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GENETICS

PEX10, SIRPA-SIRPG, and SOX5 gene polymorphisms are strongly associated with nonobstructive azoospermia susceptibility

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Abstract

Purpose Male infertility is a multifactorial syndrome encompassing a wide variety of disorders. A previous Chinese genome-wide single-nucleotide polymorphism (SNP) association studies have identified four SNPs (rs12097821 in *PRMT6* gene, rs2477686 in *PEX10* gene, rs6080550 in *SIRPA-SIRPG*, and rs10842262 in *SOX5* gene) as being significantly associated with risk factors for nonobstructive azoospermia (NOA). However, the results were not fully repeated in later studies, which calls for further investigations.

Methods We here performed a case-control study in a central Chinese population to explore the association between the four SNPs and male infertility, which included 631 infertile men (NOA and oligozoospermia) and 720 healthy fertile men. The genotyping was performed using the polymerase chain reaction–restriction fragment length polymorphism and confirmed by sequencing.

Results The results showed that rs12097821 and rs10842262 were strongly associated with the risk of NOA but not total male infertility or oligozoospermia, while rs2477686 and rs6080550 were not associated with the risk of total male infertility, NOA, or oligozoospermia. To improve the statistical strength, a meta-analysis was conducted. The results suggested that rs2477686, rs6080550, and rs10842262 were significantly associated with male infertility, especially with NOA, while rs12097821 was only found to be associated with total male infertility.

Conclusions Collectively, the rs2477686, rs6080550, and rs10842262 may indeed be the genetic risk factors for NOA, which requires further investigation using larger independent sets of samples in different ethnic populations.

Keywords Single-nucleotide polymorphism (SNP) · Male infertility · Han Chinese · Meta-analysis

Introduction

Male infertility is a major problem worldwide that affects approximately 10–15% of couples and roughly half of these cases are due to male-factor etiology. The main cause of male infertility is spermatogenic failure such as nonobstructive azoospermia (NOA) and oligozoospermia [1]. Interestingly, genetic factors have been regarded as contributing to male infertility [2], and few genetic variations, such as chromosome number defects, Y chromosome microdeletions, and autosomal chromosome variations, have been found to be associated with male infertility [3, 4]. However, the genetic basis of male infertility remains largely unknown.

Recently, Hu et al. performed a GWAS (genome-wide association study) of NOA in Han Chinese men by genotyping 906,703 SNPs in 1000 individuals with NOA (cases) and 1703 male controls using Affymetrix Genome-Wide Human SNP Array 6.0 chips [5]. They identified that the four SNPs (rs1207821 in *PRMT6* gene, rs2477686 in *PEX10* gene, rs6080550 in *SIRPA-SIRPG*, and rs10842262 in *SOX5* gene) were significantly associated with NOA. However, the follow-up studies in Japanese and Chinese men failed to replicate these results [6–9], except for that Zou et al. repeatedly

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found that rs10842262 in *SOX5* gene was significantly associated with NOA risk in Chinese men [10]. In this view, the association between the four SNPs and NOA still needs further investigation. On the other side, genetic associations in male infertility, such as NOA and/or oligozoospermia, are still not very clear. The same disease symptoms may share the same mechanisms or pathways in different ethnic groups. Therefore, the previous GWAS-linked SNPs with NOA (rs12097821, rs2477686, rs10842262, and rs6080550) may also contribute to the genetic susceptibility to oligozoospermia, which is subject to verification. To this end, a hospital-based case-control study was carried out to evaluate the association between the four SNPs and susceptibility to NOA and oligozoospermia in a Chinese population of Hubei province.

Nowadays, meta-analysis has been a popular method for resolving discrepancies in genetic association studies. Specifically, meta-analysis combines results from different studies on the same topic and thus increases statistical strength and precision in estimating effects [11]. Hence, a meta-analysis was performed, combining the results from previously published literatures and present case-control study, to provide a more precise estimation of the association between the four SNPs and male infertility.

Material and methods

Participants

This study was approved by the Ethical Committees of Huazhong University of Science and Technology, and written informed consent for the genetics analysis was obtained from all participants or their guardians.

In this study, we recruited a total of 630 infertile males (301 NOA cases and 330 oligozoospermia cases) and 720 normal controls. The infertile males recruited in this study were strictly screened by clinicians from Wuhan Tongji Reproductive Medicine Hospital. The control group consisted of 720 fertile males, who had fathered at least one child. All of the fertile controls had normal semen parameters. Men who exhibited normal semen parameters but with unknown fertilization status were not included in this study.

To diagnose the NOA and oligozoospermia, a standard clinical examination procedure was performed, including medical history, physical examination, semen analysis, serum hormone analysis, ultrasound evaluation, and genetic examination (e.g., karyotype testing and Y chromosome microdeletion detection). Then, patients were excluded if they had testicular dysplasia, hormonal abnormalities, infections, history of diseases affecting fertility (e.g., varicocele), genital tract pathologies, karyotyping anomalies, or Y-chromosome deletions. Semen samples were collected by masturbation and examined after liquefaction for 30 min at 37 °C. Semen analysis was performed using the computer-assisted semen analysis system (WLJY-9000; Weili New Century Science and Tech Development, Beijing, China) with semen parameters determined according to the guidelines of World Health Organization 2010 [12].

