ORIGINAL RESEARCH

Association between *GRK4* and *DRD1* gene polymorphisms and hypertension: a meta-analysis

He Zhang
Zhao-qing Sun
Shuang-shuang Liu
Li-na Yang

Department of Cardiology, Shengjing Hospital of China Medical University, Shenyang, People's Republic of China

Abstract: The role of *GRK4* and *DRD1* genes in hypertension remains controversial. We performed a meta-analysis to determine whether *GRK4* and *DRD1* polymorphisms influence the risk of hypertension and examined the relationship between the genetic variances and the etiology of hypertension. Relevant case-control studies were retrieved by database searches and selected according to established inclusion criteria. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the strength of the associations. Meta-regression, subgroup analysis, and sensitivity analysis were performed. A total of 15 articles containing 29 studies were finally included. In the dominant model, rs4532 locus of *DRD1* gene was related to hypertension with a pooled OR of 1.353 (95% CI =1.016–1.802, P=0.038). Subgroup analysis for ethnicity showed that rs1024323 locus of *GRK4* gene was associated with hypertension in Caucasians (OR =1.826, 95% CI =1.215–2.745, P=0.004) but not in East Asians and Africans. Rs4532 locus was associated with hypertension in East Asians (OR =1.833, 95% CI =1.415–2.376, $P<0.001$) but not in Caucasians. These data provide possible references for future case-control studies in hypertension.

Keywords: *GRK4*, *DRD1*, hypertension, meta-analysis, gene polymorphism

Introduction

Hypertension is a common complex disease affected by the mutual influence of multiple genetic and environmental factors.[1,2] It is a major risk factor for many disorders, such as renal failure, stroke, and cardiovascular disease,[3,4] and its heritability ranges from 30% to 50%.[5,6] However, the definite genetic background of hypertension is difficult to determine and remains inconclusive. Molecular genetics research has been attempting to ascertain the inherited susceptible genes of hypertension. Numerous efforts have been concentrated on the abnormal renal handling of sodium chloride metabolism in the pathogenesis of hypertension, considering that the kidney plays an important role in the long-term control of blood pressure and is the major organ involved in the regulation of sodium homeostasis.[7,8] Therefore, *GRK4* and *DRD1* genes have been assessed as the potential candidates.

*GRK*s are a seven-member family of serine/threonine protein kinases distinguished by their ability to specifically recognize, phosphorylate – and desensitize agonist-activated *GPCR*s.[9] The sodium retention in hypertension is on account of increased sodium transport and/or deficiency to respond appropriately to signs that decrease sodium transport.[10] The process of sodium transport is regulated by natriuretic and anti-natriuretic hormones, which exert their effects by *GPCR*s. Thus, *GRK*-mediated receptor phosphorylation is one of the well-distinguished mechanisms for *GPCR* desensitization. Interestingly, *GRK4*, different from the other *GRK*s in tissue distribution, is abundantly expressed in the kidney and seems to play a vital role in the regulation of

Correspondence: He Zhang
Department of Cardiology, Shengjing Hospital of China Medical University, 36 Sanhao Street, Heping District, Shenyang, 110004, People's Republic of China
Email zhanghe_77@163.com

sodium metabolism.[11] *GRK4* gene is located in chromosome 4 (4p16.3) and four splice variants (*GRK4*α, β, γ, δ) have been identified in humans.[12] An increasing number of studies show that *GRK4* gene is associated with hypertension and blood pressure in different ethnic populations.[13–16] Among them, rs2960306 (R65L), rs1024323 (A142V), and rs1801058 (A486V) polymorphisms have attracted the most attention.

