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Quantitative assessment of the association of polymorphisms in the metallothionein 2A gene with cancer risk

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Abstract

Objective: The aim of the study was to quantitatively assess the association of metallothionein 2A (*MT2A*) polymorphisms rs28366003 and rs1610216 with cancer risk.

Methods: Crude odd ratios (OR) with 95% confidence intervals (CI) were used to estimate associations of the polymorphisms with cancer risk.

Results: Six eligible case-control studies with 1899 cases and 2437 controls focused on rs28366003, and three of those six studies, with 548 cases and 926 controls, additionally focused on rs1610216. Pooled analysis showed that *MT2A* rs28366003 and rs1610216 were associated with cancer risk: (AG + GG) vs. AA, OR = 2.67; GG vs. (AG + AA), OR = 5.97; GG vs. AA, OR = 6.80; AG vs. AA, OR = 2.46; G vs. A, OR = 2.67 for rs28366003; and CC vs. (TC+TT), OR = 2.51; CC vs. TT, OR = 2.42 for rs1610216. Subgroup analysis based on ethnicity showed a significant association of rs28366003 with cancer risk in Asian and Caucasian populations. However, a significant association of rs1610216 with cancer risk was found only in the Asian population.

Conclusion: *MT2A* rs28366003 and rs1610216 polymorphisms were associated with cancer risk and might serve as genetic biomarkers for predicting cancer risk. However, larger studies are needed to confirm these findings.

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Metallothionein 2A, polymorphism, cancer, risk, meta-analysis, ethnicity

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Introduction

Cancer is a major public health problem and one of the major causes of mortality worldwide. Its etiology is complex, involving environmental factors and individual genetic background. Some single nucleotide polymorphisms (SNPs) in cancer-related genes have been found to affect cancer risk.[1–3]

The metallothionein 2A (*MT2A*) gene located at chromosome 16q13 is a member of the metallothionein gene family.[4] The protein encoded by *MT2A* can interact with that encoded by the homeobox containing 1 gene (*HMBOX1*) in vascular endothelial cells, controlling intracellular zinc levels and affecting apoptotic and autophagy pathways.[5] In addition, MT2A plays an important role in tumorigenesis and progression of multiple cancer types.[6–9] For instance, *MT2A* expression was associated with cell proliferation in breast cancer.[6] MT2A can inhibit growth of gastric cancer cells through apoptosis and G_2/M arrest.[7]

In the last 10 years, several studies have focused on the association of the rs28366003 A>G and rs1610216 T>C SNPs in *MT2A* with cancer risk. Both SNPs are located in the promoter region of *MT2A*. Moreover, rs28366003 A>G might be associated with the risk of multiple cancers, including stomach adenocarcinoma, breast cancer, inverted papilloma, laryngeal cancer, and prostate cancer.[10–15] The rs1610216 T>C SNP might only be associated with the risk of stomach adenocarcinoma.[10] However, previous case-control studies had small sample sizes and each focused on only a single type of cancer, and lacked comprehensive assessment of the association of *MT2A* rs28366003 and rs1610216 SNPs with cancer risk. Hence, in this study, our aim was to quantitatively assessed these associations by meta-analysis as a means to identify genetic biomarkers for predicting cancer risk.

Methods

Ethics statement

Ethical permission was not required because all data were obtained from public databases.

Literature search

Two investigators independently searched for eligible articles in the following databases: PubMed, Embase, CNKI, and Web of Science. The last update was on March 18, 2020. Search terms were as follows: "metallothionein 2A OR MT2A", "polymorphism OR variant OR rs28366003 OR rs1610216" and "cancer OR carcinoma OR neoplasm". The reference lists of all relevant articles were also retrieved. During the literature search, the publication language was limited to English or Chinese. Eligible studies were selected according to the following standards: (a) case-control study focusing on the association of *MT2A* rs28366003, rs1610216, or both with cancer risk, and (b) the genotype frequency distribution of polymorphisms in controls conformed to Hardy–Weinberg equilibrium (HWE). The exclusion criteria for literature selection were as follows: letters, reviews, and case reports. In addition, if multiple studies had overlapping data, only those with complete data were

included. The PRISMA guidelines were followed and the PRISMA flowchart is shown in Figure 1.

