Association between the endothelial nitric oxide synthase gene Glu298Asp polymorphism and coronary heart disease: A meta-analysis of 39 case-control studies

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Abstract. Numerous studies have indicated that the human endothelial nitric oxide synthase (eNOS) gene Glu298Asp polymorphism is associated with coronary heart disease (CHD) susceptibility, however, their conclusions are inconsistent. The present meta-analysis aimed to evaluate the precise result by searching the PubMed database and using 39 case-control studies comprising 7489 cases and 7051 controls.Each study tested the association between the eNOS Glu298Asp polymorphism and CHD. A meta-analysis was then conducted using the Comprehensive Meta Analysis 2.2 software to calculate the pooled odds ratios (ORs) of five genetic models with 95% confidence intervals (CIs). Publication bias was also explored. The meta-analysis showed a significant association between the eNOS Glu298Asp polymorphism and CHD susceptibility for all the genetic models [Asp vs. Glu, OR 1.26, 95% CI 1.14-1.40, P<0.001; Asp/Asp vs. Glu/Glu, OR 1.58, 95% CI 1.23-2.02, P<0.001; Glu/Asp vs. Glu/Glu, OR 1.12, 95% CI 1.03-1.22, P=0.001; (Glu/Asp+Asp/Asp) vs. Glu/Glu, OR 1.17, 95% CI 1.07-1.27, P<0.001; Asp/Asp vs. (Glu/Glu+Glu/Asp), OR 1.59, 95% CI 1.25-2.03, P<0.001]. Subgroup and sensitivity analyses indicated that the result was robust. A weak publication bias was detected. The results indicated that the eNOS Glu298Asp polymorphism is a risk factor for developing CHD, particularly in the Asian population.

Introduction

Coronary heart disease (CHD) is one of the main public health issues worldwide (1) and also a major cause of morbidity and mortality in developed and developing societies (2). As the onset of 50% of all first coronary events is asymptomatic (3), the clinical task of finding the CHD risk factors, which are able to accurately identify high-risk individuals, is important in order to implement primary prevention therapy and lifestyle changes. CHD is a complex disease. Epidemiology studies have suggested that the etiology of CHD involves interactions of genetic and environmental factors (4). Genetic factors contribute approximately half of the variability of the major risk factors in the pathogenesis of CHD (5).

In the past decades, a number of linkages and candidate-gene studies have been performed to identify the genes characteristic of CHD. The Glu298Asp polymorphism of the human endothelial nitric oxide synthase (eNOS) gene, which is located on chromosome 7q35-q36 comprises 26 exons, spans 21 kb and encodes an mRNA of 4052 nucleotides (6), is thought to be one of the genes associated with CHD. A variant of the eNOS gene has been identified within exon 7; a G to T transversion at nucleotide position 894 of the eNOS cDNA, resulting in a change of Glu 298 (GAG) to Asp (GAT) (7). In 1998, Shimasaki *et al* (7) first reported that the eNOS gene Glu298Asp polymorphism appeared to be an independent risk factor for myocardial infarction (MI). Since then, numerous studies focusing on the association between the eNOS gene Glu298Asp polymorphism and CHD have been published (7,16-52). Of these, certain studies further confirmed this association, while others came to negative or even contrary conclusions.

In 2010, Li *et al* (8) performed a meta-analysis of 20 studies involving a non-Asian population and 3 studies involving an Asian population in order to detect the eNOS gene Glu298Asp polymorphism and risk of CHD. A significant association was found between the Asp (T) allele in the eNOS Glu298Asp (G894T) polymorphism and CHD in the non-Asian population, but not in the Asian population. In 2012, Zhang *et al* (9) performed a meta-analysis of 18 case-control studies to determine whether the eNOS Glu298Asp polymorphism was associated with an increased risk of CHD among Asian populations. The analysis conluded that the eNOS Glu298Asp polymorphism may play a significant role in the development of CHD among Asian populations. However, the meta-analysis included certain irrelevant studies and thus contained certain

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limitations. There have been 39 relevant studies published thus far.

For these reasons, a comprehensive meta-analysis was required to provide an updated result on the overall correlation between the eNOS Glu298Asp polymorphism and CHD. A comprehensive search was consequently conducted and the present meta-analysis was performed according to the proposed PRISMA (preferred reporting items for systematic reviews and meta-analyses) guidelines (10), in order to clarify whether an association existed between the eNOS gene Glu298Asp polymorphism and the risk of CHD.

Subgroup analyses were also performed between the Asian and non-Asian populations to investigate any ethnicity-specific effects, to quantify heterogeneity between the individual studies and to investigate the existence of any potential publication bias.

