# Lack of association between the *connexin 37* C1019T gene polymorphism and coronary artery disease in a Chinese population: Meta-analysis of 2,206 subjects

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**Abstract.** The *connexin 37* (*Cx37*) C1019T gene polymorphism has been suggested to be correlated with increased coronary artery disease (CAD) risk, but research results remain inconsistent. To explore the relationship between the *Cx37* C1019T gene polymorphism and CAD in a Chinese population, the current meta-analysis of 6 individual studies involving 1,244 CAD patients and 962 controls was conducted. The pooled odds ratios (ORs) as well as the corresponding 95% confidence intervals (CIs) were estimated using a random- or fixed-effect model. No significant association was found between *Cx37* C1019T gene polymorphism and CAD in the Chinese population under the allelic (OR=0.96; 95% CI=0.59-1.56, P=0.87), recessive (OR=0.77, 95% CI=0.28-2.08, P=0.60), dominant (OR=0.990, 95% CI=0.773-1.266, P=0.934), additive (OR=1.000, 95% CI=0.736-1.359, P=1.000), homozygous (OR=1.062, 95% CI=0.598-1.887, P=0.836) or heterozygous (OR=1.017, 95% CI=0.802-1.291, P=0.888) genetic models. *Cx37* C1019T gene polymorphism was not suggested to be associated with CAD susceptibility in the Chinese population. In conclusion, no association was found between *Cx37* C1019T gene polymorphism and CAD in the Chinese population.

## Introduction

Coronary artery disease (CAD), a frequently encountered illness, poses a public health concern. CAD is considered to be a polygenic illness resulting from both environmental and hereditary factors (1). Genomic research is regarded as one of the four epoch-making revolutions and has become a study hotspot in exploring CAD pathogenesis from the molecular hereditary perspective (2).

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*Connexin 37* (*Cx37*), a gap junction protein expressed in vascular endothelial cells, regulates and controls the growth, proliferation, senium, and regeneration of the vascular endothelium by mediating the signal transduction pathway between smooth muscle and endothelial cells (3). In 2009, Derouette *et al* found *Cx37* gene knockout mice to have the tendency to develop endarterium atheromatous plaques, suggesting that *Cx37* is involved in the generation and progression of atherosclerosis as a protective landmark in the plaque formation process following endothelial injury (4). Thus, *Cx37* affects the development and progression of CAD and myocardial infarction (MI).

The *Cx37* gene, located in 1p35.1, spans 2.727 kb and contains two exons and one intron encoding 333 amino acids (5). The variation of thymine (T base) substituting for cytosine (C base) in the 1,019th base of the *Cx37* gene contributes to the supplantation of proline by serine in the 319th base of the corresponding amino acid sequence. This rs1764391 mutation affects the tertiary structure formation of the gap junction protein. Moreover, the altered amino acid transforms the phosphorylation locus and further affects protein synthesis, transport, fabrication and signal transduction. Therefore, the function of vascular endothelial cells is affected to different extents, thus contributing to individual differences in CAD susceptibility (6).

Results also differed among the studies carried out in China. Zhang *et al* reported the T allele to be the hereditary risk factor for CAD (9). In contrast with Zhang's work, Han *et al* found that the C allele was probably correlated with CAD occurrence in the Chinese northern Han population (10). However, Zhang *et al* observed no association between *Cx37* C1019T gene polymorphism and CAD (11).

Considering the results of controversial studies on *Cx37* C1019T gene polymorphism and CAD, the present meta-analysis involving 2,206 subjects was conducted to deduce a reasonable conclusion on the association between *Cx37* C1019T gene polymorphism and CAD in the Chinese population.

## Materials and methods

*Publication search and inclusion criteria.* Selected studies published between 2001 and 2010 were obtained using the MeSH terms 'coronary artery disease' or 'coronary heart disease', 'polymorphism', 'connexin 37', 'gene,' and 'Chinese'

in searching the electronic databases of PubMed, Embase, and Web of Science, as well as the China Biological Medicine Database and the China National Knowledge Infrastructure (last research updated on November 20, 2012).

