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# Association between Thrombomodulin Polymorphisms and Coronary Artery Disease Risk: A Meta-Analysis

Authors' Contribution:  
Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
Funds Collection G

ABCDEF **Shuai Zhang\***  
ABCDEF **Zhe Zhang\***  
ABCDEF **Feng Zhang\***  
CD **Chuanxiang Wei**  
CD **Ying Bu**  
AE **Siliang Zheng**  
AE **Dexing Su**

Emergency Center, Ji'nan Sixth People's Hospital, Zhangqiu, Shandong Province, China

\* These authors contributed equally to this work

**Corresponding Author:** Siliang Zheng, e-mail: zhengsl0531@163.com  
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**Background:** The associations between the thrombomodulin (TM) polymorphisms and coronary artery disease (CAD) risk remain controversial. The aim of this study was to evaluate the association of TM polymorphisms with CAD susceptibility using a meta-analysis approach.





**Material/Methods:** All eligible studies were identified through a search of PubMed, EMBASE, and China National Knowledge Infrastructure (CNKI) before February 2014. The associations between the TM polymorphisms and CAD risk was assessed by odds ratios (ORs) and 95% confidence intervals (CIs).

**Results:** A total of 14 case-control studies, including 5493 cases and 8297 controls, were eventually collected. There was a significant association between TM -33G/A polymorphism and CAD risk (OR=1.61; 95% CI, 1.35–1.92;  $I^2=15\%$ ). The TM Ala455Val polymorphism was also associated with a significantly increased CAD risk (OR=1.14; 95% CI, 1.05–1.24;  $I^2=0\%$ ). These results remained statistically significant when the adjusted ORs were combined.

**Conclusions:** Our results suggest that TM-33G/A and Ala455Val polymorphisms are risk factors for CAD.

**MeSH Keywords:** **Coronary Artery Disease • Genetics • Meta-Analysis • Thrombomodulin**

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## Background

Coronary artery disease (CAD) is the most common type of heart disease induced by the narrowing or blockage of the coronary arteries resulting from atherosclerosis, which is characterized by gradual build-up of fatty material and plaque inside the wall of arteries [1]. Although several traditional coronary risk factors, such as hypertension, diabetes mellitus, smoking, alcohol intake, family history, and obesity played roles in the development of CAD, there is an increased awareness of the contribution of polymorphic variants of genes as risk factors [2].

Thrombomodulin (TM) is a vascular endothelial cell-bound glycoprotein, which has been identified as a natural anticoagulant, and its major function is thrombin binding. The association of thrombin with TM significantly inhibits thrombin's procoagulant activity and decreases the production of thrombin itself [3]. TM also plays an important role in the protein C anticoagulation pathway. Wu et al. showed that a high level of soluble thrombomodulin (sTM) signified protection and a lower risk of CAD [4]. In addition, mouse models of TM gene mutation (TM<sup>pro/pro</sup> mouse) exhibited greatly reduced ability to generate activated protein C (APC) within the circulation, leading to thrombosis and hypercoagulable state [5]. Therefore, TM may play a critical role in the pathogenesis of CAD.

The intronless TM gene is located on human chromosome number 20p11.2. Several polymorphisms have been found in the TM gene, such as -33G/A, Ala455Val, and Ala25Thr [6–19]. During the last 2 decades, studies have investigated the correlation between these polymorphisms and CAD risk. However, the results were largely inconclusive, owing to insufficient sample size. Meta-analysis is a useful method for investigating associations between genetic factors and diseases, because a quantitative approach is used to combine the results from different studies on the same topic, thereby providing more reliable conclusions. Therefore, we conducted a meta-analysis of all available case-control studies to determine whether TM polymorphisms contributed to the pathogenesis of CAD.

## Material and Methods

### Publication search

Systematic literature searches were conducted before February 2014 in PubMed, EMBASE, and the Chinese National Knowledge Infrastructure (CNKI) databases without restrictions. Combinations of the following terms were applied: 'coronary heart disease' OR 'coronary artery disease' OR 'myocardial infarction' OR 'acute coronary syndrome' OR 'ischemic heart disease' OR 'cardiovascular disease' OR 'major adverse cardiac event' OR 'CHD' OR 'CAD' OR 'MI' OR 'ACS' OR 'IHD' OR 'MACE'; 'thrombomodulin'

OR 'TM'; 'polymorphism' OR 'variant' OR 'genetic' OR 'mutation'. We also conducted a manual search to find other articles based on references identified in the individual articles.

### Inclusion criteria and data extraction

We included articles if they met all the following criteria: (1) evaluation of TM polymorphisms and CAD susceptibility, (2) using a case-control design, and (3) genotype distributions in both cases and controls were available or the odds ratios (ORs) with 95% confidence intervals (CIs) were reported. After rigorous searching, we reviewed all papers in accordance with the criteria defined above for further analysis.