Materials and methods

Literature search. A comprehensive electronic search covering the period between 1st January, 1998 and 30th August, 2012 was conducted in the PubMed database to search for any studies attempting to detect an association between the eNOS Glu298Asp polymorphism and CHD. Published and unpublished studies were searched to ensure the analysis was as exhaustive as possible. Keywords were used with the Boolean operators 'OR' and 'AND' combined with the following MeSH or text words: ('endothelial nitric oxide synthase' OR 'eNOS') AND ('polymorphism' OR 'genetic' OR 'mutation' OR 'variant') AND ('myocardial infarction' OR 'myocardial infarct' OR 'coronary artery disease' OR 'coronary heart disease' OR 'CHD' OR 'ischemic heart disease' OR 'myocardial ischemia' OR 'angina' OR 'CAD'). No restrictions were imposed and the bibliographies of the included studies and previous meta-analysis were checked for other relevant publications. As this study ia a meta-analysis of primary studies, no specific ethical approval is required.

Study selection. The eligibility of all studies retrieved from the databases were independently evaluated by two authors according to the following criteria: i) The study must have a case-control study design; ii) the study must have investigated the association between the eNOS Glu298Asp polymorphism and CHD susceptibility; iii) only cases of CHD, without other diseases (e.g., diabetes mellitus), were considered, the controls must have been from a healthy population, and in the cases and controls, the diagnosis must have been based on angiographic or clinical criteria with clearly reported details; and iv) enough information must have been provided to calculate the odds ratios (ORs) and the relevant 95% confidence intervals (CIs). Disagreements between the two authors were resolved by discussion.

In addition, the following exclusion criteria were also used: i) no abstracts or reviews; ii) no studies in which the genotype frequencies were not reported; and iii) no repeated or overlapping publications. For studies with the same case series by the same authors or the same institution in a same period, the most recently published studies or the studies with the largest number of subjects were included.

Data extraction. The following data were extracted by two authors from all eligible publications: first author's last name, year of publication, ethnicity, age of included subjects, source of controls, matching criteria, number of cases and controls, number of different genotypes in cases and controls, Hardy-Weinberg equilibrium (HWE) and minor allele frequency in controls. Any disagreements were resolved by consensus. CHD was defined as myocardial infarction (MI), angina pectoris or other ischemic heart disease (IHD).

Statistical analysis. A pooled OR and 95% CI was computed for the risk allele using the Comprehensive Meta Analysis software (version 2.2; Biostat, Englewood, NJ, USA) (11) to generate forest plots, in order to determine whether there was a statistical association between cases and controls and to assess the heterogeneity of the included studies. The Hardy-Weinberg equilibrium (HWE) was tested by the Chi-square test at a significance level of $P<0.05$. Heterogeneity was evaluated using the Cochran's Q (12) and I^2 statistics, the I^2 statistic yielded results ranging from 0 to 100% (0-25%, no heterogeneity; 25-50%, moderate heterogeneity; 50-75%, large heterogeneity; and 75-100%, extreme heterogeneity) (13). If heterogeneity existed, the random effects model was used. If no heterogeneity existed, the random effects model was considered to have replaced the fixed effects model, therefore, all the analyses of present study used the random effects model.

In addition, the effect of a single study on the overall risk estimate was studied by the removal of each study in turn. To test the robustness of the main results, subgroup analysis was also conducted if significant heterogeneity was identified. Potential publication bias was assessed by a visual inspection of the funnel plots and by the Egger weighted regression ($P<0.05$ was considered to indicate a statistically significant difference) (14) and 'trim and fill' methods (15).

Results

Study identification. Of the 337 records initially identified, 38 articles involving 39 case-control studies were included in the present meta-analysis (7,16-52). A detailed flowchart of the selection process is shown in Fig. 1.

Study characteristics. The major characteristics of the 39 case-control studies are shown in Table I. All the information from the studies was available. The number of subjects ranged from 54 to 755 in the case group and from 43 to 624 in the control group, comprising a total of 7,489 cases and 7,051 controls. A total of 18 studies were conducted in the Asian population (7,16,20,21,23,25,26,33-37,41,42,45,48, 51, 52) and 21 in the non-Asian population (17-19,22,24,27-32,38-40, 43,44,46,47,49-50). In terms of the source of the controls, 15 were from hosptial-based (HB) studies (16,17,20,24,25,28, 30,34,35,37,41,43,45,47,51) and 24 were from population-based (PB) studies (7,18,19,21-23,26,27,29,31-33,36,38-40,42, 44,46,48-50,52). The genotyping methods included polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays (36 studies) and PCR-single strand conformation polymorphism (PCR-SSCP) assays (3 studies) (18,19). The genotype distributions in the controls of all studies were in accordance with the HWE.