The relevant publications were required to meet the following inclusion criteria: i) evaluation of *Cx37* C1019T gene polymorphism and CAD in the Chinese population and ii) CAD diagnosis based on the examination results of coronary arteriography, clinical symptoms with electrocardiogram, echocardiography, treadmill exercise test, and myocardial perfusion imaging in emission computed tomography.

*Data extraction*. The data were extracted using a standard protocol. In the present meta-analysis, studies considered to be repeated publications, studies violating the inclusion criteria, or research providing little information were excluded. If the same data appeared in different manuscripts, only one study result was used. The drawn data comprised the following items: the first author's name, publication year, region, number of genotypes, genotyping method, study design, matching criteria, and total number of cases and controls.

*Statistical analysis*. The allelic (distribution of T allelic frequency of *Cx37* C1019T gene polymorphism), recessive (TT vs. CC + CT), dominant (CC vs. CT + TT), additive (T vs. C), homozygous (TT vs. CC), and heterozygous (CT vs. CC) genetic models were used. An odds ratio (OR) corresponding to a 95% confidence interval (CI) was used to compare the association of *Cx37* C1019T and CAD. The Chi-square-based Q-test was applied to determine heterogeneity among studies (significance was set at the $P<0.05$ level) (12). The variation attributed to heterogeneity was assessed by calculating the inconsistency index $I^2$. As there was significant heterogeneity for all of the genetic models, the random-effect model (DerSimonian and Laird method) was applied for the above-mentioned models (13). The fixed-effect model was not used (the Mantel-Haenszel method) in the current meta-analysis (14). The pooled OR was determined by the Z test, and significance was set at $P<0.05$.

The Hardy-Weinberg equilibrium (HWE) was assessed by Fisher's exact test, and significance was set as $P<0.05$. The funnel plot was adopted to assess potential publication bias. The funnel plot asymmetry on the natural logarithm scale of the OR was assessed by Egger's linear regression test (significance was set at the $P<0.05$ level) (15). Stata 11.0 software (StataCorp, College Station, TX, USA) was used for statistical analysis.

**Results**

*Studies and populations*. A total of 17 publications were derived from the literature search, from which 6 complied with the research inclusion criteria. Of the 11 excluded studies, 2 were published repeatedly, 2 were reviews, 3 were unassociated with *Cx37* C1019T gene polymorphism, and 3 had foreign subjects. One study was excluded for deviating from HWE. Data were collected from a total of 1,244 CAD patients and 962 controls of the Han ethnicity (Table I) (9-11,16-18). The studies were conducted in 6 investigated regions: Taiwan, Zhejiang, Liaoning, Anhui, Shanxi and Guangdong.

Table I. Characteristics of the investigated studies of the association between *connexin 37* C1019T gene polymorphism and CAD.

| Author (Year) | Region | CAD | | | Control | | | Matching criteria | Sample size (CAD/control) | Refs. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CC | CT | TT | CC | CT | TT | | | |
| Yeh, *et al* (2001) | Taiwan | 141 | 34 | 2 | 66 | 31 | 5 | Age, gender, ethnicity, BMI, smoker (%), DM (%) | 177/102 | (16) |
| Xie, *et al* (2006) | Shanxi | 89 | 47 | 14 | 75 | 34 | 8 | Age, ethnicity | 150/117 | (17) |
| Zhang, *et al* (2007) | Zhejiang | 78 | 68 | 27 | 90 | 46 | 12 | Age, gender, ethnicity, BMI, smoker, BP | 173/148 | (9) |
| Han, *et al* (2008) | Liaoning | 110 | 268 | 124 | 76 | 187 | 147 | Ethnicity, BMI | 502/410 | (10) |
| Zhang, *et al* (2008) | Guangdong | 60 | 54 | 22 | 96 | 42 | 9 | Ethnicity | 136/147 | (11) |
| Feng, *et al* (2010) | Anhui | 41 | 42 | 23 | 6 | 16 | 16 | Gender, ethnicity, BMI, DM (%) | 106/38 | (18) |

Polymerase chain reaction-restriction fragment length polymorphism geno/typing method and case-control study design have been adopted in all of the studies. The ethnicity in all of the studies was Han. CAD, coronary artery disease; BMI, body mass index; DM, diabetes mellitus; BP, blood pressure.