GRK4 mainly exerts its function by *DRD1* and its impaired coupling of *DRD1* and its G-protein effector enzyme complex has been linked to the dopamine-mediated sodium dysregulation.[10] Likewise, *DRD1*-deficient mice develop hypertension.[17] Therefore, *DRD1* seems to be a risk factor in the development of hypertension, considering that dopaminergic and cholinergic neurotransmitters mainly regulate blood pressure by binding to their respective receptors.[18] Many efforts have been made to screen the latent pathogenic variations of *DRD1* gene. Among them, rs4532 (A48G), localizing the promoter region, was found to be associated with hypertension in Japanese and Chinese.[19,20] This single nucleotide polymorphism may affect the expression of *DRD1* by impacting micro RNA-mediated posttranscriptional regulation.[21]

Recently, many molecular epidemiological studies[22–31] were performed to investigate the association between the four aforementioned loci and hypertension. Unfortunately, the results were conflicting or inconsistent, most likely due to small sample size, diverse genetic backgrounds, and potential confounding bias. Meta-analysis is a widely used statistical method in medical study, particularly for a subject being extensively investigated while controversial results are being reported.[32] One meta-analysis was performed in 2012 to evaluate the association of three polymorphisms (rs2960306, rs1024323, and rs1801058) of *GRK4* gene with hypertension.[33] The pooled result showed that rs1801058 was associated with hypertension in East Asians and Europeans and there was a significant association between rs2960306 and hypertension among Europeans. However, Liu and Xi's meta-analysis only included five studies and the literature search was updated in September 2011. Additionally, new molecular epidemiological studies have recently been conducted to investigate the role of *GRK4* gene variations in the occurrence of hypertension in different populations and provide new evidences that were not included in the previous meta-analysis. Furthermore, the association of rs4532 polymorphism of *DRD1* gene in the disease has not been clarified in the meta-analysis. Consequently, we carried out a meta-analysis of studies examining these single nucleotide polymorphisms to provide a more comprehensive assessment of the association of *GRK4* and *DRD1* polymorphisms with hypertension.

Materials and methods
Identification and eligibility of relevant studies
To identify studies eligible for inclusion in this meta-analysis, five online electronic databases (PubMed, Embase, and Web of Science in English; and China National Knowledge Infrastructure and Wanfang Database in Chinese) were searched (the last search update was August 2015). The following keywords were used in the literature search: G-protein coupled receptor kinase 4, *GRK4*, dopamine receptor 1, *DRD1*, hypertension, and preeclampsia. Reference lists from retrieved articles and potentially relevant review articles were also hand-searched for additional studies. Studies met the following inclusion criteria: 1) case-control design, regardless of sample size, using hospital-based or population-based controls; 2) patients with hypertension; and 3) presented available allele or genotype frequencies. For duplicate publication, the most recent or largest articles were included. Study authors were contacted for additional details (eg, allele or genotype frequencies or sample characteristics) if we needed to retrieve additional data which were not stated in the original report.

Data extraction
Based on the inclusion criteria, two reviewers (He Zhang and Zhao-qing Sun) independently extracted the information from all qualified literatures. Disagreements were resolved through discussion until the two reviewers reached a consensus. The following data were extracted from each study: first author's last name, publication year, region, and counts of alleles and genotypes between cases and controls. To delineate potential moderating influences on the effects obtained from the case-control studies under consideration, we also included the following variables: 1) ethnicity of the sample population; 2) source of the controls; 3) mean age of the control and case group; 4) sex proportion; and 5) definition of hypertension and controls.

Quality assessment
Two authors (He Zhang and Shuang-shuang Liu) independently assessed the quality of the included studies according to the Newcastle–Ottawa Scale (www.ohri.ca/programs/clinical_epidemiology/oxfprd.asp). This scale consists of three components related to sample selection, comparability, and ascertainment of exposure. A score of 5 or more (maximum of 9) was regarded as "high quality"; studies with scores from 0 to 4 were considered "low quality".[34]

Statistical analysis

All statistical tests were two-sided, and $P<0.05$ was considered statistically significant. The meta-analysis was performed using Stata version 10.0 (Stata Corp LP, College Station, TX, USA). Hardy–Weinberg equilibrium (HWE) in the genotype distribution of controls was calculated again in our meta-analysis. The chi-square goodness of fit was used to test deviation from HWE.