The genotyping of the four SNPs

The peripheral blood samples (5 ml per participant) were collected into blood vacuum tubes containing EDTA and stored at 4 °C. Then, genomic DNA was extracted from blood samples using the TIANamp Blood DNA Kit (DP348; TianGen Biotech, Beijing, China) according to the manufacturer's instructions, and stored at − 20 °C before use. Next, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was conducted to genotype the four candidate polymorphisms. The primers and restriction enzymes used in this study were previously described in the study of Sato et al. [6]. For quality control, the PCR-RFLP assay was repeated twice for all subjects, and the results were 100% concordant. Moreover, 20% randomly selected PCR-amplified DNA samples were examined by DNA sequencing, the results were also 100% concordant.

Statistical analysis

All the statistical analysis was performed using Statistical Program for Social Sciences (SPSS, version 15.0, Chicago, IL, USA). All numerical data (age, abstinence time, and semen parameters) were presented as means ± SD (standard deviation of the mean). Differences in these numerical data were assessed by a one-way ANOVA (analysis of variance). Genotypic frequencies of the four SNPs (rs12097821, rs2477686, rs10842262, and rs6080550) in normal controls were tested for departure from HWE (Hardy-Weinberg equilibrium). Logistic regression analysis was used to estimate the association between the four SNPs and male infertility risk. *P* values less than 0.05 were considered significant for all statistical analyses. To adjust for multiple comparisons, we applied the Bonferroni method [13], which is made by dividing the *P* value threshold by the number of comparisons made (0.05/*n*).

Meta-analysis

We performed a comprehensive literature search updated to September of 2018 in PubMed, EMBASE, ISI Web of Science, and CNKI and Wanfang databases without language restriction. The search terms used were as follows: "PRMT6, rs12097821, and cancer/tumor/carcinoma", "PEX10, rs2477686, and cancer/tumor/carcinoma", "SIRPA-SIRPG, rs6080550, and cancer/tumor/

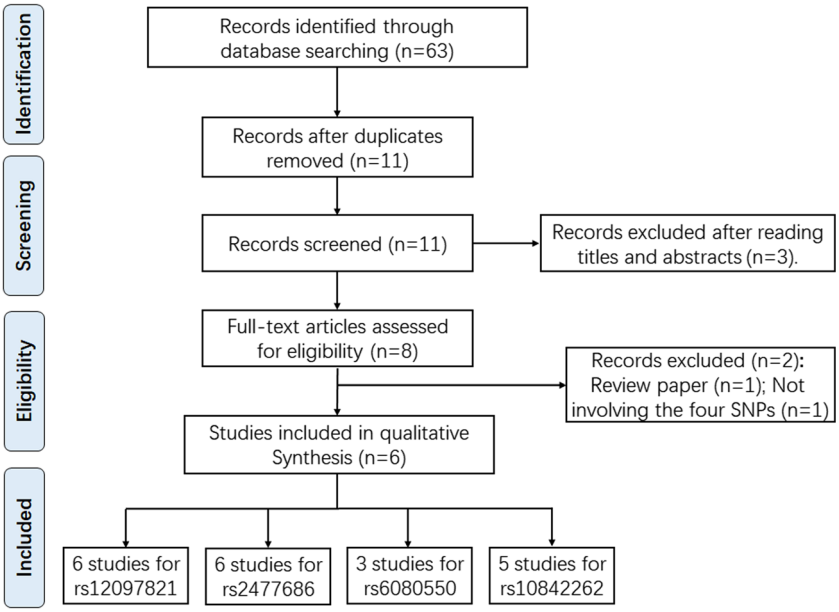

Fig. 1 Flow diagram of the literature review process for *PRMT6* rs1207821 polymorphism, *PEX10* rs2477686 polymorphism, *SIRPA-SIRPG* rs6080550 polymorphism, and *SOX5* rs10842262 polymorphism and male infertility susceptibility

carcinoma", and "SOX5, rs10842262, and cancer/tumor/carcinoma". References listed in retrieved articles were also checked for missing information. Enrolled studies should meet the following criteria: (1) studies on humans; (2) investigation of the PRMT6 rs12097821, PEX10 rs2477686, SIRPA-SIRPG rs6080550 or SOX5 rs10842262, and male infertility risk; (3) case-control study design; (4) sufficient data (allele and genotype frequencies) were accessible to estimate the OR and its 95%CI; and (5) HWE equilibrium should be established in controls. Figure 1 showed us the flowchart of the search strategy and article selection for this meta-analysis. Different ethnicity descents were categorized as Asian and Caucasian. STATA 14.0 (Stata Corp, College Station, TX) was employed to calculate all the statistical analyses. The Cochran's Q test and I^2 were used to assess the heterogeneity of included studies [14]. If $P_{\text{heterogeneity}} \geq 0.1$, the fixed-effect model was applied to calculate the combined OR [15], otherwise, random-effects model was conducted [16]. The significance of combined OR was determined by the Z test. A P value < 0.05 was considered significantly, and the Bonferroni correction for multiple testing was applied.

Results

Participants

As shown in Table 1, there were no statistical differences between case group and control group in individual characteristics, including age, duration of marriage, and

Table 1 Comparison of individual characteristics and semen parameters in study groups

Characteristics	Patients ($n = 631$)			Control ($n = 720$)
	Total cases ($n = 631$)	NOA[a] ($n = 301$)	Oligozoospermia ($n = 330$)	
Age, year	33.16 ± 4.85	32.86 ± 5.47	34.06 ± 6.85	33.87 ± 5.76
Duration of marriage, year	7.16 ± 5.37	7.85 ± 3.87	6.26 ± 6.97	6.97 ± 5.63
Abstinence, day	5.85 ± 2.37	6.23 ± 2.87	5.16 ± 3.24	5.35 ± 2.63
Semen parameters				
Ejaculate volume, ml	2.73 ± 1.37	2.25 ± 1.87	2.89 ± 2.23	2.64 ± 1.18
Total sperm/ejaculate × 10^6	–	0 ± 0	27.67 ± 13.85[2]	207.43 ± 131.61
Sperm concentration, × 10^6/ml	–	0 ± 0	12.84 ± 4.67[b]	85.36 ± 47.65