Table II. Summary of meta-analysis of association of *connexin 37 (Cx37)* C1019T gene polymorphism and CAD risk in the Chinese population.

| Genetic model | Outcome | Pooled OR (95% CI) | P-value | Literature number | CAD size | Control size | $P_{heterogeneity}$ |
|---|---|---|---|---|---|---|---|
| Allelic | Distribution frequency of T allele | 0.96 (0.59-1.56) | 0.87 | 6 | 1,244 | 962 | P<0.00001[a] |
| Recessive | TT vs. CT + CC | 0.77 (0.28-2.08) | 0.60 | 6 | 1,244 | 962 | P<0.00001[a] |
| Dominant | CC vs. CT + TT | 0.990 (0.773-1.266) | 0.934 | 6 | 1,244 | 962 | $1.0 \times 10^{-10a}$ |
| Additive | T vs. C | 1.000 (0.736-1.359) | 1.000 | 6 | 1,244 | 962 | $1.0 \times 10^{-10a}$ |
| Homozygous | TT vs. CC | 1.062 (0.598-1.887) | 0.836 | 6 | 1,244 | 962 | $1.0 \times 10^{-10a}$ |
| Heterozygous | CT vs. CC | 1.017 (0.802-1.291) | 0.888 | 6 | 1,244 | 962 | $1.0 \times 10^{-10a}$ |

[a]$P<0.05$. OR, odds ratio; CI, confidence interval; CAD, coronary artery disease; CAD size, total number of CAD cases; control size, total number of control group.

*Combined analyses.* No significant association was observed between *Cx37* C1019T gene polymorphism and CAD in the Chinese population under the allelic (OR=0.96, 95% CI=0.59-1.56, P=0.87, $P_{heterogeneity}$<0.00001, $I^2$=91.1%); recessive (OR=0.77, 95% CI=0.28-2.08, P=0.60, $P_{heterogeneity}$<0.00001, $I^2$=90.1%); dominant (OR=0.990, 95% CI=0.773-1.266, P=0.934, $P_{heterogeneity}$=$1.0 \times 10^{-10}$, $I^2$=84.7%); additive (OR=1.000, 95% CI=0.736-1.359, P=1.000, $P_{heterogeneity}$=$1.0 \times 10^{-10}$, $I^2$=91.0%); homozygous (OR=1.062, 95% CI=0.598-1.887, P=0.836, $P_{heterogeneity}$=$1.0 \times 10^{-10}$, $I^2$=86.0%) and heterozygous genetic models (OR=1.017, 95% CI=0.802-1.291, P=0.888, $P_{heterogeneity}$=$1.0 \times 10^{-10}$, $I^2$=77.9%) (Table II, Figs. 1 and 2).

*Bias diagnostics.* The funnel plot and Egger's test were adopted to assess publication bias. No visual publication bias was found in the funnel plot (Fig. 3). The difference was not statistically significant in the Egger's test, suggesting low publication bias in this meta-analysis (allelic genetic model, T=1.36, P=0.247).

## Discussion

In the present meta-analysis, *Cx37* C1019T gene polymorphism was not found to be associated with CAD susceptibility under the allelic (OR=0.96), recessive (OR=0.77), dominant (OR=0.990), additive (OR=1.000), homozygous (OR=1.062), and heterozygous genetic models (OR=1.017).