The strength of the association between the target locus and hypertension was estimated by odds ratios (ORs) with 95% confidence intervals (CIs). Pooled effect sizes across studies were performed by a random effects model.[35] Overall pooled ORs were calculated using the allele contrast model, dominant model, and recessive model. Comparisons of OR_1 (AA vs aa), OR_2 (Aa vs aa) and OR_3 (AA vs Aa) were explored with A as the risk allele.[32] The aforementioned pairwise differences were used to determine the most appropriate genetic model. If $OR_1 = OR_3 \neq 1$ and $OR_2 =1$, then a recessive model was selected. If $OR_1 = OR_2 \neq 1$ and $OR_3 =1$, then a dominant model was selected. If $OR_2 =1/OR_3 \neq 1$ and $OR_1 =1$, then a complete overdominant model was selected. If $OR_1 > OR_2 >1$ and $OR_1 > OR_3 >1$ (or $OR_1 < OR_2 <1$ and $OR_1 < OR_3 <1$), then a codominant model was selected.[36]

The degree of heterogeneity was determined by Q-statistic, and P-value and I^2 were used to evaluate the heterogeneity among different studies.[37–39] Subgroup analysis was performed by ethnicity (East Asian, African, and Caucasian) and source of controls (hospital-based and population-based). Meta-regression was employed to explore the potential sources of heterogeneity including publication date, ethnicity, source of controls, mean age of control and case group, and sex. An estimate of publication bias was assessed by funnel plot and Egger's test.[40] Sensitivity analysis was also performed to weight the potential influences of every single study on the pooled effect size.[41]

Results

After the removal of overlapping articles and those that did not meet the inclusion criteria, a total of 15 articles including 29 studies were finally included in our meta-analysis.[19,20,22–31,42–44] The main characteristics of the included studies were presented in Table 1. For *GRK4* gene, seven studies with 1,704 cases and 1,705 controls dealt with rs1801058, six studies with 1,598 cases and 1,611 controls dealt with rs2960306, and nine studies with 1,973 cases and 1,946 controls dealt with rs1024323. For *DRD1* gene, seven studies with 2,083 cases and 1,383 controls dealt with rs4532. Of the total 29 studies, Sanada et al' study concerning rs1801058 and rs2960306 presented significant deviation from HWE.[19] In light of Newcastle–Ottawa Scale, eleven articles are high quality and four articles are low quality. Genotype and allele frequencies, HWE, and sample size information are described in Tables 2–5.

Quantitative synthesis and heterogeneity analysis

Association of rs1801058 locus (*GRK4* A486V) with hypertension

We analyzed seven studies with 1,704 cases and 1,705 controls dealt with the association between rs1801058

Table 1 Baseline characteristics of qualified studies in this meta-analysis

Author	Year	Country	Ethnicity	Controls source	Mean age of control group (years)	Mean age of case group (years)	Sex index	NOS scores
Sato et al[43]	2000	Japan	East Asians	Population-based	49.00	49.00	1.69	6
Bengra et al[22]	2002	Italy	Caucasians	Hospital-based	–	–	–	3
Yuan et al[42]	2002	People's Republic of China	East Asians	Hospital-based	56.48	57.50	0.74	5
Beige et al[30]	2004	Canada	Caucasians	Hospital-based	30.70	54.60	1.50	7
Speirs et al[24]	2004	Australia and UK	Caucasians	Population-based	47.00	54.00	1.99	8
Williams et al[23]	2004	Ghana	Africans	Hospital-based	–	–	–	5
Wang et al[25]	2006	People's Republic of China	East Asians	Population-based	53.51	53.57	1.01	6
Xu et al[20]	2006	People's Republic of China	East Asians	Hospital-based	49.28	50.19	0.88	6
Cao et al[28]	2007	People's Republic of China	East Asians	Hospital-based	–	–	–	3
Martinez Cantarin et al[26]	2010	USA	Africans	Population-based	36.00	40.00	0.61	9
Sun and Zhang[27]	2010	People's Republic of China	East Asians	Hospital-based	30.36	29.13	–	6
Orun et al[44]	2011	Turkey	Caucasians	Population-based	36.70	58.12	2.26	5
Cipolletta et al[31]	2012	Italy	Caucasians	Hospital-based	–	56.01	–	4
Kimura et al[29]	2012	Brazil	Africans	Population-based	32.00	55.70	1.14	4
Sanada et al[19]	2015	Japan	East Asians	Population-based	57.50	56.20	0.86	8