Table I. Characteristics of the studies included in the meta-analysis.

First author, year	Ethnicity (country)	Source of controls	End points	Sample size		Genotype distribution						Genotyping method	HWE P-value
						Case			Control				
				Case	Control	Glu/Glu	Glu/Asp	Asp/Asp	Glu/Glu	Glu/Asp	Asp/Asp		
Yoshimura, 1998	Asian (Japan)	HB	CHD	113	100	89	23	1	91	9	0	PCR-RFLP	0.64
Shimasaki, 1998	Asian (Japan)	PB	MI	285	607	225	59	1	526	80	1	PCR-RFLP	0.25
Cai, 1999	Non-Asian (Australia)	HB	CHD	605	158	286	249	70	66	70	22	PCR-RFLP	0.62
Hingorani, 1999	Non-Asian (UK)	PB	CHD	298	138	120	71	107	66	58	14	PCR-SSCP	0.81
Poirier, 1999	Non-Asian (France)	PB	MI	368	421	163	156	49	148	219	54	PCR-SSCP	0.051
Poirier, 1999	Non-Asian (Ireland)	PB	MI	163	155	55	76	32	58	72	25	PCR-SSCP	0.74
Yoon, 2000	Asian (Korea)	HB	CHD	110	128	94	15	1	110	18	0	PCR-RFLP	0.39
Yoshimura, 2000	Asian (Japan)	PB	CHD	201	345	162	38	1	301	44	0	PCR-RFLP	0.21
Granath, 2001	Non-Asian (Australia)	PB	CHD	573	624	260	248	63	270	287	66	PCR-RFLP	0.42
Wang, 2001	Asian (China)	PB	CHD	218	218	178	38	2	177	38	3	PCR-RFLP	0.56
Colombo, 2002	Non-Asian (Italy)	HB	CHD	201	114	91	78	32	48	59	7	PCR-RFLP	0.07
Wei, 2002	Asian (China)	HB	CHD	106	108	84	19	3	98	10	0	PCR-RFLP	0.61
Colombo, 2003	Non-Asian (Italy)	PB	CHD	268	147	109	116	43	65	72	10	PCR-RFLP	0.09
Chang, 2003	Asian (Korea)	PB	CHD	108	112	80	19	3	108	10	0	PCR-RFLP	0.63
Agema, 2004	Non-Asian (The Netherlands)	PB	CHD	755	574	343	333	79	216	270	88	PCR-RFLP	0.81
Afrasyap, 2004	Non-Asian (Turkey)	HB	CHD	250	150	114	103	33	74	62	14	PCR-RFLP	0.85
Cam, 2005	Non-Asian (Turkey)	HB	CHD	115	83	44	37	34	57	24	2	PCR-RFLP	0.78
Kerkeni, 2006	Non-Asian (Tunisia)	PB	CHD	100	120	45	44	11	72	43	5	PCR-RFLP	0.65
Jaramillo, 2006	Non-Asian (Chile)	PB	CHD	112	72	73	31	8	51	20	1	PCR-RFLP	0.54
Ji, 2007	Asian (China)	PB	CHD	165	190	125	39	1	157	32	1	PCR-RFLP	0.64
Wang, 2007	Asian (China)	HB	CHD	58	43	25	21	12	21	15	7	PCR-RFLP	0.15
Kim, 2007	Asian (Korea)	HB	CHD	147	222	119	28	0	181	38	0	PCR-RFLP	0.16
Vasilakou, 2008	Non-Asian (Greece)	PB	CHD	209	161	109	85	15	76	74	11	PCR-RFLP	0.21
Mathew, 2008	Asian (India)	PB	CHD	100	100	72	26	2	79	18	3	PCR-RFLP	0.14
Zakrzewski-Jakubiak, 2008	Non-Asian (Canada)	PB	CHD	58	111	22	23	12	40	57	14	PCR-RFLP	0.36
Tamemoto, 2008	Asian (Japan)	HB	CHD	54	283	38	13	3	249	32	2	PCR-RFLP	0.39
Alp, 2009	Non-Asian (Turkey)	PB	CHD	146	122	76	59	11	71	40	11	PCR-RFLP	0.14
Angeline, 2010	Asian (India)	PB	MI	100	100	56	30	14	67	31	2	PCR-RFLP	0.46
Alkharfy, 2010	Asian (Saudi Arabia)	HB	CHD	142	145	65	67	10	98	40	7	PCR-RFLP	0.28
Bor-Kucukatay, 2010	Non-Asian (Turkey)	HB	CHD	83	74	48	28	7	50	21	3	PCR-RFLP	0.68
Velloso, 2010	Non-Asian (Brazil)	PB	CHD	100	103	49	47	4	36	51	16	PCR-RFLP	0.77
Isordia-Salas, 2010	Non-Asian (Mexico)	PB	MI	180	180	104	62	14	134	42	4	PCR-RFLP	0.74

Table I. Continued.