Atherosclerosis refers to the chronic inflammation of the arterial wall. Endothelial dysfunction is crucial in the early stages of atherosclerosis (19). Cell-to-cell interactions are required to maintain endothelial cell integrity. The cell gap junction is an important channel for message exchange between cells (20). In 2006, Wong *et al* found that *Cx37* knockout mononuclear phagocytes adhered to the endothelium more strongly than *Cx37*-deficient cells (21). This characteristic was associated with the adenosine triphosphate (ATP) release between cells. Extracellular ATP release reduced the normally expressed mononuclear phagocytes of *Cx37*. By contrast, *Cx37* knockout and inhibition of the *Cx37* semi-pathway decreased extracellular ATP release causing mononuclear phagocytes to adhere to the matrix. This condition suggests that the *Cx37* semi-pathway releases ATP, consequently prohibiting monocyte adhesion. Alteration of the cell adhesion characteristic contributes to mononuclear phagocyte aggregation in the atherosclerotic plaque and accelerates atherosclerotic progression (22). Therefore, *Cx37* plays an important role in maintaining the structural and functional integrity of the vascular endothelium, preventing thrombosis, and inhibiting foam cell generation, which are closely correlated with the suppression of atherosclerotic progression.

In 1998, Yeh *et al* noted that *Cx37* existed in coronary artery endothelial cells through immunological detection with a *Cx37* antibody (23). Moreover, the mutation of *Cx37* C1019T was associated with carotid artery atherosclerosis, acute MI, and CAD (16,24,25). No consensus exists on the relationship between *Cx37* C1019T polymorphism and CAD. In 2001, Yeh *et al* found that the C allele of the *CX37* gene potentially plays a role in the manifestation of coronary atherosclerosis in Taiwan (16). In 2010, Feng *et al* reported a similar result in male subjects from Anhui Province, China (18). By

Review: Connexin 37 gene polymorphism and coronary artery disease in Chinese population: a meta-analysis
Comparison: 01 CAD group versus control group
Outcome: 01 Distribution of T allelic frequency of Connexin 37gene C1019T polymorphism

| Study or sub-category | CAD group n/N | Control group n/N | OR (random) 95% CI | Weight % | OR (random) 95% CI |
|---|---|---|---|---|---|
| Yeh H 2001 | 38/354 | 41/204 | | 15.88 | 0.48 [0.30, 0.77] |
| Xie XJ 2006 | 75/300 | 50/234 | | 16.59 | 1.23 [0.82, 1.84] |
| Zhang SR 2007 | 122/346 | 70/296 | | 17.14 | 1.76 [1.24, 2.49] |
| Han YL 2008 | 516/1004 | 481/820 | | 18.27 | 0.75 [0.62, 0.90] |
| Zhang CL 2008 | 98/272 | 60/294 | | 16.88 | 2.20 [1.51, 3.20] |
| Feng W 2010 | 88/212 | 48/76 | | 15.25 | 0.41 [0.24, 0.71] |
| Total (95% CI) | 2488 | 1924 | | 100.00 | 0.96 [0.59, 1.56] |

Total events: 937 (CAD group), 750 (Control group)
Test for heterogeneity: Chi?= 56.12, df = 5 (P < 0.00001), I?= 91.1%
Test for overall effect: Z = 0.17 (P = 0.87)

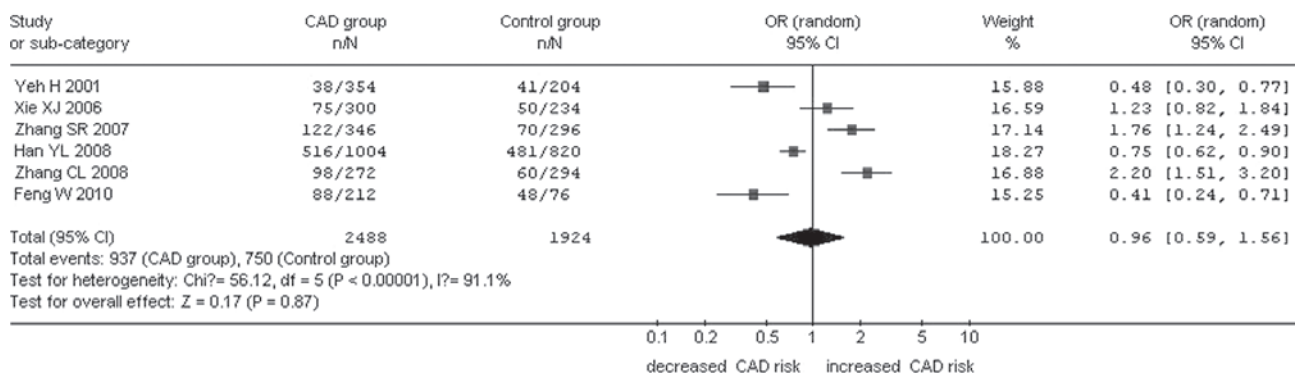0.1  0.2  0.5  1  2  5  10
decreased CAD risk   increased CAD risk