Note: Sex index = (female cases/male cases)/(female controls/male controls).
Abbreviation: NOS, Newcastle–Ottawa Scale.

Table 2 Distribution of genotype and allele frequencies of the *GRK4* rs1801058 (*GRK4* A486V) locus

Author	Genotype distribution						P_{HWE}	Allele frequency				Sample size	
	Cases, n			Controls, n				Cases, %		Controls, %		Case	Control
	GG	GT	TT	GG	GT	TT		G	T	G	T		
Bengra et al[22]	24	23	13	28	25	7	0.6966	59.2	40.8	67.5	32.5	60	60
Williams et al[23]	31	64	29	12	25	14	0.8972	50.8	49.2	48.0	52.0	124	51
Speirs et al[24]	57	77	26	117	166	29	0.0058	59.7	40.3	64.1	35.9	160	312
Wang et al[25]	400	97	6	372	109	9	0.7575	89.2	10.8	87.0	13.0	503	490
Martinez Cantarin et al[26]	49	90	26	60	115	31	0.0459	57.0	43.0	57.0	43.0	165	206
Sanada et al[19]	424	153	10	433	45	5	0.0036	85.3	14.7	94.3	5.7	587	483
Sun and Zhang[27]	31	63	11	35	53	15	0.4809	59.5	40.5	59.7	40.3	105	103

Note: P_{HWE} represents the *P*-value of Hardy–Weinberg equilibrium test in the genotype distribution of controls.

Table 3 Distribution of genotype and allele frequencies of the *GRK4* rs2960306 (*GRK4* R65L) locus

Author	Genotype distribution						P_{HWE}	Allele frequency				Sample size	
	Cases, n			Controls, n				Cases, %		Controls, %		Case	Control
	CC	CT	TT	CC	CT	TT		C	T	C	T		
Bengra et al[22]	27	22	11	28	25	7	0.6966	63.3	36.7	67.5	32.5	60	60
Williams et al[23]	12	62	51	8	23	20	0.7470	34.4	65.6	38.2	61.8	125	51
Speirs et al[24]	60	84	24	76	92	21	0.3818	60.7	39.3	64.6	35.4	160	312
Wang et al[25]	344	143	16	309	156	25	0.3621	82.6	17.4	79.0	21.0	503	490
Martinez Cantarin et al[26]	53	82	27	73	111	30	0.2359	58.0	42.0	60.0	40.0	162	214
Sanada et al[19]	361	204	23	393	79	12	0.0018	78.7	21.3	89.4	10.6	588	484

Note: P_{HWE} represents the *P*-value of Hardy–Weinberg equilibrium test in the genotype distribution of controls.

Table 4 Distribution of genotype and allele frequencies of the *GRK4* rs1024323 (*GRK4* A142V) locus

Author	Genotype distribution						P_{HWE}	Allele frequency				Sample size	
	Cases, n			Controls, n				Cases, %		Controls, %		Case	Control
	CC	CT	TT	CC	CT	TT		C	T	C	T		
Bengra et al[22]	15	29	16	25	26	9	0.6039	49.2	50.8	63.3	36.7	60	60
Williams et al[23]	99	21	4	42	9	0	0.4895	88.3	11.7	91.2	8.8	124	51
Speirs et al[24]	31	84	30	79	134	35	0.0683	50.3	49.7	58.9	41.1	145	248
Wang et al[25]	169	218	116	96	226	168	0.2055	55.3	44.7	42.7	57.3	503	490
Martinez Cantarin et al[26]	128	37	3	140	65	5	0.4248	87.2	12.8	82.1	17.9	168	210
Sanada et al[19]	145	286	157	181	227	77	0.6788	49.0	51.0	60.7	39.3	588	485
Sun and Zhang[27]	68	34	3	72	29	2	0.6376	81.0	19.1	84.0	16.0	105	103
Cao et al[28]	19	50	33	28	48	17	0.6510	43.1	56.9	55.9	44.1	102	93
Kimura et al[29]	98	72	8	117	72	17	0.2200	75.3	24.7	74.3	25.7	178	206

