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Association between KITLG Gene Polymorphisms and Testicular Germ Cell Tumors: A Systematic Review and Meta-analysis*

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Summary: It has been reported that c-KIT ligand (KITLG) gene polymorphisms may be associated with testicular germ cell tumors (TGCT). Owing to mixed and inconclusive results, we conducted a systematic review and meta-analysis to summarize and clarify this association. A systematic search of studies on the association between KITLG gene polymorphisms and TGCT susceptibility was conducted in databases. Odds ratios and 95% confidence intervals were used to pool the effect size. Six articles were included in our systematic review and meta-analysis. Compared with adenine (A), KITLG rs995030 guanine (G) might be associated with increased risk of TGCT. There are insufficient data to fully confirm the association between KITLG rs4474514 and TGCT susceptibility. Well-designed studies with larger sample size and more subgroups are required to validate the risk identified in the current meta-analysis.
Key words: c-KIT ligand; single nucleotide polymorphisms; testicular germ cell tumors; meta-analysis

More than 90% of cancers of the testicle develop in germ cells. Seminomas and nonseminomas are two main types of testicular germ cell tumor (TGCT) in men[1]. Several loci associated with TGCTs have been detected by genome-wide association studies (GWAS), and single nucleotide polymorphisms (SNPs) in KITLG gene were found as a strong genetic risk factor[2]. The c-KIT ligand (KITLG) gene encodes the ligand for the membrane bound receptor tyrosine kinase KIT. Survival, proliferation and migration of germ cells might be regulated by the KITLG/KIT signaling system[3]. Some KITLG gene polymorphisms can join in the development of TGCT by affecting the KITLG/KIT signaling system. SNP rs995030, which resides in a functional p53-binding site, can influence the transcription of KITLG gene[4]. Association between KITLG gene polymorphisms and TGCTs has been studied in several SNPs of KITLG and in several populations. Sample sizes in some of these studies are relatively small. Therefore, we decided to perform a systematic review and meta-analysis to estimate it.

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1 MATERIALS AND METHODS

1.1 Identification of Eligible Studies

A systematic search in PubMed, Embase, Cochrane Library, clinicaltrials.gov, China National Knowledge Infrastructure (CNKI), Wanfang databases were carried out by two independent investigators. The following terms were used: "KITLG" AND "cancer of testis OR carcinoma of testis OR testicular cancer OR testis cancer OR ball cancer OR testicular germ cell tumors OR TGCT" AND "polymorphisms OR polymorphism", without any limitation applied. The last search update was performed on Oct 22, 2018. References of related studies and reviews were also manually searched for additional studies.

1.2 Inclusion and Exclusion Criteria

Studies selected in this systematic review and meta-analysis must meet the following inclusion criteria: (1) evaluation of the association between KITLG gene polymorphisms and TGCT susceptibility; (2) case-control study; (3) studies focusing on tissues of human beings; (4) detailed genotype data could be acquired to calculate the odds ratios (ORs) and 95% confidence intervals (95%CIs). Exclusion criteria were as follows: (1) duplication of previous publications (When there were multiple publications from the same population, only the largest study was included); (2)

comment, review and editorial; (3) study without detailed genotype data; (4) GWAS; (5) studies focusing on cell lines. Dissertation theses were included in the analysis.

Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria by screening the title, abstract and full-text. Any dispute was solved by discussion.

1.3 Data Extraction

Two investigators extracted data of the eligible studies independently. In the case of a conflict, an agreement was reached by discussion. If the dissent still existed, the third investigator would be involved to adjudicate the disagreements. And we tried to contact the author by email for detailed genotype data.

The following contents were collected: first author's surname, year of publication, the characteristics of cases and controls, source of control groups, country of origin, the detective sample, ethnicity, genotyping method, Hardy-Weinberg equilibrium, number of cases and controls for each genotype.

1.4 Methodological Quality Assessment

The qualities of included studies were evaluated independently by two investigators according to Newcastle-Ottawa Scale (NOS)[5] and the most important factor was "age, gender and country". Quality scores range from 0 to 9, and higher scores means better quality of the study. Disagreement was resolved through discussion.

1.5 Statistical Analysis

Our meta-analysis was conducted according to the PRISMA checklists[6]. Hardy-Weinberg equilibrium (HWE) was evaluated for each study by Chi-square test in control groups, and $P<0.05$ was considered as a significant departure from HWE. OR and 95% CIs were calculated to evaluate the strength of the association between KITLG gene polymorphisms and TGCT susceptibility. Pooled ORs were obtained from combination of single studies by allelic comparison (A *vs.* B), dominant model (BA+AA *vs.* BB), recessive model (AA *vs.* BB+BA), homozygote comparison (AA *vs.* BB) and heterozygote comparison (BA *vs.* BB), respectively. The statistical significant level was determined by Z-test with P value less than 0.05.