Data extraction

Two investigators independently selected the relevant articles and extracted the following data: author's name, publication year, country, ethnicity, cancer type, genotyping method, number of cases and controls, and genotype and allele frequencies. The *P*-value of HWE in controls (P_{HWE}) was calculated in each study. Any disagreement was resolved by discussion with a third author.

Bioinformatics analysis

The effects of rs28366003 and rs1610216 on binding of transcription factors were explored using the SNPinfo tool (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html).

Statistical analysis

The goodness-of-fit chi-square test was used to evaluate whether genotype frequency distribution of SNPs in controls conformed to HWE. $P_{HWE} > 0.05$ was considered to conform to HWE. The association of each SNP with cancer risk was analyzed by calculating the odds ratio (OR) and 95% confidence interval (95% CI). A *P*-value of the Z-test (Pz) < 0.05 was considered to indicate a significant association. Chi-square-based Q test and I^2 index were used to examine between-study heterogeneity. Heterogeneity was considered to exist if the heterogeneity *P*-value (P_H) < 0.05 in

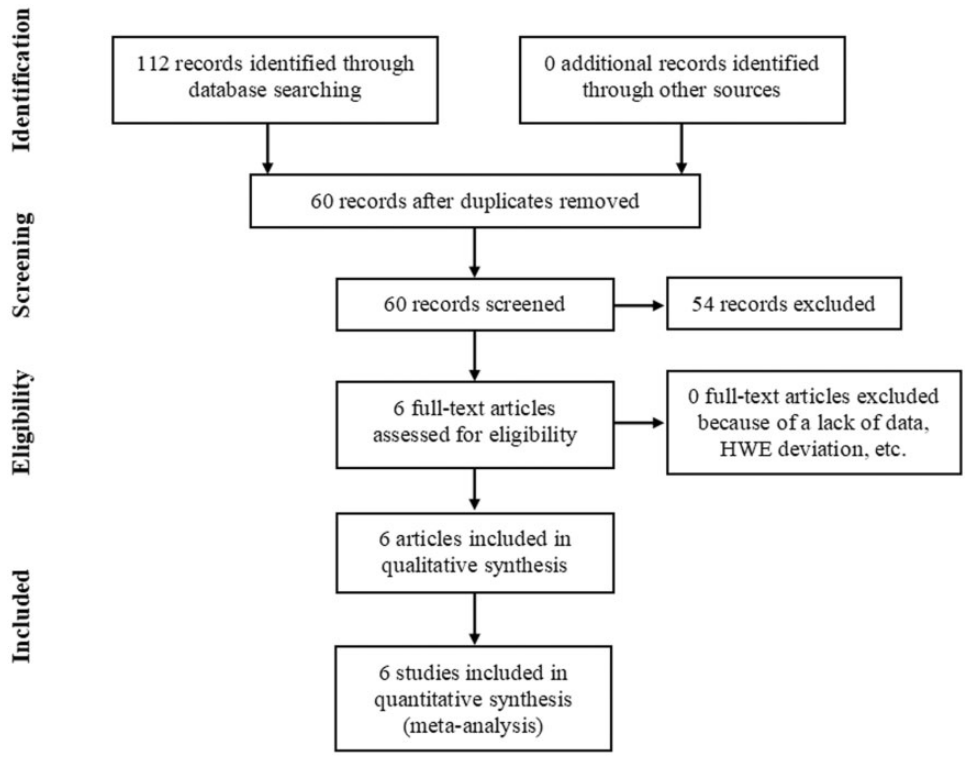

Figure 1. The PRISMA flowchart for the selection of studies.

the chi-square-based Q test and/or the I^2 index was >50%. When no significant between-study heterogeneity existed, the fixed-effect model was selected; otherwise, the random-effect model was applied. Sensitivity analysis was performed by sequentially omitting one study at a time to evaluate whether the pooled results were robust. Publication bias was assessed using Begg's and Egger's tests. When *P*-value was < 0.05 in the Begg's and Egger's tests, significant publication bias was considered to exist. Trial sequential analysis (TSA) was also applied to quantify the statistical reliability of the meta-analysis. All analyses were conducted by using STATA 12.0 (StataCorp., College Station, TX, USA) and TSA 0.9.5.10 (http://www.ctu.dk/tsa/downloads.aspx) software.

