	<0.001 0.108	– 0.152	– 0.533
AG vs GG	10 [12, 14, 17, 19, 21, 26] b7 [14, 17, 19, 21, 26]	0.80 (0.71~0.91) 0.85 (0.80~0.90)	3.52 5.50	0.001 <0.001	87.3% 36.7%	<0.001 0.148	– 0.424	– 1.000
AA vs GG	10 [12, 14, 17, 19, 21, 26] b7 [14, 17, 19, 21, 26]	0.77 (0.54~1.10) 0.81 (0.67~0.97)	1.46 2.32	0.144 0.020	88.1% 39.0%	<0.001 0.131	– 0.158	– 0.230
rs266882								
A allele vs G allele	4 [15, 18, 27, 28] c2 [18, 28]	1.26 (0.97~1.64) 1.00 (0.91~1.10)	1.71 0.01	0.087 0.995	83.7% 0.0%	<0.001 0.978	– –	– –
AG vs GG	4 [15, 18, 27, 28] d3 [15, 18, 28]	1.40 (0.92~2.13) 1.20 (0.82~1.76)	1.56 0.95	0.119 0.340	68.2% 57.1%	0.024 0.097	– 0.622	– 0.296
AA vs GG	4 [15, 18, 27, 28] d3 [15, 18, 28]	1.45 (0.92~2.29) 1.18 (0.80~1.72)	1.59 0.84	0.112 0.402	74.4% 62.9%	0.008 0.068	– 0.277	– 0.296
rs174776								
T allele vs C allele	3 [16, 18, 21]	0.86 (0.80~0.93)	3.74	<0.001	0.0%	0.619	0.326	1.000
CT vs CC	2 [18, 21]	0.87 (0.79~0.97)	2.58	0.010	0.0%	0.844	–	–
TT vs CC	2 [18, 21]	0.77 (0.55~1.06)	1.62	0.106	0.0%	0.436	–	–
rs17632542								
T allele vs C allele	4 [17, 22] a3 [17, 22]	0.61 (0.43~0.86) 0.72 (0.64~0.82)	2.79 4.99	0.005 <0.001	95.0% 50.2%	<0.001 0.134	– 0.659	– 1.000
TC vs CC	3 [17] a2 [17]	0.57 (0.37~0.87) 0.72 (0.59~0.87)	2.61 3.43	0.009 0.001	95.4% 72.2%	<0.001 0.058	– –	– –
TT vs CC	3 [17] a2 [17]	0.32 (0.14~0.75) 0.50 (0.30~0.83)	2.62 2.68	0.009 0.007	73.1% 0.0%	0.024 0.819	– –	– –

Table 3. Meta-analysis of associations between SNPs and PCa risk – cont.

SNP	Number of studies	Test for overall effect			Test for heterogeneity		Test for publish bias	
		OR (95% CI)	Z-score	p-value	I ²	p-value	P _{egger's}	P _{egg's}
rs266849								
G allele vs A allele	8 [17, 19, 26] e5 [17, 19, 26]	0.81 (0.71~0.92) 0.94 (0.89~0.98)	3.16 2.68	0.002 0.007	92.2% 17.0%	<0.001 0.306	– 0.289	– 0.462
GA vs AA	7 [17, 19, 26] e4 [17, 19, 26]	0.80 (0.70~0.91) 0.91 (0.85~0.98)	3.40 2.55	0.001 0.011	90.1% 43.8%	<0.001 0.149	– 0.927	– 0.734
GG vs AA	7 [17, 19, 26] e4 [17, 19, 26]	0.73 (0.55~0.97) 0.98 (0.86~1.10)	2.15 0.39	0.032 0.699	87.2% 4.4%	<0.001 0.371	– 0.812	– 1.000
rs266878								
G allele vs C allele	2 [18, 21]	0.86 (0.78~0.94)	3.23	0.001	0.0%	0.410	–	–
GC vs CC	2 [18, 21]	0.87 (0.78~0.97)	2.60	0.009	0.0%	1.000	–	–
GG vs CC	2 [18, 21]	0.72 (0.52~0.98)	2.10	0.036	0.0%	0.354	–	–
rs266876								
C allele vs T allele	2 [18, 21]	0.83 (0.63~1.08)	1.42	0.157	90.9%	0.001	–	–
CT vs TT	2 [18, 21]	0.99 (0.90~1.08)	0.22	0.825	0.0%	0.703	–	–
CC vs TT	2 [18, 21]	0.77 (0.65~0.91)	3.02	0.003	0.0%	0.784	–	–
rs1058274								
G allele vs A allele	2 [18, 21]	0.98 (0.92~1.05)	0.56	0.578	0.0%	0.669	–	–
GA vs AA	2 [18, 21]	1.01 (0.92~1.11)	0.15	0.878	0.0%	0.926	–	–
GG vs AA	2 [18, 21]	0.94 (0.82~1.09)	0.81	0.419	0.0%	0.658	–	–
rs2569735								
A allele vs G allele	2 [18, 21]	0.90 (0.82~0.99)	2.14	0.032	8.4%	0.296	–	–
AG vs GG	2 [18, 21]	0.92 (0.83~1.02)	1.61	0.108	0.0%	0.464	–	–
AA vs GG	2 [18, 21]	0.72 (0.52~0.99)	2.03	0.042	0.0%	0.583	–	–