Heterogeneity was evaluated by Q-test and I^2 index[7]. When Q-test's P-value was less than 0.10 and/or I^2 index was more than 50%, the random-effects model (DerSimonian and Laird method) was used; otherwise, the fixed-effects model (Mantel and Haenszel method) was conducted[8]. Sensitivity analyses were performed towards each genetic model to evaluate effect of each study on combined ORs by sequentially excluding each study in total and in any subgroup including more than two studies. Potential publication bias was checked by Begg's funnel[9] plots and Egger's test[10]. An asymmetric plot, the P value of Begg's test (P_B) less than 0.05, and the P value of Egger's test (P_E) less than 0.05 was considered a significant publication bias. All statistical analyses were performed with Stata 12.0 software (StataCorp, College Station, USA). A two-tailed $P<0.05$ was considered significant except for specified conditions, where a certain P value was declared.

2 RESULTS

2.1 Characteristics of Studies

A total of 102 articles were acquired from databases [PubMed=27, Embase=52, Cochrane=0, clinicaltrials.gov=0, CNKI=20, Wanfang=3, other sources (from manually search)=0]. The selection process is shown in fig. 1. Eight full-text articles were excluded (1 duplicate study[11], 7 without detailed genotype data). The duplicate study was from the same population but smaller than the study by Dantsev IS[17]. Finally, 6 articles[12–17] were included in our systematic review and meta-analysis. The characteristics of each study are shown in table 1. Different genotyping methods were utilized including PCR-RFLP, Mass-Array, TaqMan SNP Genotyping Assay, Pyrosequencing and Sequencing. Blood samples were used for genotyping in all studies except for cases in studies by Poynter JN[12] and Grasso C[15]. GCT tissue and paired normal adjacent tissue were studied in cases reported by Poynter JN[12]. Saliva was studied by Grasso C[15]. The control group in study by Dantsev IS[17] had shown significant departure from HWE.

In the systematic review, eligible case-control studies about SNP rs995030, rs4474514, rs3782179, rs2046971, rs1472899, rs1508595 of KITLG gene were included. However, only single study about rs2046971, rs1472899, rs1508595 was found, and rs3782179 showed strong linkage disequilibrium with rs4474514. Then we conducted the meta-analysis only in KITLG SNP rs995030 and rs4474514.

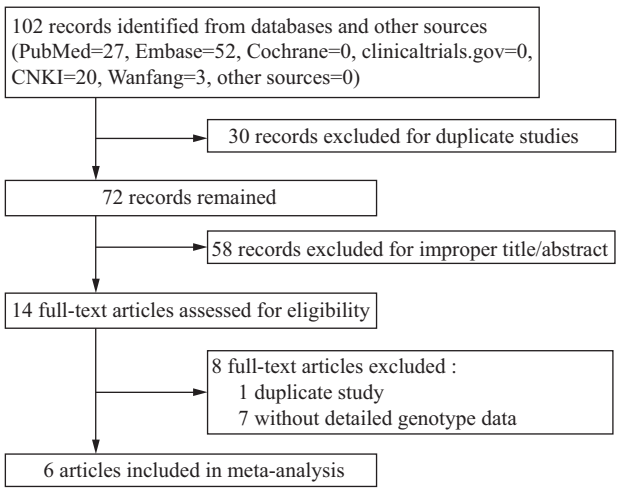

Fig. 1 Flow chart of study selection

Table 1 Characteristics of studies included in the systematic review and meta-analysis