Figure 1. Forest plot of coronary artery disease (CAD) associated with *connexin 37* C1019T gene polymorphism under an allelic genetic model (distribution frequency of T allelic of *Cx37* C1019T gene).

Review: Connexin 37 gene polymorphism and coronary artery disease in Chinese population: a meta-analysis (Version 01)
Comparison: 01 CAD group versus control group
Outcome: 02 TT vs. CC+CT

| Study or sub-category | CAD group n/N | Control group n/N | OR (random) 95% CI | Weight % | OR (random) 95% CI |
|---|---|---|---|---|---|
| Yeh H 2001 | 2/175 | 5/97 | | 12.71 | 0.21 [0.04, 1.12] |
| Xie XJ 2006 | 14/136 | 8/109 | | 16.88 | 1.45 [0.58, 3.59] |
| Zhang SR 2007 | 27/146 | 12/136 | | 17.78 | 2.34 [1.14, 4.84] |
| Han YL 2008 | 124/378 | 147/263 | | 19.22 | 0.39 [0.28, 0.53] |
| Zhang CL 2008 | 22/114 | 9/138 | | 17.32 | 3.43 [1.51, 7.78] |
| Feng W 2010 | 23/83 | 16/22 | | 16.09 | 0.14 [0.05, 0.41] |
| Total (95% CI) | 1032 | 765 | | 100.00 | 0.77 [0.28, 2.08] |

Total events: 212 (CAD group), 197 (Control group)
Test for heterogeneity: Chi?= 50.33, df = 5 (P < 0.00001), I?= 90.1%
Test for overall effect: Z = 0.52 (P = 0.60)

0.1  0.2  0.5  1  2  5  10
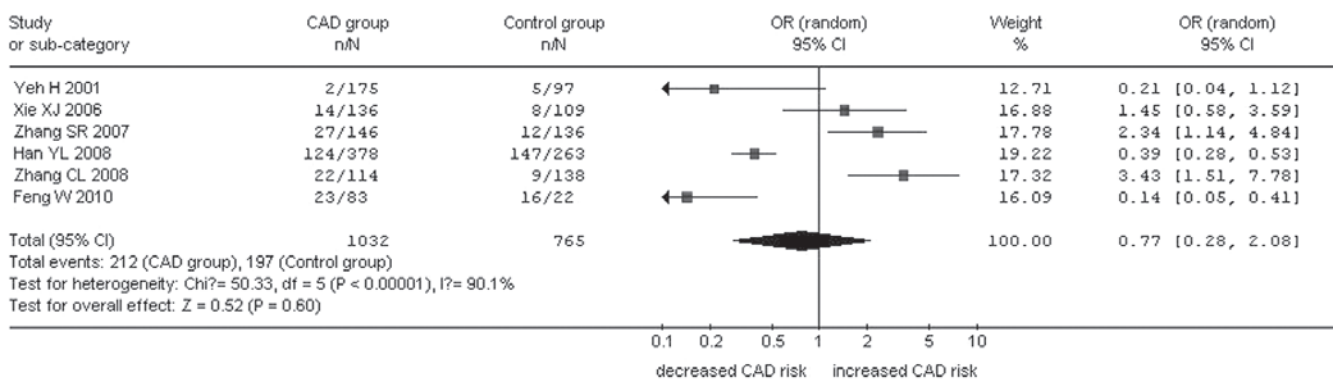decreased CAD risk   increased CAD risk

Figure 2. Forest plot of coronary artery disease (CAD) associated with *connexin 37* C1019T gene polymorphism under a recessive genetic model (TT vs. CC + CT).