All data were presented as means ± SD (standard deviation of the mean)

[a] NOA, nonobstructive azoospermia

[b] $P < 0.001$ compared with the control group by one-way ANOVA (analysis of variance)

Table 2 Genotype and allele distributions of SNPs and the association with the risk of male infertility

Genotype	1. Total cases	2. NOA[a]	3. Oligozoospermia	4. Normal controls	*HWE*[b]	Logistic Regression [*P*, OR(95% CI)][c]			
						Genetic model	1 vs. 4	2 vs. 4	3 vs. 4
PRMT6 rs12097821 polymorphism									
G	925 (73.3%)	425 (70.6%)	500 (75.8%)	1110 (77.1%)		G vs. T	0.023, 0.82 (0.69–0.97)	0.002, 0.71 (0.58–0.88)	0.505, 0.93 (0.75–1.15)
T	337 (26.7%)	177 (29.4%)	160 (24.2%)	330 (22.9%)		GG vs. TT	0.085, 0.68 (0.42–1.06)	0.014, 0.53 (0.31–0.90)	0.686, 0.90 (0.49–1.56)
GG	340 (53.9%)	150 (49.8%)	190 (57.6%)	428 (59.4%)	0.968	GG vs. GT	0.085, 0.80 (0.63–1.05)	0.019, 0.73 (0.50–0.97)	0.616, 0.91 (0.70–1.21)
GT	246 (40.0%)	125 (41.5%)	121 (36.7%)	254 (35.3%)		GT vs. TT	0.398, 0.80 (0.53–1.31)	0.234, 0.71 (0.40–1.25)	0.873, 0.97 (0.51–1.70)
TT	45 (7.1%)	26 (8.7%)	19 (5.8%)	38 (5.3%)		GG vs. GT + TT	0.040, 0.78 (0.65–0.97)	0.005, 0.66 (0.50–0.87)	0.568, 0.91 (0.70–1.24)
						GG + GT vs. TT	0.158, 0.75 (0.49–1.15)	0.045, 0.61 (0.37–0.98)	0.750, 0.93 (0.50–1.58)
PEX10 rs2477686 polymorphism									
G	146 (10%)	85 (14.1%)	61 (9.2%)	171 (11.9%)		G vs. C	0.103, 0.82 (0.65–1.04)	0.163, 1.22 (0.92–1.61)	0.075, 0.76 (0.56–1.03)
C	1316 (90%)	517 (85.9%)	599 (90.8%)	1269 (88.1%)		GG vs. CC	0.969, 1.04 (0.40–2.55)	0.430, 1.50 (0.53–4.20)	0.465,0.61 (0.16–2.25)
GG	9 (1.4%)	6 (2%)	3 (1%)	10 (1.4%)	0.957	CG vs. CC	0.758, 0.97 (0.72–1.24)	0.228, 1.23 (0.90–1.70)	0.096, 0.73 (0.50–1.07)
GC	128 (20.3%)	73 (24.2%)	55 (16.7%)	151 (21.0%)		GG vs. CG	0.900, 1.07 (0.40–2.70)	0.687, 1.25 (0.45–3.56)	0.774, 0.80 (0.21–3.13)
CC	494 (78.3%)	222 (73.8%)	272 (82.3%)	559 (77.6%)		GG vs. GC + CC	0.954, 1.01 (0.40–2.55)	0.481, 1.45 (0.50–4.03)	0.517, 0.67 (0.20–2.35)
						GG + CG vs. CC	0.774, 0.98 (0.75–1.27)	0.182, 1.22 (0.90–1.71)	0.073, 0.73 (0.51–1.05)
SIRPA-SIRPG rs6080550 polymorphism									
A	368 (29.2%)	172 (28.6%)	196 (54.4%)	363 (25.0%)		A vs. G	0.021, 1.22 (1.03–1.45)	0.115, 1.19 (0.96–1.47)	0.031, 1.25 (1.02–1.54)
G	894 (70.8%)	430 (71.4%)	464 (45.6%)	1077 (75.0%)		AA vs. GG	0.076, 1.49 (0.99–2.25)	0.247, 1.36 (0.80–2.34)	0.081, 1.58 (0.97–2.60)
AA	53 (8.4%)	24 (8.0%)	29 (8.8%)	46 (6.4%)	0.961	AA vs. AG	0.414, 1.22 (0.80–1.85)	0.611, 1.17 (0.69–1.95)	0.410, 1.27 (0.74–2.08)
AG	261 (41.4%)	123 (40.9%)	138 (41.8%)	271 (37.6%)		AG vs. GG	0.078, 1.20 (0.99–1.55)	0.233, 1.21 (0.93–1.60)	0.100, 1.25 (0.97–1.68)
GG	317 (50.2%)	154 (51.1%)	163 (49.4%)	403 (56.0%)		AA vs. AG + GG	0.182, 1.31 (0.86–2.01)	0.362, 1.25 (0.79–2.15)	0.163, 1.44 (0.89–2.31)
						AA + AG vs. GG	0.035, 1.25 (1.00–1.58)	0.160, 1.20 (0.95–1.61)	0.047, 1.33 (1.01–1.67)
SOX5 rs10842262 polymorphism									
G	437 (34.6%)	222 (36.9%)	215 (32.5%)	436 (30.3%)		G vs. C	0.017, 1.22 (1.04–1.43)	0.004, 1.35 (1.10–1.64)	0.301, 1.11 (0.91–1.35)
C	826 (65.4%)	380 (63.1%)	446 (67.5%)	1004 (69.7%)		GG vs. CC	0.032, 1.50 (1.02–2.17)	0.008, 1.80 (1.15–2.80)	0.356, 1.21 (0.75–1.99)
GG	76 (12.0%)	41 (13.6%)	35 (10.6%)	66 (9.2%)	0.991	GG vs. GC	0.272, 1.20 (0.81–1.75)	0.181, 1.37 (0.90–2.05)	0.648, 1.15 (0.74–1.79)
GC	285 (45.2%)	140 (46.5%)	145 (44.0%)	304 (42.2%)		GC vs. CC	0.092, 1.20 (0.95–1.50)	0.045, 1.31 (1.00–1.75)	0.446, 1.09 (0.81–1.45)
CC	270 (42.8%)	120 (39.9%)	150 (45.4%)	350 (48.6%)		GG vs. GC + CC	0.086, 1.33 (0.92–1.90)	0.035, 1.59 (1.01–2.34)	0.463, 1.15 (0.79–1.80)
						GG + GC vs. CC	0.032, 1.25 (1.05–1.59)	0.011, 1.42 (1.10–1.85)	0.342, 1.11 (0.85–1.40)