Results

A total of 112 articles were retrieved through literature search. One hundred and six articles were excluded by reading titles and abstracts. Finally, six case-control studies[10–15] were included in our meta-analysis (Figure 1). The characteristics of eligible studies are presented in Table 1 and Table 2. Six studies with 1899 cases and 2437 controls focused on rs28366003, and three of those six studies, with 548 cases and 926 controls, additionally focused on rs1610216. The pooled analysis showed that *MT2A* rs28366003 SNP was associated with cancer risk: (AG + GG) vs. AA, OR = 2.67, $P_Z < 0.01$; GG vs. (AG + AA), OR = 5.97, $P_Z < 0.01$; GG vs. AA, OR = 6.80, $P_Z < 0.01$; AG vs. AA, OR = 2.46, $P_Z < 0.01$; G vs. A, OR = 2.67, $P_Z < 0.01$ (Table 3 and Figure 2). The *MT2A* rs1610216 SNP was also associated with cancer risk: CC vs. (TC+TT), OR = 2.51, $P_Z = 0.03$; CC vs. TT, OR = 2.42, $P_Z = 0.04$ (Table 4 and Figure 2). Subsequently, we performed a stratified analysis based on ethnicity. Results showed that *MT2A* rs28366003 was significantly associated with cancer risk, in both the Asian subpopulation [(AG + GG) vs. AA, OR = 2.15, $P_Z < 0.01$; AG vs. AA, OR = 1.95, $P_Z < 0.01$; G vs. A, OR = 2.20, $P_Z < 0.01$], and in the Caucasian subpopulation [(AG + GG) vs. AA, OR = 3.06, $P_Z < 0.01$; GG vs. (AG + AA), OR = 6.11, $P_Z < 0.01$; GG vs. AA, OR = 6.84, $P_Z < 0.01$; AG vs. AA, OR = 2.90, $P_Z < 0.01$; G vs. A, OR = 3.01, $P_Z < 0.01$] (Table 3). However, the association of *MT2A* rs1610216 with cancer risk was found only in the Asian subpopulation [CC vs. (TC+TT), OR = 4.35, $P_Z = 0.01$; CC vs. TT, OR = 4.28, $P_Z = 0.02$] (Table 4).

Table 1. The main characteristics of eligible studies.

Study	Country	Ethnicity	Cancer type	Genotyping method	Cases	Controls
Shokrzadeh et al., 2019[10]	Iran	Asian	Stomach adenocarcinoma	PCR-RFLP	95	90
Liu et al., 2017[11]	China	Asian	Breast cancer	Sequenom MassARRAY	459	549
Starska et al., 2015[13]	Poland	Caucasians	Sinonasal inverted papilloma	PCR-RFLP	130	418
Starska et al., 2014[14]	Poland	Caucasians	Laryngeal cancer	PCR-RFLP	323	418
Krześlak et al., 2014[12]	Poland	Caucasians	Breast cancer	PCR-RFLP	534	556
Forma et al., 2012[15]	Poland	Caucasians	Prostate cancer	PCR-RFLP	358	406

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Table 2. Genotype frequency distribution of metallothionein 2A (*MT2A*) rs28366003 and rs1610216 polymorphisms.

	rs28366003								
Study	AA	GA	GG	Total	AA	GA	GG	Total	P_{HWE}
Shokrzadeh et al., 2019[10]	44	39	12	95	52	34	4	90	0.60
Liu et al., 2017[11]	378	70	11	459	508	41	0	549	0.36
Starska et al., 2015[13]	98	31	1	130	401	17	0	418	0.67
Starska et al., 2014[14]	288	32	3	323	401	17	0	418	0.67
Krześlak et al., 2014[12]	465	66	3	534	516	40	0	556	0.38
Forma et al., 2012[15]	275	75	8	358	361	43	2	406	0.56
	rs1610216								
	TT	TC	CC	Total	TT	TC	CC	Total	P_{HWE}
Shokrzadeh et al., 2019[10]	43	36	16	95	46	40	4	90	0.20
Starska et al., 2015[13]	96	33	1	130	309	106	3	418	0.06
Starska et al., 2014[14]	237	84	2	323	309	106	3	418	0.06

P_{HWE}, *P*-value for Hardy–Weinberg equilibrium.