Note: P_{HWE} represents the *P*-value of Hardy–Weinberg equilibrium test in the genotype distribution of controls.

Table 5 Distribution of genotype and allele frequencies of the *DRD1* rs4532 locus

Author	Genotype distribution						P_{HWE}	Allele frequency				Sample size	
	Cases, n			Controls, n				Cases, %		Controls, %		Case	Control
	AA	GA	GG	AA	GA	GG		A	G	A	G		
Sanada et al[19]	6	100	482	8	107	368	0.9447	9.5	90.5	12.7	87.3	588	483
Beige et al[30]	188	253	52	78	107	24	0.1601	63.8	36.2	62.9	37.1	493	209
Cipolletta et al[31]	33	126	94	12	51	36	0.3464	37.9	62.1	37.9	62.1	253	100
Xu et al[20]	211	105	14	147	44	4	0.7410	79.9	20.2	86.7	13.3	330	195
Yuan et al[42]	111	68	8	115	38	3	0.9460	77.5	22.5	85.9	14.1	187	156
Sato et al[43]	93	35	3	113	23	0	0.2814	84.4	15.6	91.5	8.5	131	136
Orun et al[44]	47	36	18	46	44	14	0.5051	64.4	35.6	65.4	34.6	101	104

Note: P_{HWE} represents the *P*-value of Hardy–Weinberg equilibrium test in the genotype distribution of controls.

Table 6 Summarized ORs with 95% CIs for the association of *GRK4* and *DRD1* polymorphisms with hypertension

Polymorphism	Genetic model	n	Statistical model	OR	95% CI	P_z	I^2 (%)	P_h	P_e
Rs1801058 (A486V)									
	Allele contrast	7	Random	1.203	0.858–1.688	0.284	85.4	<0.001	0.886
	Homozygous codominant	7	Random	1.213	0.856–1.719	0.277	15.1	0.315	0.716
	Heterozygous codominant	7	Random	1.223	0.760–1.969	0.407	85.6	<0.001	0.848
	Dominant	7	Random	1.243	0.789–1.958	0.349	85.5	<0.001	0.864
	Recessive	7	Random	1.155	0.815–1.639	0.418	28.5	0.211	0.687
Rs2960306 (R65L)									
	Allele contrast	6	Random	1.219	0.851–1.747	0.279	87.2	<0.001	0.940
	Homozygous codominant	6	Random	1.280	0.858–1.911	0.226	39.7	0.141	0.430
	Heterozygous codominant	6	Random	1.278	0.768–2.126	0.344	87.3	<0.001	0.882
	Dominant	6	Random	1.304	0.793–2.145	0.295	88.0	<0.001	0.971
	Recessive	6	Random	1.144	0.858–1.525	0.360	8.3	0.363	0.504
Rs1024323 (A142V)									
	Allele contrast	9	Random	1.161	0.830–1.625	0.383	90.0	<0.001	0.620
	Homozygous codominant	9	Random	1.413	0.671–2.975	0.362	89.0	<0.001	0.609
	Heterozygous codominant	9	Random	1.120	0.796–1.577	0.515	77.7	<0.001	0.630
	Dominant	9	Random	1.190	0.785–1.805	0.413	86.7	<0.001	0.581
	Recessive	9	Random	1.257	0.745–2.123	0.391	82.3	<0.001	0.593
Rs4532									
	Allele contrast	7	Random	1.303	1.055–1.610	0.014	62.0	0.015	0.156
	Homozygous codominant	7	Random	1.271	0.886–1.824	0.192	8.3	0.365	0.002
	Heterozygous codominant	7	Random	1.287	0.976–1.696	0.073	44.8	0.093	0.947
	Dominant	7	Random	1.353	1.016–1.802	0.038	52.2	0.051	0.751
	Recessive	7	Random	1.282	1.039–1.582	0.021	0.0	0.445	0.289