[a] NOA, nonobstructive azoospermia

[b] Genotypic frequency of SNPs in normal controls was tested for departure from Hardy-Weinberg equilibrium (HWE) using the χ^2 test

[c] The *P* value was calculated using two-sided χ^2 test

OR (95% CI) was estimated by logistic regression analysis, and adjusted for age, marriage, abstinence time and semen parameters

Table 3 Characteristics of previous studies and the present study

References (author, year)	Country (ethnicity)	Male infertility	Genotyping assay	Case, control (*n*)			HWE
				Total	Allele	Genotype	
PRMT6 rs12097821 polymorphism					G/T	GG/GT/TT	
Sato et al. 2013[6]	Japan (Asian)	NOA[a]	PCR-RFLP	490, 1167	795/185, 1917/417	322/151/17, 788/341/38	0.881
Hu et al., 2012[12]	China (Asian)	NOA	SNP Array Chip	2920, 5727	4106/1734, 8562/2892	1456/1194/270, 3205/2152/370	0.731
Zou et al., 2014[10]	China (Asian)	NOA	Sequencing	525, 512	735/315, 731/293	265/205/55, 255/221/36	0.200
Tu et al., 2015[7]	China (Asian)	NOA	TaqMan assay	545, 632	786/304, 905/359	280/226/39, 317/271/44	0.172
Liu-1 et al., 2017[8]	China (Asian)	Male infertility	Snapshot PCR	184, 51	266/102, 78/24	99/68/17, 29/20/2	0.522
		NOA	Snapshot PCR	97, 51	136/58, 78/24	48/40/9, 29/20/2	0.522
		Oligozoospermia	Snapshot PCR	87, 51	130/44, 78/24	51/28/8, 29/20/2	0.522
Liu-2 et al., 2017[9]	China (Asian)	Male infertility	MassARRAY	134, 454	182/86, 657/251	61/60/13, 232/193/29	0.182
		Asthenozoospermia	MassARRAY	95, 454	130/60, 657/251	45/40/10, 232/193/29	0.182
		Oligoasthenozoospermia	MassARRAY	21, 454	25/17, 657/251	6/13/2, 232/193/29	0.182
		Oligozoospermia	MassARRAY	18, 454	27/9, 657/251	10/7/1, 232/193/29	0.182
This study, 2018	China (Asian)	Male infertility	RFLP-PCR	631, 720	925/337, 1110/330	340/246/45, 428/254/38	0.968
		NOA	RFLP-PCR	301, 720	425/177, 1110/330	150/125/26, 428/254/38	0.968
		Oligozoospermia	RFLP-PCR	330, 720	500/160, 1110/330	190/121/19, 428/254/38	0.968
PEX10 rs2477686 polymorphism					G/C	GG/GC/CC	
Sato et al., 2013[6]	Japan (Asian)	NOA	PCR-RFLP	485, 1161	131/839, 313/2009	9/113/363, 18/277/866	0.436
Hu et al., 2012[12]	China (Asian)	NOA	SNP Array Chip	2882, 5729	837/4927, 1225/10233	88/661/2133, 76/1073/4580	0.146
Zou et al., 2014[10]	China (Asian)	NOA	Sequencing	524, 518	138/910,117/919	13/112/399,11/95/412	0.054
Tu et al., 2015[7]	China (Asian)	NOA	TaqMan assay	545, 632	154/936, 160/1104	14/126/405, 11/138/483	0.753
Liu-1 et al., 2017[8]	China (Asian)	Male infertility	Snapshot PCR	183, 51	36/330, 17/85	1/34/148, 0/17/34	0.153
		NOA	Snapshot PCR	96, 51	22/170, 17/85	1/20/75, 0/17/34	0.153
		Oligozoospermia	Snapshot PCR	87, 51	14/160, 17/85	0/14/73, 0/17/34	0.153
Liu-2 et al., 2017[9]	China (Asian)	Male infertility	MassARRAY	135, 456	38/232, 114/798	7/24/104, 5/104/347	0.363
		Asthenozoospermia	MassARRAY	95, 456	22/168, 114/798	3/16/76, 5/104/347	0.363
		Oligoasthenozoospermia	MassARRAY	22, 456	13/31, 114/798	4/5/13, 5/104/347	0.363
		Oligozoospermia	MassARRAY	18, 456	3/33, 114/798	0/3/15, 5/104/347	0.363
This study, 2018	China (Asian)	Male infertility	PCR-RFLP	631, 720	146/1316, 171/1269	9/128/494, 10/151/559	0.957
		NOA	PCR-RFLP	301, 720	85/517, 171/1269	6/73/222, 10/151/559	0.957
		Oligozoospermia	PCR-RFLP	330, 720	61/599, 171/1269	3/55/272, 10/151/559	0.957
SIRPA-SIRPG rs6080550 polymorphism					A/G	AA/AG/GG	
Sato et al., 2013[6]	Japan (Asian)	NOA	RFLP-PCR	481, 1158	134/828, 333/1983	8/118/355, 30/273/855	0.148
Hu et al.,2012[12]	China (Asian)	NOA	SNP Array Chip	2923, 5731	1340/4506, 2177/9285	154/1032/1737, 215/1747/3769	0.478
Liu-2 et al., 2017[9]	China (Asian)	Male infertility	MassARRAY	135, 454	113/157, 402/506	27/59/49, 84/234/136	0.343
		Asthenozoospermia	MassARRAY	95, 454	85/105, 402/506	21/43/31, 84/234/136	0.343
		Oligoasthenozoospermia	MassARRAY	22, 454	17/27, 402/506	2/13/7, 84/234/136	0.343
		Oligozoospermia	MassARRAY	18, 454	11/25, 402/506	4/3/11, 84/234/136	0.343
This study, 2018	China (Asian)	Male infertility	RFLP-PCR	631, 720	368/894, 363/1077	53/261/317, 46/271/403	0.961
		NOA	RFLP-PCR	301, 720	172/430, 363/1077	24/123/154, 46/271/403	0.961
		Oligozoospermia	RFLP-PCR	330, 720	196/464, 363/1077	29/138/163, 46/271/403	0.961
SOX5 rs10842262 polymorphism					G/C	GG/GC/CC	
Sato et al. 2013[6]	Japan (Asian)	NOA	PCR-RFLP	487, 1155	373/601, 827/1483	81/211/195, 146/535/474	0.794
Hu et al., 2012[12]	China (Asian)	NOA	SNP Array Chip	2887, 5711	2121/3653, 3656/7766	338/1445/1104, 581/2494/2636	0.802
Zou et al., 2014[10]	China (Asian)	NOA	sequencing	522, 529	351/693, 291/767	65/221/236, 41/209/279	0.831