Table 3. Association of metallothionein 2A (*MT2A*) rs28366003 polymorphism with cancer risk.

		Heterogeneity				
Comparison	Subgroup	P_H	I^2	Effect model	OR [95% CI]	P_Z
(AG+GG) vs. AA	Overall	<0.01	69.9%	Random	2.67 [1.86, 3.83]	<0.01
	Asian	0.15	51.3%	Random	2.15 [1.31, 3.53]	<0.01
	Caucasian	<0.01	78.2%	Random	3.06 [1.80, 5.18]	<0.01
GG vs. (AG+AA)	Overall	0.76	0.0%	Fixed	5.97 [2.76, 12.91]	<0.01
	Asian	0.13	56.7%	Random	6.67 [0.73, 61.28]	0.09
	Caucasian	0.96	0.0%	Fixed	6.11 [1.90, 19.64]	<0.01
GG vs. AA	Overall	0.77	0.0%	Fixed	6.80 [3.11, 14.87]	<0.01
	Asian	0.14	54.9%	Random	7.45 [0.85, 65.76]	0.07
	Caucasian	0.96	0.0%	Fixed	6.84 [2.12, 22.07]	<0.01
AG vs. AA	Overall	<0.01	71.5%	Random	2.46 [1.68, 3.59]	<0.01
	Asian	0.16	49.3%	Fixed	1.95 [1.39, 2.74]	<0.01
	Caucasian	<0.01	78.4%	Random	2.90 [1.69, 4.96]	<0.01
G vs. A	Overall	<0.01	70.8%	Random	2.67 [1.90, 3.75]	<0.01
	Asian	0.06	71.3%	Random	2.20 [1.26, 3.82]	<0.01
	Caucasian	<0.01	76.8%	Random	3.01 [1.84, 4.91]	<0.01

P_H, heterogeneity *P*-value; P_Z, *P*-value of Z-test.

Sensitivity analysis for rs28366003 showed that no individual study significantly changed the pooled ORs. However, sensitivity analysis for rs1610216 showed a significant change in the pooled ORs after the study of Shokrzadeh et al.[10] was removed (Figure 3). Potential publication bias was evaluated by two test methods