^a The heterogeneity test showed that the data of Kote-Jarai et al.¹⁷ (stage 1) was heterogeneous. After excluding it, the heterogeneity was eliminated;

^b The heterogeneity test showed that the data of Kote-Jarai et al.¹⁷ (stage 1), Eeles et al.²⁶ (stage 1) and Hu et al.¹² was heterogeneous. After excluding it,

the heterogeneity was eliminated; ^c The heterogeneity test showed that the data of Soni et al.¹⁵ and Lai et al.²⁷ was heterogeneous. After excluding it,

the heterogeneity was eliminated; ^d The heterogeneity test showed that the data of Lai et al.²⁷ was heterogeneous. After excluding it, the heterogeneity was

eliminated; ^e The heterogeneity test showed that the data of Kote-Jarai et al.¹⁷ (stage 1), Kote-Jarai et al.¹⁷ (stage 3) and Eeles et al.²⁶ (stage 1) was heterogeneous. After excluding it, the heterogeneity was eliminated.

SNP – single nucleotide polymorphism; PCa – prostate cancer; 95% CI – 95% confidence interval. Kote-Jarai et al.¹⁷ was identified as 3 studies (stage 1, stage 2 and stage 3); Eeles et al.²⁶ was also identified as 3 studies (stage 1, stage 2 UK and stage 2 Australia).

associated with the risk of PCa (rs1058205 C vs T allele: OR = 0.79, 95% CI = 0.73~0.87, p-value <0.001; rs2735839 A vs G allele: OR = 0.78, 95% CI = 0.71~0.86, p-value <0.001; rs174776 T vs C allele: OR = 0.86, 95% CI = 0.80~0.93, p-value <0.001; rs17632542 T vs C allele: OR = 0.61, 95% CI = 0.43~0.86, p-value = 0.005; rs266849 G vs A allele: OR = 0.81, 95% CI = 0.71~0.92, p-value = 0.002; rs266878 G vs C allele: OR = 0.86, 95% CI = 0.78~0.94, p-value = 0.001; rs2569735 A vs G: OR = 0.90, 95% CI = 0.82~0.99, p-value = 0.032). For the genotypes, the pooled results showed that the genotype TC (TC vs TT: OR = 0.79, 95% CI = 0.72~0.86, p-value <0.001) and CC (CC vs TT: OR = 0.62, 95% CI = 0.49~0.77, p-value <0.001) of rs1058205, the genotype AG (AG vs GG: OR = 0.80, 95% CI = 0.71~0.91, p-value = 0.001) of rs2735839, the genotype CT (CT vs CC: OR = 0.87, 95% CI = 0.79~0.97, p-value = 0.010) of rs174776, the genotype TC (TC vs CC: OR = 0.57, 95% CI = 0.37~0.87, p-value = 0.009) and TT (TT vs CC: OR = 0.32, 95% CI = 0.14~0.75, p-value = 0.009) of rs17632542, the genotype GA (GA vs AA: OR = 0.80,

95% CI = 0.70~0.91, p-value = 0.001) and GG (GG vs AA: OR = 0.73, 95% CI = 0.55~0.97, p-value = 0.032) of rs266849, the genotype GC (GC vs CC: OR = 0.87, 95% CI = 0.78~0.97, p-value = 0.009) and GG (GG vs CC: OR = 0.72, 95% CI = 0.52~0.98, p-value = 0.036) of rs266878, the genotype CC (CC vs TT: OR = 0.77, 95% CI = 0.65~0.91, p-value = 0.003) of rs266876, and the genotype AA (AA vs GG: OR = 0.72, 95% CI = 0.52~0.99, p-value = 0.042) of rs2569735 were statistically associated with PCa risk, while there was no significance for the genotype AA of rs2735839, the genotype AG and AA of rs266882, the genotype TT of rs174776, the genotype CT of rs266876, the genotype GA and GG of rs1058274, or the genotype AG of rs2569735.