Table 3 (continued)

References (author, year)	Country (ethnicity)	Male infertility	Genotyping assay	Case, control (*n*)			HWE
				Total	Allele	Genotype	
Tu et al., 2015[7]	China (Asian)	NOA	TaqMan assay	545, 632	385/705, 401/863	58/269/218, 65/271/296	0.798
Liu-1 et al., 2017[8]	China (Asian)	Male infertility	Snapshot PCR	177, 51	89/265, 28/74	15/59/103, 6/16/29	0.129
		NOA	Snapshot PCR	96, 51	55/137, 28/74	9/37/50, 6/16/29	0.129
		Oligozoospermia	Snapshot PCR	81, 51	34/128, 28/74	6/22/53, 6/16/29	0.129
This study, 2018	China (Asian)	Male infertility	PCR-RFLP	631, 720	437/826, 436/1004	76/285/270, 66/304/350	0.998
		NOA	PCR-RFLP	301, 720	222/380, 436/1004	41/140/120, 66/304/350	0.998
		Oligozoospermia	PCR-RFLP	330, 720	215/446, 436/1004	35/145/150, 66/304/350	0.998

[a] NOA, nonobstructive azoospermia

abstinence time. However, semen concentration and total semen count per ejaculate were significantly lower in oligozoospermia group.

The association between the four SNPs and male infertility susceptibility

In this study, we performed comparisons of allele and genotype frequencies of the four SNPs and applied six genetic model (allele model, carrier model, homozygote model, heterozygote model, recessive model, dominant model) analysis between infertile males (total cases, NOA cases or oligozoospermia cases) and normal controls (Table 2). There were no deviations from HWE observed within the control group for all studied SNPs. After Bonferroni correction ($P < 0.0084$, 0.05/6), we found that *PRMT6* rs12097821 polymorphism and *SOX5* rs10842262 polymorphism were strongly associated with risk of NOA, but not with risk of total male infertility or oligozoospermia. For SNP 12097821, allele G was a significant predisposition allele of NOA (G vs. T, $P = 0.002$, OR = 0.71, 95% CI = 0.58–0.88), and individuals with genotype GG had a higher risk for NOA compared with GT + TT (GG vs. GT + TT, $P = 0.005$, OR = 0.66, 95% CI = 0.50–0.87). For SNP rs10842262, G allele had a higher risk for NOA than those carrying the C allele (G vs. C, $P = 0.004$, OR = 1.35, 95% CI = 1.10–1.64), and GG genotype conferred higher risk for NOA relative to CC genotype (GG vs. CC, $P = 0.008$, OR = 1.80, 95% CI = 1.15–2.80). Moreover, we observed that *PEX10* rs2477686 polymorphism and *SIRPA-SIRPG* rs6080550 polymorphism were not associated with the susceptibility to total male infertility, NOA, or oligozoospermia under none of the six genetic models.