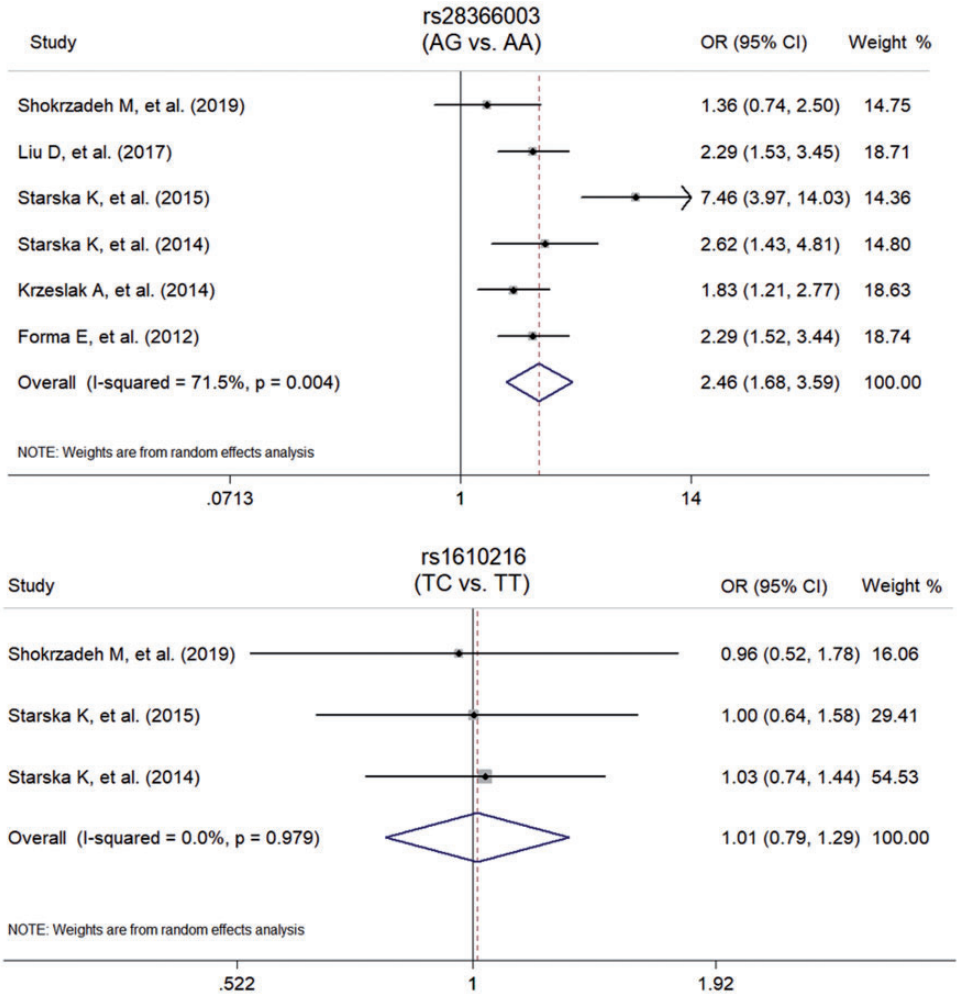

Figure 2. Representative forest plots of metallothionein 2A (*MT2A*) rs28366003 and rs1610216 polymorphisms.

(Table 5 and Figure 4). Begg's test did not show any significant publication bias. However, Egger's test showed significant publication bias under GG vs. (AG + AA) and GG vs. AA of the rs28366003 SNP, and TC vs. TT of the rs1610216 SNP. TSA showed that sample size was insufficient to obtain a significant result; thus, more studies are required (Figure 5).

Bioinformatics analysis showed that rs28366003 could affect binding of transcription factors ZF5, PAX, and PPARG, and that rs1610216 could affect binding of transcription factors CP2, TBX5, and HEN1.

Discussion

Forma et al. analyzed 358 prostate cancer cases and 406 population controls in a Polish population and found that the *MT2A* rs28366003 SNP was associated

Table 4. Association of metallothionein 2A (*MT2A*) rs1610216 polymorphism with cancer risk.

Comparison	Subgroup	Heterogeneity		Effect model	OR [95% CI]	P_Z
		P_H	I^2			
(TC+CC) vs. TT	Overall	0.80	0.0%	Fixed	1.06 [0.83, 1.35]	0.64
	Asian	–	–	–	1.26 [0.71, 2.25]	0.43
	Caucasian	0.93	0.0%	Fixed	1.02 [0.78, 1.33]	0.88
CC vs. (TC+TT)	Overall	0.25	28.6%	Fixed	2.51 [1.11, 5.69]	0.03
	Asian	–	–	–	4.35 [1.40, 13.58]	0.01
	Caucasian	0.88	0.0%	Fixed	0.94 [0.23, 3.85]	0.93
CC vs. TT	Overall	0.27	24.4%	Fixed	2.42 [1.05, 5.58]	0.04
	Asian	–	–	–	4.28 [1.33, 13.81]	0.02
	Caucasian	0.89	0.0%	Fixed	0.94 [0.23, 3.88]	0.93
TC vs. TT	Overall	0.98	0.0%	Fixed	1.01 [0.79, 1.29]	0.92
	Asian	–	–	–	0.96 [0.52, 1.78]	0.90
	Caucasian	0.92	0.0%	Fixed	1.02 [0.78, 1.34]	0.87
C vs. T	Overall	0.29	21.9%	Fixed	1.12 [0.90, 1.38]	0.31
	Asian	–	–	–	1.53 [0.98, 2.39]	0.06
	Caucasian	0.96	0.0%	Fixed	1.02 [0.80, 1.29]	0.91

P_H, heterogeneity *P*-value; P_Z, *P*-value of Z-test.