First author, year	Ethnicity (country)	Source of controls	End points	Sample size		Genotype distribution						Genotyping method	HWE P-value
						Case			Control				
				Case	Control	Glu/Glu	Glu/Asp	Asp/Asp	Glu/Glu	Glu/Asp	Asp/Asp		
Salimi, 2010	Asian (Iran)	HB	CHD	241	261	112	103	26	160	84	17	PCR-RFLP	0.20
Saini, 2011	Asian (India)	PB	CHD	60	50	45	15	0	44	6	0	PCR-RFLP	0.65
Motawi, 2011	Non-Asian (Egypt)	HB	CHD	100	50	46	34	20	19	28	3	PCR-RFLP	0.80
Rahimi, 2012	Asian (Iran)	HB	CHD	105	92	61	33	11	67	24	1	PCR-RFLP	0.47
da Costa Escobar Piccoli, 2012	Non-Asian (Brazil)	PB	CHD	135	115	58	52	18	62	44	7	PCR-RFLP	0.83
Rai, 2012	Asian (India)	PB	CHD	253	174	159	84	10	119	50	5	PCR-RFLP	0.93
Gad, 2012	Non-Asian (Egypt)	PB	MI	104	101	52	47	5	59	34	8	PCR-RFLP	0.33

HWE, Hardy-Weinberg equilibrium; HB, hospital-based; PB, population-based; CHD, coronary heart disease; MI, myocardial infarction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-SSCP, PCR-single strand conformation polymorphism.

Figure 1. Flowchart of the study selection process.

Meta-analyses. Table II shows the main results and the results of the heterogeneity test on the meta-analyses. Overall, substantial heterogeneity existed that all the genetic models used the random-effects models. A significant association was identified between the eNOS Glu298Asp polymorphism and CHD susceptibility for all the genetic models, with a lower Asp allele frequency shown in the cases than in the controls [Asp vs. Glu, OR 1.26, 95% CI 1.14-1.40, P<0.001, Fig. 2; Asp/Asp vs. Glu/Glu, OR 1.58, 95% CI 1.23-2.02, P<0.001; Glu/Asp vs. Glu/Glu, OR 1.12, 95% CI 1.03-1.22, P=0.001; (Glu/Asp+Asp/Asp) vs. Glu/Glu, OR 1.17, 95% CI 1.07-1.27, P<0.001; Asp/Asp vs. (Glu/Glu+Glu/Asp), OR 1.59, 95% CI 1.25-2.03, P<0.001].

In the stratified analysis, a marked and exact association was identified between the eNOS Glu298Asp polymorphism and CHD susceptibility in the Asian population, hospital-based, CHD and PCR-RFLP subgroups of all the genetic models; however, the results of the non-Asian population and population-based subgroups were inconsistent. In contrast, the MI and PCR-SSCP subgroups showed no association with the eNOS Glu298Asp polymorphism.

Sensitivity analysis. A sensitivity analysis was carried out by omitting each study included in the overall meta-analysis in turn. The results in any of the genetic models were not materially altered, which showed that the results were robust (Asp vs. Glu model, Fig. 3).

Publication bias. A funnel plot was created and the Egger's test was performed to assess any possible publication bias. The funnel plot for the genetic Asp vs. Glu model showed that a weak publication bias was detected (blank circles in Fig. 4) and the 'trim and fill' method estimated that there were 17 possible missing studies (black spots in Fig. 4). This finding was also confirmed by the results of the Egger's test [Asp vs. Glu, Asp/Asp vs. Glu/Glu, Glu/Asp vs. Glu/Glu and (Glu/Asp+Asp/Asp) vs. Glu/Glu, P<0.001; Asp/Asp vs. (Glu/Glu+Glu/Asp), P=0.001].

Table II. Pooled ORs and 95% CIs of the association between eNOS Glu298Asp polymorphism and CHD.