Meta-analysis of the four SNPs and male infertility susceptibility

The characteristics of included studies for this meta-analysis were presented in Table 3. In the control groups of included studies, the genotypic frequencies of rs12097821, rs2477686, rs6080550, and rs10842262 were all concordant with the HWE. For the four SNPs, their association with male infertility susceptibility was evaluated in total group of infertile men, as well as in subtypes of infertile men (NOA and oligozoospermia).

Meta-analysis of PRMT6 rs12097821 polymorphism

A sum of seven publications for rs12097821 that met the inclusion criteria were finally retrieved. As shown in Table 4, we found that rs12097821 significantly decreased male infertility risk under two models: G vs. T, OR = 0.84, 95% CI = 0.80–0.89, $P < 0.001$; GG vs. GT, OR = 0.87, 95% CI = 0.81–0.94, $P < 0.001$. However, our data indicated that

Table 4 Meta-analysis of associations between PRMT6 rs12097821 polymorphism and male infertility susceptibility

Genetic model	Heterogeneity test			Summary OR (95% CI)	Hypothesis test		Studies (*n*)
	Q	*P*	I^2		*Z*	*P*	
rs12097821 and male infertility							
G vs. T	9.22	0.162	34.9%	0.84 (0.80–0.89)	6.20	< 0.001	7
GG vs. TT	167.09	< 0.001	96.4%	1.05 (0.92–1.19)	0.05	0.957	7
GG vs. GT	8.66	0.194	30.7%	0.87 (0.81–0.94)	3.71	< 0.001	7
GT vs. TT	120.01	< 0.001	95.0%	1.13 (1.00–1.30)	0.16	0.873	7
GG vs. GT + TT	80.75	< 0.001	92.6%	0.91 (0.85–0.97)	0.05	0.963	7
GG + GT vs. TT	168.80	< 0.001	96.4%	0.91 (0.89–0.94)	0.02	0.988	7
rs12097821 and nonobstructive azoospermia (NOA)							
G vs. T	11.49	0.043	56.5%	0.86 (0.77–0.97)	2.57	0.010	6
GG vs. TT	168.50	< 0.001	97.0%	1.00 (0.38–2.66)	0.01	0.995	6
GG vs. GT	10.11	0.072	50.5%	0.90 (0.79–1.03)	1.49	0.136	6
GT vs. TT	118.93	< 0.001	95.8%	1.13 (0.48–2.61)	0.27	0.784	6
GG vs. GT + TT	83.72	< 0.001	94.0%	1.00 (0.70–1.43)	0.02	0.987	6
GG + GT vs. TT	169.03	< 0.001	97.0%	1.05 (0.41–2.68)	0.09	0.925	6
rs12097821 and oligozoospermia							
G vs. T	0.28	0.869	0%	0.94 (0.77–1.14)	0.62	0.533	3
GG vs. TT	0.79	0.673	0%	0.83 (0.50–1.40)	0.69	0.488	3
GG vs. GT	0.72	0.699	0%	0.98 (0.76–1.26)	0.16	0.875	3
GT vs. TT	1.29	0.524	0%	0.85 (0.50–1.43)	0.62	0.533	3
GG vs. GT + TT	0.38	0.828	0%	0.96 (0.75–1.22)	0.35	0.724	3
GG + GT vs. TT	1.00	0.607	0%	0.84 (0.50–1.39)	0.69	0.487	3

Table 5 Meta-analysis of associations between PEX10 rs2477686 polymorphism and male infertility susceptibility

Genetic model	Heterogeneity test			Summary OR (95% CI)	Hypothesis test		Studies (*n*)
	Q	*P*	I^2		*Z*	*P*	
rs2477686 and male infertility							
G vs. C	30.65	< 0.001	80.4%	1.058 (0.86–1.30)	0.55	0.585	7
GG vs. CC	9.88	0.130	39.3%	1.973 (1.55–2.51)	5.51	< 0.001	7
CG vs.CC	19.14	0.004	68.6%	1.026 (0.85–1.24)	0.27	0.788	7
GG vs. CG	7.77	0.256	22.7%	1.662 (1.29–2.13)	3.99	< 0.001	7
GG + CG vs.CC	21.49	0.002	72.1%	1.06 (0.88–1.29)	0.64	0.524	7
GG vs. GC + CC	9.29	0.158	35.4%	1.904 (1.50–2.42)	5.24	< 0.001	7
rs2477686 and nonobstructive azoospermia (NOA)							
G vs. C	14.55	0.012	65.6%	1.167 (0.99–1.37)	1.86	0.063	6
GG vs. CC	5.66	0.341	11.6%	1.982 (1.55–2.54)	5.38	< 0.001	6
CG vs.CC	10.38	0.065	51.8%	1.138 (0.97–1.33)	1.61	0.106	6
GG vs.CG	2.93	0.710	0%	1.612 (1.25–2.09)	3.64	< 0.001	6
GG + CG vs.CC	12.83	0.025	61.0%	1.169 (1.08–1.27)	1.72	0.085	6
GG vs. GC + CC	5.00	0.416	0%	1.898 (1.48–2.43)	5.06	< 0.001	6
rs2477686 and oligozoospermia							
G vs. C	1.75	0.417	0%	0.698 (0.53–0.92)	2.53	0.011	3
GG vs. CC	0.54	0.463	0%	0.712 (0.22–2.35)	0.56	0.577	2
CG vs.CC	2.20	0.333	9.0%	0.680 (0.50–0.92)	2.47	0.014	3
GG vs.CG	0.49	0.483	0%	0.943 (0.28–3.20)	0.09	0.925	2
GG + CG vs.CC	2.12	0.346	5.7%	0.690 (0.59–0.81)	2.59	0.010	3
GG vs. GC + CC	0.57	0.450	0%	0.752 (0.23–2.48)	0.47	0.640	2