Meta-analysis of associations between SNPs of *KLK3* and the Gleason score of PCa

Only rs2735839 was involved in the meta-analysis of associations between SNPs of *KLK3* and the Gleason score

Table 4. Meta-analysis for associations between SNPs and the GS of PCa

SNP	Number of studies	Test for overall effect			Test for heterogeneity		Test for publish bias	
		OR (95% CI)	Z-score	p-value	I ²	p-value	P _{egger's}	P _{egg's}
rs2735839 GS < 7 vs GS ≥ 8								
A allele vs G allele	3 [12, 24]	0.598 (0.465~0.770)	3.99	<0.001	0.0%	0.806	–	–
AG/GG vs AA	2 [11,12]	2.731 (0.622~12.00)	1.33	0.183	75.5%	0.043	–	–
rs2735839 GS < 8 vs control								
G allele vs A allele	2 [19, 21]	0.841 (0.796~0.889)	6.14	<0.001	0.0%	0.883	–	–
rs2735839 GS ≥ 8 vs control								
G allele vs A allele	2 [19, 21]	1.09 (0.991~1.201)	1.77	0.077	0.0%	0.517	–	–
rs2735839 GS ≥ 4+3 vs GS ≤ 3+4								
G allele vs A allele	2 [11, 24]	1.413 (1.257~1.588)	5.80	<0.001	0.0%	0.360	–	–

SNP – single nucleotide polymorphism; GS – Gleason score; PCa – prostate cancer; 95% CI – 95% confidence interval.

(GS) of PCa.^{11,12,19,21,24} As shown in Table 4, when compared with the group of GS ≥ 8 carrier, the A allele was a protective factor for the group of GS < 7 (A vs G allele: OR = 0.598, 95% CI = 0.465~0.770, p-value <0.001); when compared with the group of GS ≤ 3+4 carrier, the G allele was a risk factor for the group of GS ≥ 4+3 (G vs A allele: OR = 1.413, 95% CI = 1.257~1.588, p-value <0.001). When compared with the controls, the G allele was a protective factor for the group of GS < 8 (G vs A allele: OR = 0.841, 95% CI = 0.796~0.889, p-value <0.001), while not significantly associated with the group of GS ≥ 8 (G vs A allele: OR = 1.09, 95% CI = 0.991~1.201, p-value <0.077).

Meta-analysis for associations between SNPs of *KLK3* and fatal PCa risk

SNP rs2735839 was also involved in the meta-analysis of associations between SNPs and the risk of fatal PCa.^{13,23} The pooled result showed that there was no significance between rs2735839 and fatal PCa (G vs A allele: OR = 1.230, 95% CI = 0.725~2.088, p-value = 0.442).

Heterogeneity test and sensitivity analysis

A heterogeneity test was performed and the results showed that heterogeneity existed in the meta-analysis of associations between the risk of PCa and rs1058205, rs2735839, rs266882, rs17632542, and rs266849. Therefore, a sensitivity analysis was conducted employing the sequential omission of individual studies to find the source of the heterogeneity. As shown in Table 3, after excluding some studies, the heterogeneity was eliminated. Most pooled results were not materially altered, indicating the robustness of the results of this meta-analysis, except the meta-analysis of the genotype (AA vs GG) of rs2735839 and the genotype (GG vs AA) of rs266849. After eliminating the heterogeneity, the genotype AA of rs2735839 was significantly associated with PCa risk (AA vs GG:

OR = 0.81, 95% CI = 0.67~0.97, p-value = 0.020), while there was no significant association between the genotype GG of rs266849 and PCa risk (GG vs AA: OR = 0.98, 95% CI = 0.86~1.10, p-value = 0.699).

Publication bias assessment

Begg's funnel plot and Egger's test were performed to assess publication bias in the literature if the number of included studies was more than 3. The results of this meta-analysis showed that no evidence of publication bias was found for any of the analyses.

Discussion

The etiology and pathogenesis of PCa is still elusive. However, recently, increasing evidence suggests that genetic factors are associated with PCa susceptibility. For many years, PSA, which plays an important role in sperm motility, has been used as a biomarker for PCa screening. The PSA is also involved in the proteolytic breakdown of the extracellular matrix in PCa tumorigenesis, which contributes to tumor invasion and metastasis²⁹; high serum PSA correlates with mutations in p53 and overexpression of the B-cell lymphoma 2 protein, which inhibits apoptosis in tumor cells.³⁰ These findings strongly suggest that PSA plays a role in the etiology of PCa. The PSA protein is encoded by *KLK3*, and increasing numbers of studies have recently reported that the polymorphisms of *KLK3* associated with PSA levels may be associated with PCa. However, these results were conflicting and there was still no comprehensive analysis to clear up the confusion. Therefore, in this study, we performed a literature review and conducted a meta-analysis to explore the association between the SNPs of *KLK3* that were analyzed in more than 2 studies and the risk of PCa.