		Asp vs. Glu			Asp/Asp vs. Glu/Glu			Glu/Asp vs. Glu/Glu			(Glu/Asp+Asp/Asp) vs. Glu/Glu			Asp/Asp vs. (Glu/Glu+Glu/Asp)		
Total and subgroups	Studies	OR (95% CI)	P-value	I^2 (%)	OR (95% CI)	P-value	I^2 (%)	OR (95% CI)	P-value	I^2 (%)	OR (95% CI)	P-value	I^2 (%)	OR (95% CI)	P-value	I^2 (%)
Total	39	1.26 (1.14-1.40)	<0.001	64.73	1.58 (1.23-2.02)	<0.001	56.78	1.12 (1.03-1.22)	0.001	29.38	1.17 (1.07-1.27)	<0.001	37.77	1.59 (1.25-2.03)	<0.001	57.75
Ethnicity																
Asian	18	1.48 (1.32-1.65)	<0.001	16.74	2.15 (1.49-3.10)	<0.001	0	1.40 (1.24-1.59)	<0.001	0	1.43 (1.27-1.61)	<0.001	0	1.88 (1.31-2.69)	<0.001	0
Non-Asian	21	1.13 (1.00-1.26)	0.04	65.63	1.38 (1.04-1.84)	0.03	66.29	0.96 (0.89-1.05)	0.38	0	1.02 (0.94-1.11)	0.67	13.86	1.47 (1.10-1.97)	0.01	69.77
Source of control																
HB	15	1.43 (1.19-1.72)	<0.001	63	2.14 (1.40-3.27)	<0.001	46.34	1.40 (1.24-1.59)	0.02	31.5	1.29 (1.09-1.53)	<0.001	40.91	2.09 (1.38-3.16)	<0.001	45.96
PB	24	1.18 (1.06-1.32)	<0.001	61.84	1.34 (1.00-1.80)	0.05	57.75	0.96 (0.89-1.05)	0.38	24.88	1.11 (1.01-1.22)	0.04	29.73	1.38 (1.03-1.86)	0.03	60.82
End point																
CHD	33	1.26 (1.13-1.41)	<0.001	64.22	1.60 (1.21-2.12)	<0.001	57.47	1.11 (1.01-1.21)	0.03	23.74	1.16 (1.06-1.27)	<0.001	34.03	1.63 (1.23-2.16)	<0.001	59.75
MI	6	1.28 (0.99-1.65)	0.06	72.65	1.55 (0.83-2.88)	0.17	60.54	1.08 (0.91-1.52)	0.21	57.25	1.21 (0.95-1.55)	0.12	59.91	1.48 (0.85-2.57)	0.16	53.06
Genotyping type																
PCR-RFLP	36	1.28 (1.15-1.43)	<0.001	63.85	1.63 (1.24-2.15)	<0.001	55.67	1.15 (1.05-1.26)	<0.001	24.83	1.20 (1.09-1.31)	<0.001	37.47	1.60 (1.22-2.08)	<0.001	54.51
PCR-SSCP	3	1.13 (0.82-1.56)	0.46	79.99	1.38 (0.71-2.68)	0.34	77.36	0.86 (0.71-1.04)	0.12	0	0.97 (0.81-1.16)	0.75	10.28	1.61 (0.78-3.33)	0.19	82.78

eNOS, endothelial nitric oxide synthase; ORs, odds ratios; CIs, confidence intervals; HB, hospital-based; PB, population-based; CHD, coronary heart disease; MI, myocardial infarction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-SSCP, PCR-single strand conformation polymorphism.