Notes: n, the number of studies; P_z, P-value for association test; P_h, P-value for heterogeneity test; P_e, P-value for publication bias test.
Abbreviations: ORs, odds ratios; CIs, confidence intervals.

and hypertension. The dominant model was determined according to the principle of genetic model selection (Table 6, Figure 1).[36,45] The summary results indicated that there was no association between rs1801058 locus and the occurrence of hypertension. The pooled OR using random effects model was 1.243 (95% CI =0.789–1.958, *P*=0.349). Subgroup analysis for ethnicity indicated that the locus was not associated with hypertension among East Asians, Africans, and Caucasians (Table 7). Moreover, no association between rs1801058 locus and hypertension was
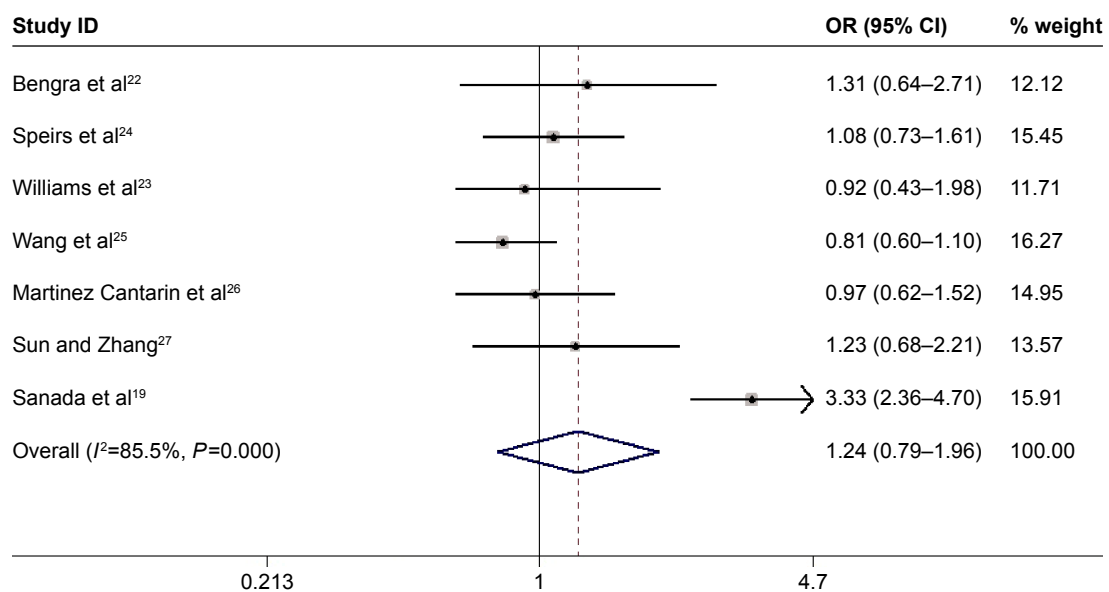

Study ID		OR (95% CI)	% weight
Bengra et al[22]		1.31 (0.64–2.71)	12.12
Speirs et al[24]		1.08 (0.73–1.61)	15.45
Williams et al[23]		0.92 (0.43–1.98)	11.71
Wang et al[25]		0.81 (0.60–1.10)	16.27
Martinez Cantarin et al[26]		0.97 (0.62–1.52)	14.95
Sun and Zhang[27]		1.23 (0.68–2.21)	13.57
Sanada et al[19]		3.33 (2.36–4.70)	15.91
Overall (I^2=85.5%, *P*=0.000)		1.24 (0.79–1.96)	100.00

0.213   1   4.7

Figure 1 Forest plot of the association between *GRK4* rs1801058 (*GRK4* A486V) locus and hypertension in dominant model (GT + TT vs GG).
Note: Weights are from random effects analysis.
Abbreviations: OR, odds ratio; CI, confidence interval.