Data were carefully extracted independently by 2 authors. If they encountered conflicting evaluations, an agreement was reached following a discussion; if agreement could not be reached, another author was consulted to resolve the debate. The following information was extracted from each study: first author, year of publication, ethnicity, disease type, sex, sample size, polymorphisms, adjustment, ORs, and the corresponding 95% CIs of CAD risk.

### Statistical analysis

The dominant model was used in this study because this genetic model was most widely used in the included studies. The strength of the association between TM polymorphisms and CAD risk was measured by OR and corresponding 95% CI. Heterogeneity between the studies was quantified using the Cochran Q test in combination with the  $I^2$  statistic, which represents the percentage of variability across studies that is attributable to heterogeneity rather than to chance. Heterogeneity among studies was considered significant when  $P$  was less than 0.1 for the Q-test or when the  $I^2$  value was greater than 50%. When the heterogeneity was low, the fixed-effects model was used; otherwise we used the random-effects model. Subgroup analyses were stratified by age, ethnicity, and CAD type. Sensitivity analysis was undertaken by removing an individual study each time to check whether any of single study could bias the overall estimate. Egger's regression test was used to assess the potential publication bias [20]. Data analysis was performed using RevMan 5.1 software (Nordic Cochrane Center, Copenhagen, Denmark) and STATA version 11 (StataCorp LP, College Station, TX, USA).

## Results

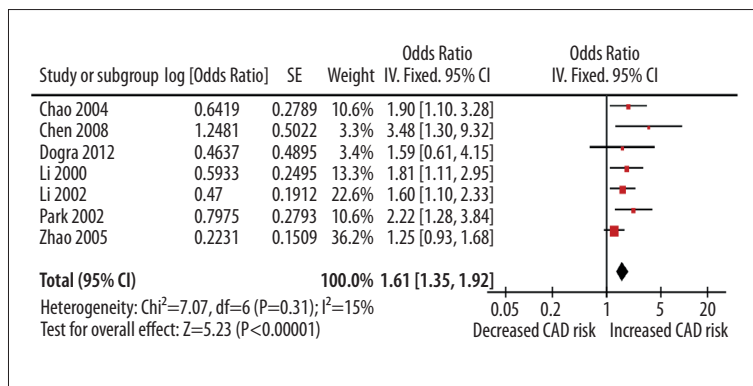
### Study characteristics

Through searching and selection, a final list of 14 eligible studies was collected for meta-analysis [6–19]. A total of 5493 cases and 8297 controls were included in this meta-analysis. Table 1

**Table 1.** Characteristics of the studies.

First author	Year	Ethnicity	Disease type	Age of patients	Sex	Case (n)	Control (n)	Polymorphisms	Adjustment
Doggen	1998	Caucasian	MI	56.2	Male	560	646	Ala25Thr	No
Li	2000	Asian	CAD	63	Mixed	320	200	-33G/A	Yes
Wu 1	2001	Caucasian	CAD	45–64	Mixed	289	356	Ala455Val	Yes
Wu 2	2001	African	CAD	45–64	Mixed	87	105	Ala455Val	Yes
Li	2002	Asian	MI	57.5	Mixed	278	450	-33G/A	Yes
Park	2002	Asian	MI	57	Mixed	85	393	-33G/A, Ala455Val	No
Ranjith	2003	Asian	MI	≤45	Mixed	195	300	Ala455Val	No
Chao	2004	Asian	MI	≤50	Mixed	143	145	-33G/A, Ala455Val	Yes
Konstantoulas	2004	Caucasian	CAD	56	Male	201	2367	Ala455Val	No
Zhao	2005	Asian	CAD*	54	Mixed	808	814	-33G/A	Yes
Chen	2006	Asian	CAD	62	Mixed	84	80	Ala455Val	No
Chen	2008	Asian	MI	69	Mixed	80	78	-33G/A	No
Guella	2011	Caucasian	MI	39.6	Mixed	1880	1880	Ala455Val	No
Dogra	2012	Asian	MI	≤40, ≥60	Mixed	350	350	-33G/A, Ala455Val	No
Shah	2012	Asian	CAD	53	Mixed	133	133	Ala455Val	No

\* Different data can be extracted separately. CAD – coronary artery disease; MI – myocardial infarction.



**Figure 1.** Forest plot of CAD risk of TM -33G/A polymorphism.

summarized the main characteristics of those included studies. There were 4 case-control studies from Caucasian populations, 10 case-control studies from Asian populations, and 1 study was from an African population. Most of the studies focused on -33G/A and Ala455Val polymorphisms, and only 1 study investigated Ala25Thr polymorphism.