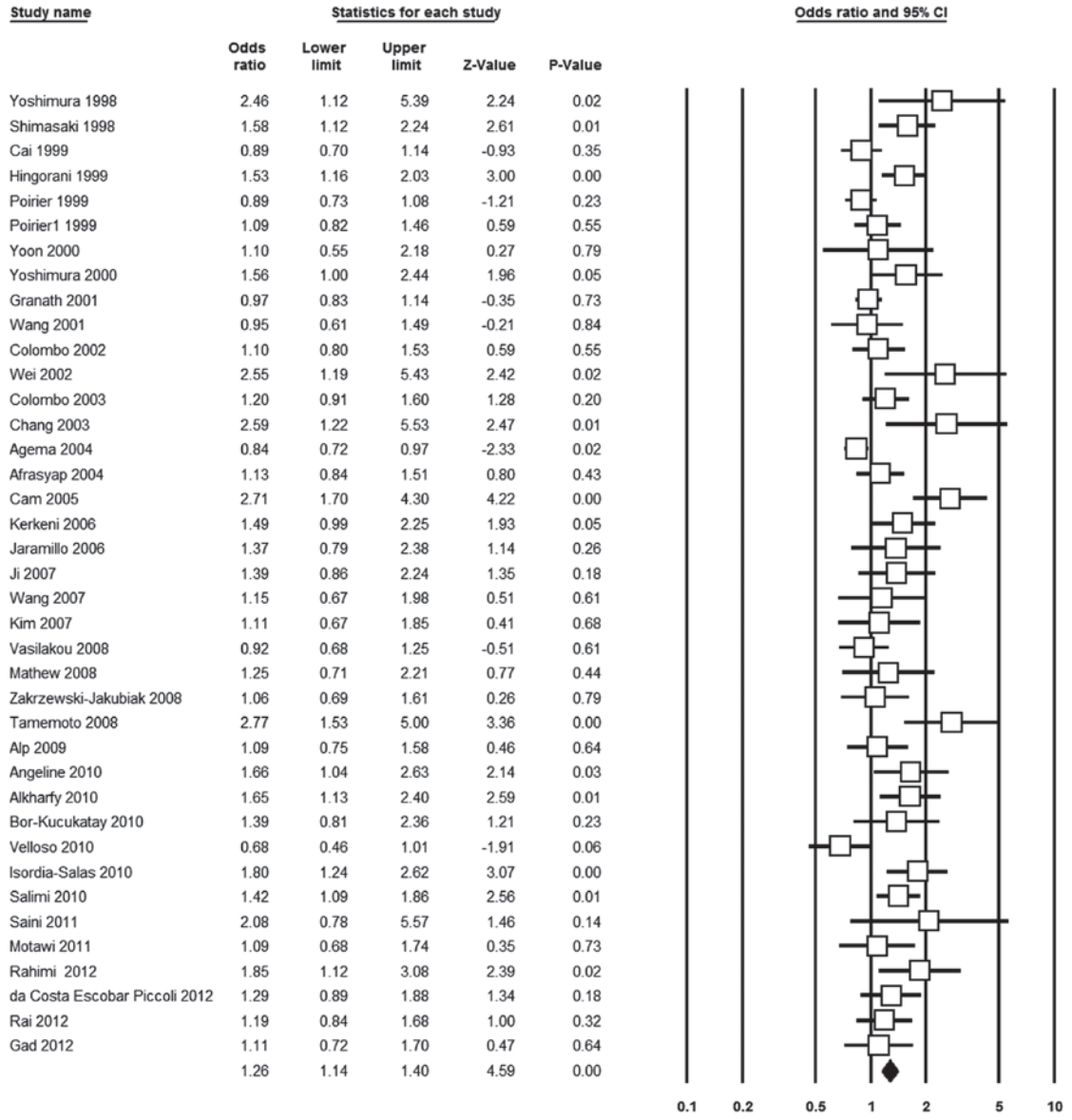

Figure 2. Pooled ORs and relevant 95% CIs comparing the Asp genotype with the Glu genotype, shown under a random-effects model. OR, odds ratio; CI, confidence interval.

Discussion

CHD often presents suddenly with little warning and easily results in mortality. Therefore, the early identification of asymptomatic but susceptible individuals is extremely important. However, traditional risk factors are inadequate for identifying these asymptomatic high-risk individuals. At present, genetic testing is a well-accepted method for the early identification of patients with subclinical CHD, as it is non-invasive, relatively simple and inexpensive when compared with non-invasive coronary imaging tools. Additionally, the genetic markers are fixed and require measuring only once in an individual's lifetime, they are able to guide therapy selection and they may be of use in family counseling (53). To identify the genetic markers of an increased CHD risk is a key aim and the eNOS gene Glu298Asp polymorphism is a novel solution.

The human eNOS gene is located on chromosome 7q35-q36 (6). A polymorphic variant of this gene in exon 7 characterized by a Glu298Asp (894G→T) polymorphism has been identified. Although the mechanism responsible for the eNOS Glu298Asp mutation that is related to endothelial dysfunction remains unknown, various studies (8,9,54-57) have determined the correlation of its genotypes and numerous associated diseases. A number of studies have identified a lower Asp allele frequency in CHD cases than in healthy controls, as the two alleles are considered to have codominant effects on eNOS levels. However, certain studies have generated results that disagreed with each other. This may be due to the studies including varying populations (e.g., varied race or country) sampling strategies or genotyping procedures or small sample sizes. The present meta-analysis was performed in all the populations and subgroup analyses were conducted according to these factors.