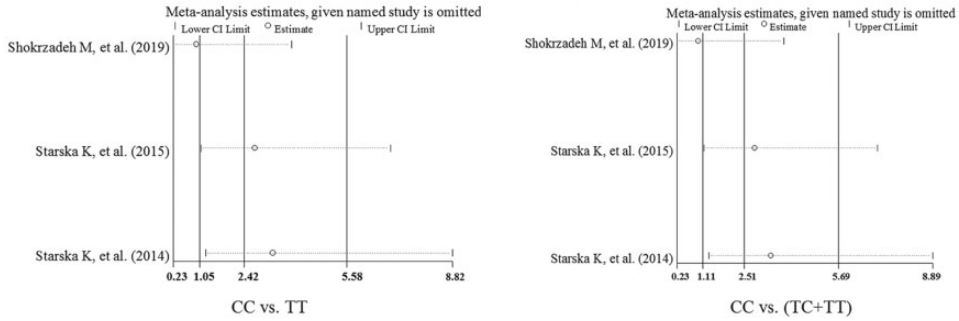

Figure 3. Sensitivity analysis for metallothionein 2A (*MT2A*) rs1610216 polymorphism under CC vs. TT and CC vs. (CT+TT).

with the risk of prostate cancer.[15] Compared with individuals with the rs28366003 AA genotype, individuals with the AG or GG genotype had a significantly increased risk of prostate cancer. Starska et al. analyzed 323 genetically unrelated individuals with squamous cell laryngeal cancer and 418 randomly selected healthy volunteers in a Polish population and found that the *MT2A* rs28366003 SNP was associated with the risk of squamous cell laryngeal cancer.[14] Compared with individuals with the AA genotype at rs28366003, individuals with the AG or GG genotype had a significantly increased risk of squamous cell laryngeal cancer. In addition, there was a significant association between rs28366003 and tumor stage and tumor front grading of squamous cell laryngeal cancer. Most carriers of the minor allele (G) had a higher stage and increased cancer aggressiveness. Based on 534 breast

Table 5. Publication bias analysis of the pooled analysis.

Polymorphism	Comparison	*P*-value of Begg's test	*P*-value of Egger's test
rs28366003	(AG+GG) vs. AA	0.45	0.38
	GG vs. (AG+AA)	0.13	0.03
	GG vs. AA	0.13	0.03
	AG vs. AA	0.70	0.45
	G vs. A	0.71	0.24
rs1610216	(TC+CC) vs. TT	1.00	0.45
	CC vs. (TC+TT)	1.00	0.27
	CC vs. TT	1.00	0.28
	TC vs. TT	0.30	<0.01
	C vs. T	0.30	0.53

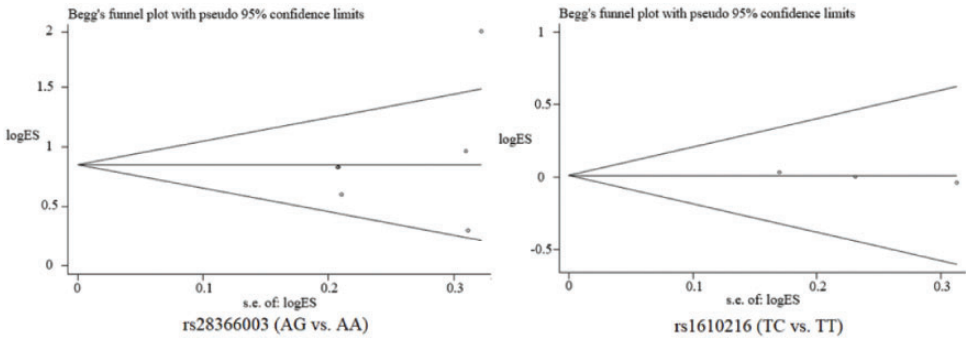

Figure 4. Representative funnel plots of metallothionein 2A (*MT2A*) rs28366003 and rs1610216 polymorphisms.