In total, 59 SNPs were mentioned in the literature, and among them, 21 SNPs were involved in more than

2 studies. Finally, 10 SNPs – rs1058205, rs2735839, rs266882, rs174776, rs17632542, rs266849, rs266878, rs266876, rs1058274, and rs2569735 – were eligible to be included in this meta-analysis. The pooled results indicated that the minor alleles of rs1058205 (C allele), rs2735839 (A allele), rs174776 (T allele), rs17632542 (T allele), rs266849 (G allele), rs266878 (G allele), and rs2569735 (A allele) were significantly associated with PCa risk. For the genotype analysis, when compared to genotypes of the common homozygotes (rs1058205: TT, rs2735839: GG, rs174776: CC, rs17632542: CC, rs266849: AA, rs266878: CC, rs266876: TT, and rs2569735: GG), the heterozygote genotype carriers of rs1058205 (CT), rs2735839 (AG), rs174776 (CT), rs17632542 (TC), rs266849 (GA), and rs266878 (GC) had a lower risk of PCa, as did the homozygotes genotype carrier of rs1058205 (CC), rs2735839 (AA), rs17632542 (TT), rs266878 (GG), rs266876 (CC), and rs2569735 (AA).

The Gleason grading system remains the most powerful prognostic predictor for PCa because it delineates the architectural patterns of tumors.³¹ It is the core value in risk-scoring systems, including the D'Amico classification system,³² which incorporates the GS, clinical stage and PSA level to stratify the risk of recurrence of localized PCa before treatment and is used to guide treatment selection. Thus, we subsequently performed the GS stratified analyses; only rs2735839 was involved in this part. The GS results range from 1 to 10, and can be divided into 3 grades: GS 1–6 is the low grade in the Gleason grading system; GS 8–10 is the high grade; and GS 7 is the intermediate grade. Our pooled results showed that when compared with GS ≥ 8 PCa (high grade), the A allele was a protective factor for GS < 7 PCa. Patients with GS 7 PCa are a heterogeneous group, consisting of 2 subtypes: GS 3+4 and GS 4+3.³³ The GS 4+3 subtype has had less favorable clinical outcomes than the GS 3+4 subtype.^{33–35} Therefore, the GS 3+4 subtype can be treated as low grade, while GS 4+3 subtype is high grade. Currently there are no reliable biomarkers to further stratify this group. Some studies have therefore stratified GS 7 PCa to explore effective biomarkers. We pooled their relevant data, and the results indicated that when compared to the GS $\leq 3+4$ PCa carrier, the G allele was a risk factor for the GS $\geq 4+3$ carrier.

Finally, we also performed a meta-analysis of associations of the SNPs of *KLK3* and fatal PCa, and again only rs2735839 was involved in this analysis. Our pooled results suggested that there was no significant association between them.

To our knowledge, this is the first study to review all of the SNPs of *KLK3* mentioned in the relevant literature and to perform meta-analyses to illuminate the association between the risk of PCa and SNPs of *KLK3* that have been involved in more than 2 studies. Although our study showed some positive results, this meta-analysis had 2 limitations that should be taken into consideration when assessing the results. First, the overall outcomes were based on unadjusted effect estimates. Among the included studies, only a few were matched for age or other factors.

Therefore, some other confounding factors could slightly modify the estimates, and a more precise evaluation would have to be adjusted for the potentially suspicious factors. Second, in some pooled analyses such as the GS analysis, the number of included studies was too small, so further relevant studies should be carried out in the future so that a stronger conclusion can be drawn.

Conclusions

A strong association was observed between rs1058205, rs2735839, rs266882, rs174776, rs17632542, rs266849, rs266878, rs266876, rs1058274 and rs2569735, and PCa. Therefore, these SNPs may be valuable as biomarkers for PCa risk. Besides, G allele of rs2735839 was noted as a risk factor for the GS < 7 PCa carrier when compared with GS ≥ 8 PCa, as well as for the GS $\geq 4+3$ carrier when compared to the GS $\leq 3+4$ PCa carrier. Considering that the quality and quantity of the reviewed articles were limited, larger well-designed studies should be conducted in the future to further confirm the association between *KLK3* genetic polymorphisms and PCa.

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