The present meta-analysis of 39 case-control studies revealed that the eNOS Glu298Asp polymorphic variant was associated with CHD susceptibility. The results also revealed

Study name	Point	Lower limit	Upper limit	Z-Value	P-Value
Yoshimura 1998	1.25	1.13	1.38	4.43	0.00
Shimasaki 1998	1.25	1.13	1.39	4.40	0.00
Cai 1999	1.28	1.16	1.42	4.75	0.00
Hingorani 1999	1.25	1.13	1.39	4.39	0.00
Poirier 1999	1.28	1.16	1.42	4.78	0.00
Poirier1 1999	1.27	1.15	1.41	4.57	0.00
Yoon 2000	1.27	1.15	1.40	4.58	0.00
Yoshimura 2000	1.26	1.14	1.39	4.43	0.00
Granath 2001	1.28	1.15	1.42	4.64	0.00
Wang 2001	1.27	1.15	1.41	4.65	0.00
Colombo 2002	1.27	1.15	1.41	4.57	0.00
Wei 2002	1.25	1.13	1.38	4.42	0.00
Colombo 2003	1.27	1.14	1.41	4.50	0.00
Chang 2003	1.25	1.13	1.38	4.42	0.00
Agema 2004	1.28	1.16	1.41	4.97	0.00
Afrasyap 2004	1.27	1.15	1.41	4.55	0.00
Cam 2005	1.23	1.12	1.36	4.31	0.00
Kerkeni 2006	1.26	1.14	1.39	4.44	0.00
Jaramillo 2006	1.26	1.14	1.40	4.50	0.00
Ji 2007	1.26	1.14	1.40	4.48	0.00
Wang 2007	1.27	1.14	1.40	4.56	0.00
Kim 2007	1.27	1.15	1.40	4.57	0.00
Vasilakou 2008	1.28	1.15	1.42	4.70	0.00
Mathew 2008	1.26	1.14	1.40	4.53	0.00
Zakrzewski-Jakubiak 2008	1.27	1.15	1.41	4.60	0.00
Tamemoto 2008	1.24	1.13	1.37	4.36	0.00
Alp 2009	1.27	1.15	1.41	4.58	0.00
Angeline 2010	1.26	1.13	1.39	4.42	0.00
Alkharfy 2010	1.25	1.13	1.39	4.39	0.00
Bor-Kucukatay 2010	1.26	1.14	1.40	4.49	0.00
Velloso 2010	1.28	1.16	1.42	4.91	0.00
Isordia-Salas 2010	1.25	1.13	1.38	4.36	0.00
Salimi 2010	1.26	1.14	1.39	4.42	0.00
Saini 2011	1.26	1.14	1.39	4.49	0.00
Motawi 2011	1.27	1.15	1.41	4.58	0.00
Rahimi 2012	1.25	1.13	1.38	4.40	0.00
da Costa Escobar Piccoli 2012	1.26	1.14	1.40	4.49	0.00
Rai 2012	1.27	1.14	1.40	4.52	0.00
Gad 2012	1.27	1.15	1.41	4.57	0.00
	1.26	1.14	1.40	4.59	0.00

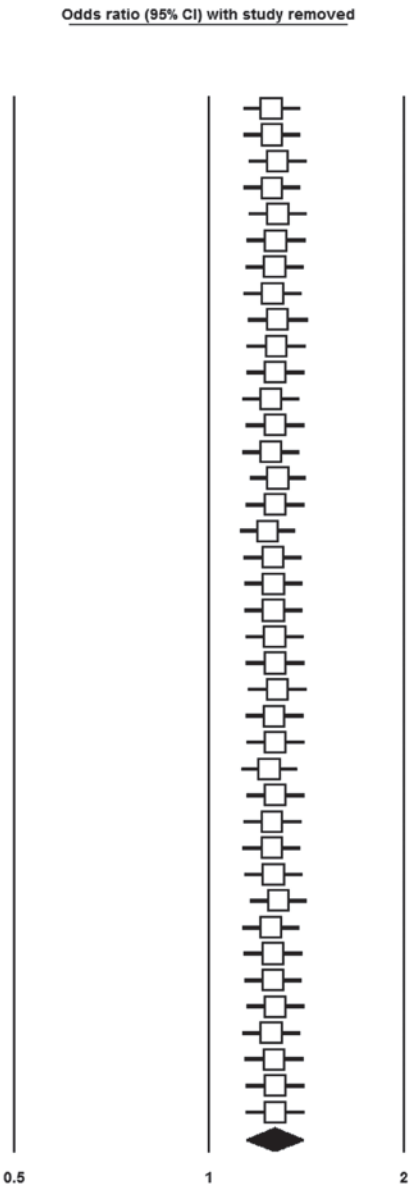

Figure 3. Forest plot of the sensitivity analysis performed by removing each study in turn (Asp vs. Glu). The pooled OR was represented by a diamond of standard height, with the width indicating the 95% CI. CI, confidence interval; OR, odds ratio.