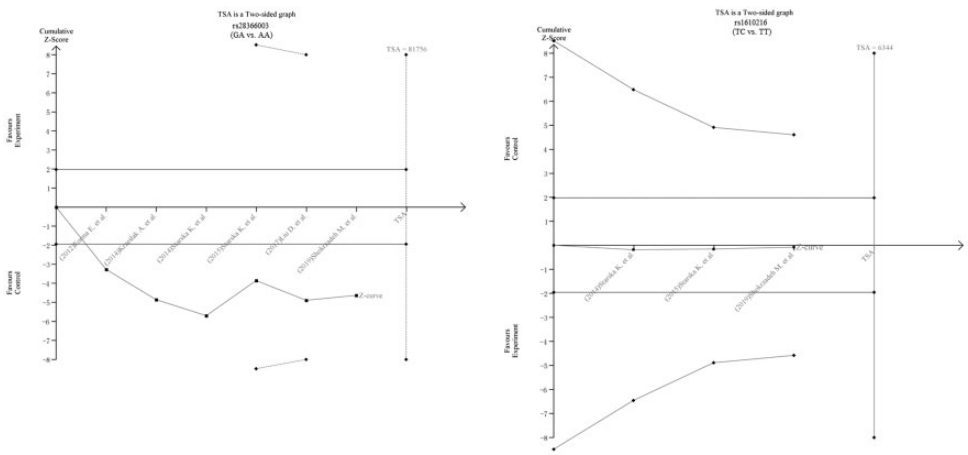

Figure 5. Trial sequential analysis of metallothionein 2A (*MT2A*) rs28366003 and rs1610216 polymorphisms.

cancer cases and 556 population controls, Krześlak et al. found that rs28366003 was associated with the risk of breast cancer in a Polish population.[12] Compared with homozygous common allele (AA) carriers, heterozygotes with the G variant had a significantly increased risk of breast cancer. Starska et al. found that rs28366003 was significantly associated with the risk of inverted papilloma in a population-based study including 130 genetically unrelated patients and 418 randomly selected healthy volunteers.[13] Compared with individuals carrying the AA genotype, individuals heterozygous or homozygous for the G variant had a significantly increased risk of inverted papilloma. Moreover, carriers of the risk allele (G) showed higher Krouse stage, diffuse tumor growth, bone destruction, and higher incidence of tumor recurrence. Liu et al. conducted a case-control study that included 459 breast cancer patients and 549 healthy controls from northwest China, and found that the minor allele G of rs28366003 was associated with an increased risk of breast cancer.[11] Shokrzadeh et al. found that, compared with individuals with the AA genotype at rs28366003, individuals with the GG genotype had a significantly increased risk of gastric cancer, based on 95 patients with gastric cancer and 90 healthy individuals in Iran.[10] In the current pooled analysis, we confirmed that *MT2A* rs28366003 was associated with the risk of cancer, including breast cancer and prostate cancer. Furthermore, we observed this association of rs28366003 and cancer risk in both Asian and Caucasian subpopulations.

For the *MT2A* rs1610216 polymorphism, Shokrzadeh et al. found that compared with the TT genotype, the CC genotype increased the risk of gastric cancer in an Iranian population.[10] Starska et al. found that rs1610216 was not associated with the risk of cancer, including inverted papilloma and squamous cell laryngeal cancer, in a Polish population.[13,14] However, we showed that rs1610216 was associated with the risk of cancer, especially in our Asian population.

The effects of rs28366003 and rs1610216 on binding of transcription factors were explored using the SNPinfo tool, and results showed that rs28366003 could affect binding of transcription factors ZF5, PAX, and PPARG, and that rs1610216 could affect binding of transcription factors CP2, TBX5, and HEN1. We found that PPARG, CP2, and HEN1 participate in the development of tumors.[16–18]

Our study has some limitations. For instance, we found an unstable result in the sensitivity analysis for the rs1610216 SNP. Significant publication bias was found in our analysis of rs28366003 and rs1610216. These limitations might result from the small number of studies in our meta-analysis. In addition, because of the lack of relevant data, the results of our study could not be adjusted according to the characteristics of included studies, such as age and sex. Hence, our results should be interpreted with caution.

In conclusion, our results suggest that significant associations exist between *MT2A* rs28366003 and rs1610216 SNPs and cancer risk. These findings allow us to better understand the effects of these two polymorphisms on cancer risk and their potential functions. However, considering the limitations mentioned above, further well-designed studies with large sample sizes, diverse ethnic groups, and different cancer types are warranted to verify our findings.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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