Table 6 Meta-analysis of associations between SIRPA-SIRPG rs6080550 polymorphism and male infertility susceptibility

Genetic model	Heterogeneity test			Summary OR (95% CI)	Hypothesis test		Studies (*n*)
	Q	*P*	I^2		*Z*	*P*	
rs6080550 and male infertility							
A vs. G	9.96	0.019	69.9%	1.12 (0.96–1.30)	1.39	0.164	4
AA vs. GG	7.35	0.062	59.2%	1.21 (0.85–1.71)	1.07	0.287	4
AA vs. AG	2.62	0.455	0.0%	1.17 (0.98–1.40)	1.74	0.081	4
AG vs. GG	8.89	0.031	66.3%	1.11 (0.92–1.35)	1.11	0.267	4
AA vs. AG + GG	4.38	0.224	31.4%	1.31 (1.10–1.55)	3.08	0.002	4
AA + AG vs. GG	10.38	0.016	71.1%	1.17 (1.09–1.26)	1.14	0.255	4
rs6080550 and non-obstructive azoospermia (NOA)							
A vs. G	5.60	0.061	64.3%	1.16 (0.99–1.36)	1.80	0.071	3
AA vs. GG	4.55	0.103	56.0%	1.27 (0.84–1.92)	1.12	0.263	3
AA vs. AG	2.50	0.287	19.9%	1.15 (0.94–1.40)	1.39	0.165	3
AG vs. GG	2.45	0.294	18.2%	1.24 (1.14–1.35)	4.99	<0.001	3
AA vs. AG + GG	3.83	0.147	47.8%	1.33 (1.10–1.61)	2.95	0.003	3
AA + AG vs. GG	4.27	0.118	53.2%	1.20 (1.02–1.41)	2.20	0.028	3
rs6080550 and oligozoospermia							
A vs. G	4.56	0.033	78.1%	0.90 (0.41–1.97)	0.27	0.791	2
AA vs. GG	2.24	0.135	55.3%	1.32 (0.83–2.07)	1.18	0.239	2
AA vs. AG	1.81	0.178	44.8%	1.38 (0.85–2.22)	1.31	0.191	2
AG vs. GG	9.48	0.002	89.5%	0.49 (0.07–3.75)	0.68	0.495	2
AA vs. AG + GG	0.03	0.855	0%	1.39 (0.89–2.17)	1.44	0.149	2
AA + AG vs. GG	9.36	0.002	89.3%	0.64 (0.14–2.95)	0.57	0.567	2

Table 7 Meta-analysis of associations between SOX5 rs10842262 polymorphism and male infertility susceptibility

Genetic model	Heterogeneity test			Summary OR (95% CI)	Hypothesis test		Studies (*n*)
	Q	*P*	I^2		*Z*	*P*	
rs10842262 and male infertility							
G vs. C	4.09	0.536	0.0%	1.22 (1.15–1.28)	7.40	< 0.001	6
GG vs. CC	4.19	0.523	0.0%	1.40 (1.24–1.57)	5.64	< 0.001	6
GG vs. CG	7.81	0.167	36.0%	1.09 (0.97–1.22)	1.42	0.156	6
CG vs. CC	9.24	0.100	45.9%	1.30 (1.20–1.40)	6.84	< 0.001	6
GG vs. CG + CC	5.69	0.338	12.1%	1.23 (1.10–1.37)	3.68	< 0.001	6
GG + CG vs. CC	6.90	0.228	27.5%	1.32 (1.23–1.41)	7.63	< 0.001	6
rs10842262 and nonobstructive azoospermia (NOA)							
G vs. C	3.68	0.597	0.0%	1.23 (1.16–1.30)	7.59	< 0.001	6
GG vs. CC	4.31	0.505	0.0%	1.42 (1.26–1.60)	5.78	< 0.001	6
GG vs. CG	8.33	0.139	40.0%	1.09 (0.97–1.23)	1.44	0.149	6
CG vs. CC	8.50	0.131	41.2%	1.31 (1.22–1.42)	7.02	< 0.001	6
GG vs. CG + CC	6.08	0.298	17.8%	1.24 (1.11–1.38)	3.77	< 0.001	6
GG + CG vs. CC	5.98	0.308	16.4%	1.33 (1.24–1.43)	7.83	< 0.001	6
rs10842262 and oligozoospermia							
G vs. C	2.17	0.140	54.0%	1.06 (0.88–1.28)	0.59	0.556	2
GG vs. CC	1.51	0.219	33.9%	1.12 (0.73–1.71)	0.52	0.602	2
GG vs. CG	0.36	0.546	0.0%	1.06 (0.69–1.63)	0.27	0.787	2
CG vs. CC	0.85	0.357	0.0%	1.07 (0.82–1.38)	0.49	0.626	2
GG vs. CG + CC	1.08	0.298	7.7%	1.09 (0.72–1.63)	0.40	0.691	2
GG + CG vs. CC	1.57	0.211	36.2%	1.07 (0.84–1.37)	0.56	0.577	2

rs12097821 was not associated with NOA or oligozoospermia under none of the six genetic models (G vs. T, GG vs. TT, GG vs. GT, GT vs. TT, GG vs. GT + TT or GG + GT vs. TT).

Meta-analysis of PEX10 rs2477686 polymorphism

A sum of seven publications for rs2477686 that met the inclusion criteria were finally retrieved. In Table 5, our results showed that GG genotype significantly increases the susceptibility to male infertility and NOA when compared with GC and/or CC genotypes (GG vs. CC, GG vs. GC, and GG vs. GC + CC). However, the significant association did not remain in oligozoospermia.