No.	Study ID / KITLG rs995030	Year	Country	Ethnicity	Control type	Genotyping method	Case			Control			*P* for HWE*	Quality
							AA	GA	GG	AA	GA	GG		
1.1	Ferlin A[13]	2012	Italy	Caucasian	PB*	Sequencing	3	73	350	24	192	398	0.888	8
1.2	Grasso C[15]	2016	Italy	Caucasian	PB	Pyrosequencing	2	39	161	15	91	227	0.140	9
1.3	Shenghua Duan[16]	2016	China	Uygur	PB	Sequencing	1	3	22	2	22	32	0.445	7
1.4	Dantsev IS[17]	2018	Russia	Caucasian	PB	PCR-RFLP	8	33	101	3	67	83	**0.011#**	9
	KITLG rs4474514						GG	AG	AA	GG	AG	AA		
2.1	Poynter JN¥[12]	2012	USA	Caucasian	PB	TaqMan SNP Genotyping Assay	0	7	8	2	25	49	0.569	6
2.2	Ferlin A[13]	2012	Italy	Caucasian	PB	Sequencing	4	75	347	29	197	388	0.536	8
2.3	Azevedo MF¥[14]	2013	USA	Caucasian	PB	PCR-RFLP and Mass-Array	0	13	81	28	192	472	0.135	6
2.4	Shenghua Duan[16]	2016	China	Uygur	PB	Sequencing	1	3	22	3	21	32	0.853	7
	KITLG rs3782179						CC	TC	TT	CC	TC	TT		
3.1	Azevedo MF¥[14]	2013	USA	Caucasian	PB	PCR-RFLP and Mass-Array	0	13	81	28	192	472	0.135	6
3.2	Shenghua Duan[16]	2016	China	Uygur	PB	Sequencing	1	3	22	3	21	32	0.853	7
	KITLG rs2046971						GG	CG	CC	GG	CG	CC		
4	Shenghua Duan[16]	2016	China	Uygur	PB	Sequencing	1	2	23	3	21	32	0.853	7
	KITLG rs1472899						CC	TC	TT	CC	TC	TT		
5	Shenghua Duan[16]	2016	China	Uygur	PB	Sequencing	1	3	22	3	21	32	0.853	7
	KITLG rs1508595						AA	GA	GG	AA	GA	GG		
6	Dantsev IS[17]	2018	Russia	Caucasian	PB	PCR-RFLP	10	35	97	20	62	71	0.275	9

*HWE: Hardy-Weinberg equilibrium; PB: population-based; NA: not available.
#Results with statistical significant difference were marked as bold.
¥Several extragonadal germ cell tumor cases were included in Poynter JN's study. All cases were familial testicular germ cell tumors in Azevedo MF' study.

2.2 Overall Analyses

Summary results in each genetic model of KITLG rs995030 and rs4474514 are listed in table 2. In allelic comparison (A *vs.* G), heterozygote comparison (GA *vs.* GG), dominant model (GA+AA *vs.* GG) of KITLG rs995030, and in all genetic models of KITLG rs4474514, significantly decreased risk of TGCT was found. No statistically significant changes of TGCT risk were found in other genetic models of KITLG rs995030.

2.3 Sensitivity Analyses

In homozygote comparison (AA *vs.* GG) and recessive model (AA *vs.* GG+GA) of KITLG rs995030, when study No. 1.4 was excluded, statistically different results were obtained. In all genetic models of KITLG rs4474514, when study No. 2.2 with/without No. 2.3 was excluded, statistically different results were obtained. Other results showed stability in sensitivity analyses (table 2).

2.4 Publication Bias

Begg's funnel plot and Egger's test were used to assess the publication bias[18]. Symmetry of funnel plot, P_B and P_E were evaluated. No significant publication bias was found in Egger's test or Begg's test in either

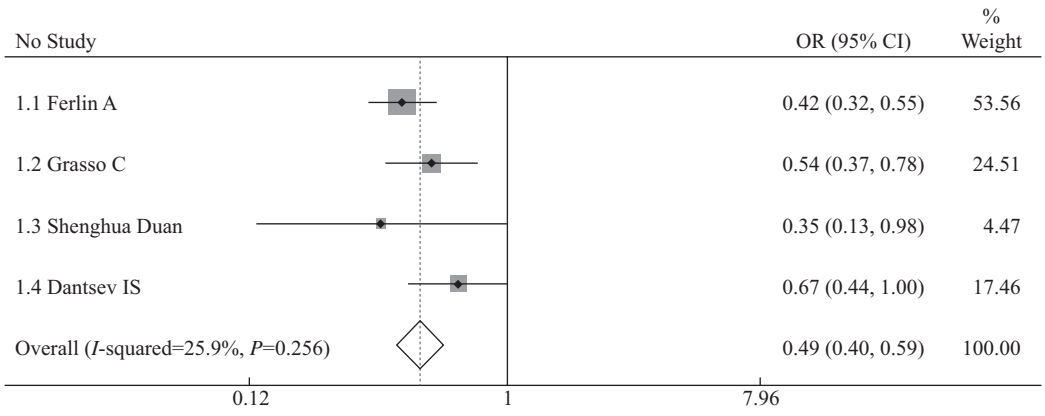

Fig. 2 Forest plot with a fixed effects model for the association between KITLG rs995030 and TGCT in allelic comparison (A *vs.* G) For each study, the estimate of OR and its 95% CI is plotted with a box and a horizontal line. Rhombus: pooled OR and its 95% CI.