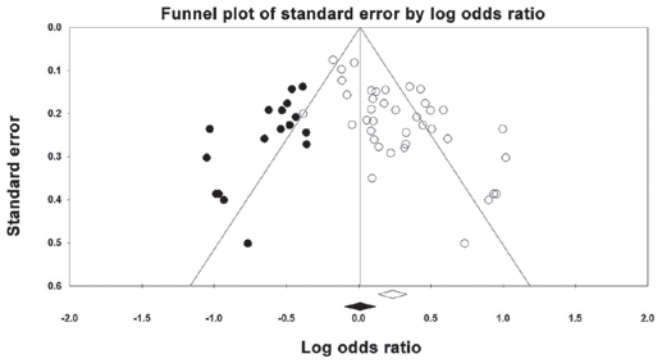

Figure 4. Filled funnel plot with pseudo-95% CIs of the results of the 39 studies (Asp vs. Glu). The log odds ratio (OR) represents the natural logarithm of the OR of individual studies; the standard error by the log OR represents the standard error in the natural logarithm of the OR of individual studies. A blank circle in the figure represents a study, while a black spot represents an unpublished study that would have to exist to negate the results of the meta-analysis. CI, confidence interval.

that a greater Asp allele frequency gave rise to an increased susceptibility to CHD, with the random effects ORs ranging from 1.12 to 1.59 for the five genetic models under investigation. The results remained statistically significant following sensitivity analyses that omitted one study at a time and confirmed the result was robust. In the subgroups analyses, the association depended on four variables: ethnicity, source of control, end point and genotying method. A strong association was identified between the eNOS Glu298Asp polymorphism and CHD in the Asian population, hospital-based, and CHD subgroups. The ORs and relevant 95% CIs of each subgroup were similar, which also confirmed that an association existed between the eNOS Glu298Asp polymorphism and CHD. However, the results of the non-Asian population and population-based analyses were inconsistent. In contrast, the the MI and PCR-SSCP subgroup showed no association with the eNOS Glu298Asp polymorphism.

Compared with a previous meta-analysis (8), the strength of the present meta-analysis was based on a more comprehensive search and the identification of a greater number of published studies, thereby providing more statistical power to detect the genetic effect estimates. A further sensitivity analysis confirmed that there was a significant association between the eNOS Glu298Asp polymorphism and the CHD risk among the Asian population. The results of the present study were also consistent with another previous meta-analysis and externally confirmed the validity of its results, which indicated that the association between the eNOS Glu298Asp polymorphism and CHD risk was significant among Asian populations (9).

There are certain limations to the present study. First, heterogeneity was detected in the present meta-analysis and although heterogeneity between studies is extremely common in the meta-analysis of genetic association studies, this should not be ignored. Heterogenity may be due to a number of reasons, including differences in the recruitment procedures of the study population, environmental backgrounds, genotyping methods or diagnostic criteria. Subgroup analyses were performed to assess these criteria, but the heterogeneity remained. Secondly, the sample sizes in the majority of the studies were relatively small. Compared with those studies with a large sample size, the studies with a small sample size may overestimate the true association, whereas a study with a large sample size may reflect a true association as it has greater statistical power. Thirdly, the present study performed a more comprehensive search, but a weak reporting bias was detected. Reporting bias is a significant threat to any literature-based review or meta-analysis. Lastly, the present results were based on unadjusted estimates and the lack of original data from the eligible studies limited the evaluation of the effects of the gene-gene and gene-environment interactions in CHD development. Undoubtedly, all these limitations may have affected the final conclusions.

The present meta-analysis may also provide certain suggestions for clinical practice and further research. Further research should clarify the association of other polymorphisms and their interactions with the eNOS Glu298Asp polymorphism and CHD. Clinically, the eNOS Glu298Asp polymorphism may be used as a basis to judge whether an Asian individual has a susceptibility to CHD or not and to provide more attention to those who are susceptible.

In conclusion, the results of the present study support the significant association of the eNOS Glu298Asp polymorphism and CHD susceptibility. The Asp allele was associated with an increased CHD susceptibility, while the Glu allele was associated with a decreased CHD susceptibility. However, large-scale studies with consideration of the gene-gene and gene-environment interactions should be conducted in the future to investigate this association.

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