Meta-analysis of SIRPA-SIRPG rs6080550 polymorphism

A sum of four publications for rs6080550 that met the inclusion criteria were finally retrieved. As shown in Table 6, rs6080550 was suggested to significantly associate with male infertility and NOA under different genetic models. Specifically, rs6080550 conferred an increased risk to male infertility under AA vs. AG + GG model (OR = 1.31, 95% CI = 1.10–1.55, $P = 0.002$), while to NOA under AG vs. GG model (OR = 1.24, 95% CI = 1.14–1.35, $P < 0.001$) and AA vs. AG + GG model (OR = 1.33, 95% CI = 1.10–1.61, $P = 0.003$). However, we observed that there was no association between rs6080550 and oligozoospermia.

Meta-analysis of SOX5 rs10842262 polymorphism

A sum of six publications for rs10842262 that met the inclusion criteria were finally retrieved. In Table 7, we found that rs10842262 was significantly associated with male infertility and NOA under the same genetic models (G vs. C, GG vs. CC, GC vs. CC, GG vs. GC + CC and GG + GC vs. CC). Whereas, no significant association was found for rs10842262 and oligozoospermia.

Discussion

Up to now, several replication studies have been conducted to validate the four GWAS-linked SNPs (rs12097821, rs2477686, rs10842262, and rs6080550) with NOA, while the results remain conflicting rather than conclusive. Sato et al. were the first group to repeat an association study between the four SNPs and NOA in a Japanese population, but showed no significant difference for these four SNPs, which is almost inconsistent with findings of the previous GWAS in the Han Chinese population by Hu et al. [5, 6]. Furthermore, 2 replication studies in the Han Chinese population showed that rs12097821 and rs2477686 were not associated with NOA [7, 10]. However, Zou et al. [10], but not Tu et al. [7], reported that there was a significant association between rs10842262 and NOA. Consistent with the finding of Tu et al., Liu et al. found that the rs12097821, rs2477686, and rs10842262 were not associated with NOA, and also were not associated with oliogozoospermia [8]. Similarly, the significant association of the four SNPs with NOA was partially repeated in the present case-control study, and only rs12097821 and rs10842262 were shown to be associated with NOA in a central Chinese population. Moreover, the association of the four SNPs with idiopathic male infertility (asthenozoospermia, oligozoospermia, and oligoasthenozoospermia) was also explored, and Liu et al. found that rs2477686, but not rs12097821, rs10842262, or rs6080550, was significantly associated with idiopathic male infertility, especially oligoasthenozoospermia [9].

The possible reasons for these discrepancies might be as follows. First, there may be a risk of false positive of the risk loci reported by the original GWAS study in Chinese population, which leads to subsequent validation studies failing to replicate the original result. Second, the interaction of these four SNPs and different genetic backgrounds among different ethnic populations may produce the variation of individual susceptibility to male infertility. Third, the insufficient sample size may cause inadequate statistical strength. In the present study, rs2477686 and rs6080550 were not significantly associated with NOA susceptibility in Chinese population. However, rs2477686 and rs6080550 displayed associations in the same direction (per-genetic comparison ORs > 1) as reported in the previous GWAS (Table 2). Therefore, the four SNPs need to be investigated using more samples in other ethnic populations or in Chinese population from different geographic regions for further confirmation.

To solve the discrepancies and improve the statistical strength, a meta-analysis followed combining results from previously published literature and the present case-control study was conducted. Interestingly, three of the four SNPs (rs2477686, rs10842262, and rs6080550) displayed significant associations with NOA in the same direction (per-allele ORs > 1) in combined cohorts as previously reported in the GWAS, while none of the four SNPs was associated with oligozoospermia. When combing the NOA and oligozoospermia as total male infertility, all the four SNPs were shown to associate with male infertility. However, one limitation of this study should be acknowledged. Since the publication bias can be evaluated for meta-analysis with sufficient numbers of included studies ($n > 10$), the assessment of publication bias was not performed. Therefore, we could not eliminate the possibility of publication bias in the present meta-analysis.

Previous knowledge suggested that PRMT6 (rs12097821) may regulate POLB and thereby function in synapsis and recombination during meiosis [17–20], PEX10 (rs2477686) has roles in male fertility (e.g., regulating spermatocyte

cytokinesis) [21], SIRPA (rs6080550) may modulate engraftment of human hematopoietic stem cells [22], and SOX5 (rs10842262) regulates gene expression during spermatogenesis [23, 24]. These findings collectively suggested that these four SNPs may serve as potential biomarkers for male infertility predisposition in Asian population. However, it cannot rule out the possibility that the four SNPs may not be the causal loci but rather be in linkage disequilibrium with the causal loci. Moreover, the effect of four SNPs on functions of PRMT6, PEX10, SIRPA-SIRPG, and SOX5 was not assessed in spermatogenesis from individuals with different genotypes (-in vivo), which should be analyzed in further functional study.

In conclusion, the rs12097821 and rs10842262 were strongly associated with NOA in Chinese men of Hubei province. In addition, the meta-analysis that followed showed that in Asian population, rs2477686, rs6080550, and rs10842262 were significantly associated with male infertility especially with NOA, while rs12097821 was only associated with total male infertility. Our current findings suggest that rs2477686, rs6080550, and rs10842262 may indeed be the genetic risk factors for NOA. However, cohort expansion and further mechanistic studies on the role of these genetic factors that influence spermatogenesis and sperm progressive motility are necessary for the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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