Table 2 Summary of pooled ORs in the meta-analysis

KITLG rs995030	Number (cases/controls)	A *vs.* G		AA *vs.* GG		GA *vs.* GG		GA+AA *vs.* GG		AA *vs.* GG+GA	
		OR* (95% CI*)	I^2 (%)	OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)	OR (95% CI)	I^2 (%)
	796/1156	**0.489 (0.405–0.590)**#	25.9	*0.431 (0.104–1.782)*#	**70.5**	**0.455 (0.365–0.566)**	15.0	**0.441 (0.357–0.546)**	0.0	*0.554 (0.124–2.487)*	**73.8**
		G *vs.* A		GG *vs.* AA		AG *vs.* AA		AG+GG *vs.* AA		GG *vs.* AA+AG	
KITLG rs4474514	561/1438	***0.454 (0.291–0.710)***	**51.9**	***0.182 (0.076–0.434)***	0.0	***0.474 (0.275–0.819)***	**57.4**	***0.435 (0.259–0.733)***	**55.8**	***0.225 (0.095–0.533)***	0.0

*OR: Odds ratio; CI: confidence interval
#Results with statistical significant difference were marked as bold. Unstable results in sensitivity analyses were marked as italic.

genetic models of KITLG rs995030 or rs4474514. However, in homozygote comparison (AA *vs.* GG, fig. 3) and recessive model (AA *vs.* GG+GA) of KITLG rs995030, study No. 1.4 extended beyond the diagonal line which represents pseudo-95%CI limits about the effect estimate in funnel plot. In allelic comparison (G *vs.* A), heterozygote comparison (AG *vs.* AA) and dominant model (AG+GG *vs.* AA) of KITLG rs4474514, study No. 2.1 extended beyond the diagonal line.

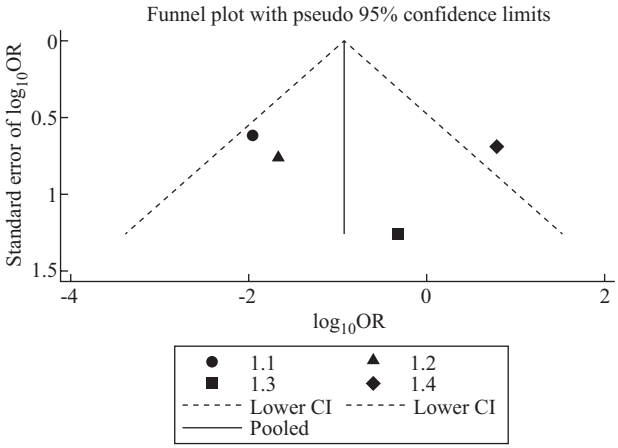

Fig. 3 Begg's funnel plot for publication bias in homozygote comparison (AA *vs.* GG) of KITLG rs995030
The vertical line represents the meta-analysis summary estimate, and the diagonal lines represent pseudo-95%CI limits about the effect estimate.

3 DISCUSSION

Above all, we found KITLG rs995030 adenine (A) was associated with decreased risk of TGCT in allelic comparison (A *vs.* G), heterozygote comparison (GA *vs.* GG), dominant model (GA+AA *vs.* GG), and the results showed stability in sensitivity analyses and no publication bias. No statistically significant changes of TGCT risk was found in other two genetic models of KITLG rs995030, however, the two results lacked stability in sensitivity analyses and in funnel plot.

Significantly decreased risk of TGCT was found in all genetic models of KITLG rs4474514, however, all of those results lacked stability in sensitivity analyses and some even showed instability in funnel plot. We found that several extragonadal germ cell tumor cases were included in Poynter JN's study[12] (**No. 2.1**) and all cases were familial testicular germ cell tumors in Azevedo MF's study[14] (**No. 2.3**), which might be responsible for part of the instability.

Meanwhile, the limitations of this meta-analysis need to be addressed. To date, the number of available studies which can be included in this meta-analysis was small. Data for subgroup analyses were too scanty to

perform. The control group of study by Dantsev IS[17] had shown significant departure from HWE. Several extragonadal germ cell tumor cases were included in study by Poynter JN[12]. All cases were familial testicular germ cell tumors in the study by Azevedo MF[14]. It was not clearly mentioned in some studies that whether the individuals with cryptorchidism, hydrocele or varicocele were excluded. Related studies published in other languages or unpublished were possibly missed. With those limitations, the study provided some insights into the potential association between KITLG gene polymorphisms and TGCT susceptibility.

In conclusion, our results suggested that compared with adenine (A), KITLG rs995030 guanine (G) might be associated with increased risk of TGCT. There are insufficient data to fully confirm the association between KITLG rs4474514 and TGCT susceptibility, and the results should be interpreted with caution. Well-designed studies with larger sample size and more subgroups are required to validate the risk identified in the current meta-analysis.

Conflict of Interest Statement

All authors declare that they have no conflict of interest.

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