Review: Connexin 37 gene polymorphism and coronary artery disease in Chinese population: a meta-analysis
Comparison: 01 CAD group versus control group
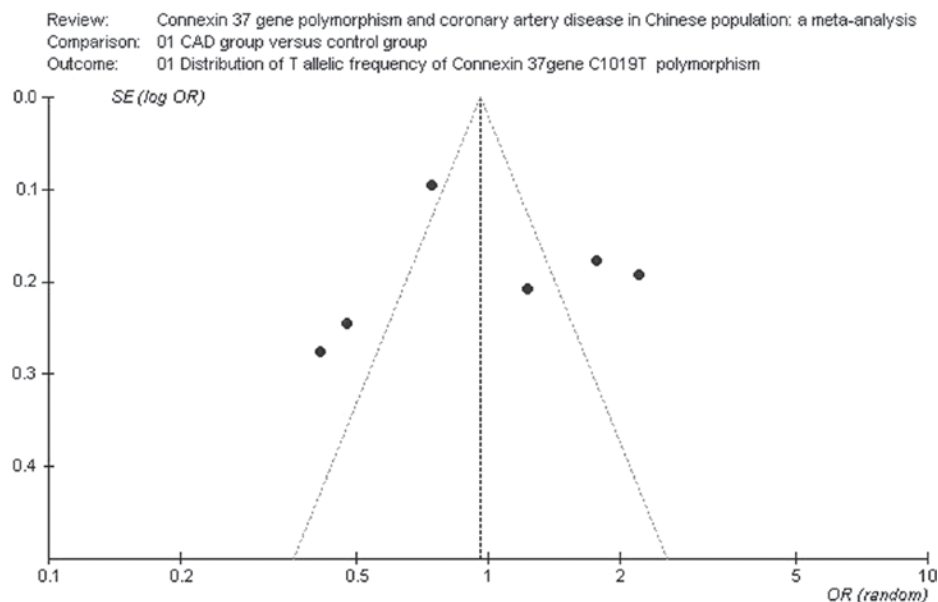Outcome: 01 Distribution of T allelic frequency of Connexin 37gene C1019T polymorphism

Figure 3. Funnel plot for studies of the association of coronary artery disease (CAD) and *connexin 37* C1019T gene polymorphism under an allelic genetic model (distribution of T allelic frequency of *connexin 37* C1019T gene polymorphism). The horizontal and vertical axis correspond to the OR and confidence limits. OR, odds ratio; SE, standard error.

contrast, Zhang SR *et al* and Zhang CL *et al* both reported different results showing that the T allele was a risk factor for CAD (9,11).

The number of meta-analyses on gene polymorphism and CAD increases annually (26-28). However, no meta-analysis on the relationship between *Cx37* C1019T gene polymorphism and CAD was retrieved. The current meta-analysis analyzed the independent studies on this subject comprehensively and quantitatively. The meta-analysis was used to analyze and summarize the collected research data using a statistical method and to provide a method to quantify the average effect to address the research question. The advantage of a meta-analysis involves its capacity to increase conclusion reliability and resolve inconsistency among study results by increasing the sample size. Therefore, the drawn conclusion should be more objective and reasonable than that of individual studies.

The present meta-analysis has a number of limitations. Sample non-uniformity in terms of gender, age and location existed among the individual studies. The case selection criteria and statistical methods differed among the independent studies. The illness phenotype was diverse. Large-scale studies on CAD and *Cx37* C1019T were inadequate. Impact factors, such as diet and lifestyle, genetic background as well as pharmaceutical intervention, were considered distinct and require further investigation.

In conclusion, the present meta-analysis suggested no association between the *Cx37* C1019T gene polymorphism and CAD susceptibility in the Chinese population. However, considering the above limitations, further studies are required to confirm these results.

## Acknowledgements

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