### Quantitative data synthesis

#### -33G/A polymorphism

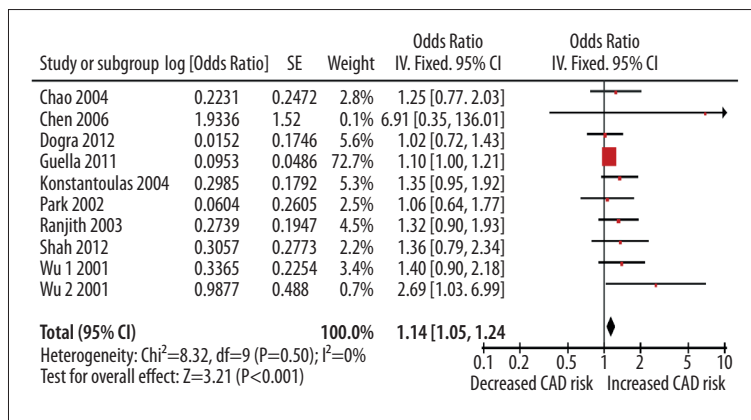
Seven studies investigated the association between the -33G/A polymorphism and CAD risk. The AA and AG genotypes were

associated with a significantly increased risk of CAD when compared with GG genotype (OR=1.61; 95%CI, 1.35–1.92;  $I^2=15\%$ ; Figure 1). Four studies reported adjusted ORs. The combination of adjusted ORs also indicated that -33G/A polymorphism was associated with increased CAD risk (OR=1.50; 95% CI, 1.23–1.82;  $I^2=0\%$ ). In the stratified analysis by ethnicity, a statistically significant association was found for studies with Asians (OR=1.61; 95% CI, 1.35–1.92;  $I^2=15\%$ ). In the subgroup analysis by age, the -33G/A polymorphism was significantly associated with early-onset CAD risk (OR=1.74; 95% CI 1.28–2.36;  $I^2=17\%$ ), but not with late-onset CAD risk (OR=1.45, 95% CI 0.97–2.18;  $I^2=64\%$ ). Six studies reported the association between -33G/A polymorphism and myocardial infarction

**Table 2.** Meta-analysis results of TM polymorphisms on coronary artery disease.

Polymorphisms	Subgroup	Association			Heterogeneity		
		OR (95% CI)	Z	P Value	$\chi^2$	P Value	$I^2$ (%)
<b>-33G/A</b>							
AA + GA vs. GG	Overall	1.61 (1.35–1.92)	5.23	<0.01	7.07	0.31	15
AA + GA vs. GG	Adjusted	1.50 (1.23–1.82)	4.04	<0.01	2.86	0.41	0
AA + GA vs. GG	Asian	1.61 (1.35–1.92)	5.23	<0.01	7.07	0.31	15
AA + GA vs. GG	Early-onset	1.74 (1.28–2.36)	3.53	<0.01	3.62	0.31	17
AA + GA vs. GG	Late-onset	1.45 (0.97–2.18)	1.80	0.07	2.81	0.09	64
AA + GA vs. GG	MI	1.61 (1.32–1.96)	4.64	<0.01	4.64	0.26	23
<b>Ala455Val</b>							
Val/Val + Val/Ala vs. Ala/Ala	Overall	1.14 (1.05–1.24)	3.21	<0.01	8.32	0.50	0
Val/Val + Val/Ala vs. Ala/Ala	Adjusted	1.57 (1.05–2.34)	2.20	0.03	1.47	0.23	32
Val/Val + Val/Ala vs. Ala/Ala	Asian	1.18 (0.97–1.43)	1.68	0.09	2.88	0.72	0
Val/Val + Val/Ala vs. Ala/Ala	Caucasian	1.13 (1.03–1.23)	2.58	0.01	2.17	0.34	8
Val/Val + Val/Ala vs. Ala/Ala	Early-onset	1.45 (1.14–1.83)	3.09	<0.01	3.66	0.30	18
Val/Val + Val/Ala vs. Ala/Ala	Late-onset	0.75 (0.50–1.11)	1.45	0.15	0.08	0.78	0
Val/Val + Val/Ala vs. Ala/Ala	MI	1.11 (1.02–1.21)	2.32	0.02	1.31	0.86	0

MI – myocardial infarction.



**Figure 2.** Forest plot of CAD risk of TM Ala455Val polymorphism.

(MI) risk, and the pooled result suggested a significant association (OR=1.61; 95% CI, 1.32–1.96;  $I^2=23\%$ ). Results of meta-analysis are shown in Table 2. We performed a sensitivity analysis to evaluate the stability of the meta-analysis. The statistical significance of the result was not altered when any single study was omitted. The Egger’s test did not reveal evidence of obvious asymmetry ( $P=0.43$ ).

**Ala455Val polymorphism**

Ten studies identified an association between the Ala455Val polymorphism and CAD risk. The Val/Val and Val/Ala genotypes

were associated with a significantly increased risk of CAD when compared with Ala/Ala genotype (OR=1.14; 95% CI, 1.05–1.24;  $I^2=0\%$ ; Figure 2). Two studies reported adjusted ORs. The combination of adjusted ORs also indicated that Ala455Val polymorphism was associated with increased CAD risk (OR=1.57; 95% CI, 1.05–2.34;  $I^2=32\%$ ). In the stratified analysis by ethnicity, a statistically significant association was found for studies with Caucasians (OR=1.13; 95% CI, 1.03–1.23;  $I^2=8\%$ ) but not with Asians (OR=1.18; 95% CI, 0.97–1.43;  $I^2=0\%$ ). In the subgroup analysis by age, this polymorphism was significantly associated with early-onset CAD risk (OR=1.45; 95% CI 1.14–1.83;  $I^2=18\%$ ) but not with late-onset CAD risk (OR=0.75, 95% CI

0.50–1.11;  $I^2=0\%$ ). Five studies reported the association between Ala455Val polymorphism and MI risk, and the pooled result suggested a significant association (OR=1.11; 95% CI, 1.02–1.21;  $I^2=0\%$ ). Table 2 shows these results. We performed a sensitivity analysis to evaluate the stability of the meta-analysis. The statistical significance of the result was not altered when any single study was omitted. Egger's test did not show evidence of obvious asymmetry ( $P=0.12$ ).

## Discussion

The present meta-analysis, including 5493 CAD cases and 8297 controls from 14 case-control studies, explored the associations of TM -33G/A and Ala455Val polymorphisms with CAD risk. We demonstrated that these 2 polymorphisms were significantly associated with increase CAD risk. Subgroup analyses stratified by ethnicity showed different results between the 2 polymorphisms and CAD risk. TM -33G/A polymorphism might play an important role in the development of CAD in Asians, while Ala455Val polymorphism might influence the risk of CAD in Caucasians. It was possible that different lifestyles, diets, and environments may account for this discrepancy. However, we were only able to include a small number of studies. More studies are needed to assess the association between TM -33G/A and Ala455Val polymorphisms and CAD risk in different ethnicities. Interestingly, in the subgroup analysis by age, we found that TM -33G/A and Ala455Val polymorphisms showed increased early-onset CAD risk but not late-onset CAD risk. This result suggests that even the same variant in the same gene may have a different effect on the pathogenesis and occurrence of CAD in different individuals. In the MI subgroup analysis, we found that patients with TM -33G/A or Ala455Val polymorphism had increased MI risk, suggesting TM -33G/A and Ala455Val polymorphisms are critical for MI development.

In the TM gene coding region, a dimorphism at nucleotide position 1418, cytosine transition to thymidine, results in an alanine (A) to valine (V) substitution at amino acid position 455 (Ala455Val). This polymorphism is located at the sixth EGF-like domain, which is responsible for the binding of thrombin and activation of protein C. Sugiyama et al. found that this polymorphism reduced plasma TM levels and may be associated with deep vein thrombosis in Japanese [21]. The -33G/A polymorphism was found in the 5'-gene regulatory elements of the TM gene. This polymorphism may cause diminished

transcription and concomitantly reduced expression of TM [22]. TM acts as an important physiological anticoagulant and deficiency of this protein could result in excessive thrombus formation. Thus, TM -33G/A and Ala455Val polymorphisms may be associated with increased CAD risk. Results of this meta-analysis support this speculation.

Our study has some strengths. First, it was the first to study the associations between TM -33G/A and Ala455Val polymorphisms and CAD risk. Second, the methodological issues for meta-analysis, such as subgroup analysis and one-way sensitivity analysis, were well investigated. Third, the main result remained statistically significant when the adjusted ORs were combined.

Results from one-way sensitivity analysis suggest high stability and reliability of our results. We must mention the importance of heterogeneity and publication bias, which might influence the results of meta-analysis. In our study, significant heterogeneity was not observed. Additionally, Egger's tests were used to find potential publication bias. The results indicated that there was no significant publication bias.

Some limitations in our meta-analysis should be mentioned. First, the number of published studies included in our meta-analysis remained insufficient for a comprehensive analysis. Second, lack of the original data of the eligible studies limited the evaluation of the effects of the gene-gene and gene-environment interactions in CAD development. Third, only published studies were included in this meta-analysis. Therefore, publication bias may have occurred, even though the use of a statistical test did not show it. Finally, other than -33G/A and Ala455Val polymorphisms, there was Ala25Thr polymorphism in the TM gene. We could not perform meta-analysis on this polymorphism due to the limited data.

## Conclusions

In conclusion, this meta-analysis showed that TM -33G/A and Ala455Val polymorphisms are risk factors of CAD. More studies with larger sample sizes are still required to provide a more comprehensive, precise, and representative statistical analysis.

## Conflict of interest

The authors declare that they have no conflict of interest.

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