Table 7 Stratified analysis for the association of *GRK4* and *DRD1* polymorphisms with hypertension under dominant model

Polymorphism	Subgroup type	Subgroup	n	OR	95% CI	P_z	I^2 (%)	P_h
Rs1801058 (A486V)								
	Ethnicity	Caucasians	2	1.133	0.800–1.604	0.481	0.0	0.650
		Africans	2	0.960	0.652–1.414	0.836	0.0	0.907
		East Asians	3	1.496	0.569–3.931	0.414	94.6	<0.001
	Source of controls	Hospital-based	3	1.162	0.786–1.718	0.451	0.0	0.782
		Population-based	4	1.302	0.663–2.557	0.443	92.6	<0.001
Rs2960306 (R65L)								
	Ethnicity	Caucasians	2	1.172	0.811–1.694	0.399	0.0	0.771
		Africans	2	1.158	0.780–1.719	0.466	0.0	0.354
		East Asians	2	1.462	0.435–4.911	0.539	97.5	<0.001
	Source of controls	Hospital-based	2	1.276	0.718–2.269	0.406	0.0	0.420
		Population-based	4	1.294	0.693–2.415	0.419	92.7	<0.001
Rs1024323 (A142V)								
	Ethnicity	Caucasians	2	1.826	1.215–2.745	0.004	0.0	0.636
		Africans	3	0.885	0.590–1.325	0.552	43.6	0.170
		East Asians	4	1.182	0.539–2.590	0.677	93.7	<0.001
	Source of controls	Hospital-based	4	1.546	1.092–2.190	0.014	0.0	0.600
		Population-based	5	1.000	0.556–1.800	0.999	92.5	<0.001
Rs4532								
	Ethnicity	Caucasians	3	0.946	0.726–1.233	0.684	0.0	0.981
		East Asians	4	1.833	1.415–2.376	<0.001	0.0	0.967
	Source of controls	Hospital-based	4	1.337	0.913–1.960	0.136	65.4	0.034
		Population-based	3	1.391	0.801–2.416	0.241	48.0	0.146

Notes: n, the number of studies; P_z, *P*-value for association test; P_h, *P*-value for heterogeneity test.
Abbreviations: OR, odds ratio; CI, confidence interval.

observed when subgroup analysis for source of controls was conducted. Significant heterogeneity was observed, thus a meta-regression was conducted subsequently to explore the heterogeneity sources. However, the results of meta-regression indicated that ethnicity (*P*=0.346), source of controls (*P*=0.776), age of the control group (*P*=0.285), age of the case group (*P*=0.200), and sex index (*P*=0.956) had no statistical significance except for publication date (*P*=0.020).

Association of rs2960306 locus (*GRK4* R65L) with hypertension

We analyzed six studies with 1,598 cases and 1,611 controls dealt with the association between rs2960306 and hypertension. The dominant model was determined according to the principle of genetic model selection (Table 6, Figure 2). The summary results indicated that there was no association between rs2960306 locus and the occurrence of hypertension. The pooled OR using random effects model was 1.304 (95% CI =0.793–2.145, *P*=0.295). Subgroup analysis for ethnicity indicated that the locus was not associated with hypertension among East Asians, Africans, and Caucasians (Table 7). Moreover, no association between rs2960306 locus and hypertension was observed when subgroup analysis for

source of controls was conducted. Significant heterogeneity was observed, thus a meta-regression was conducted subsequently to explore the heterogeneity sources. However, the results of meta-regression indicated that publication date (*P*=0.063), ethnicity (*P*=0.786), source of controls (*P*=0.963), age of the control group (*P*=0.469), age of the case group (*P*=0.236), and sex index (*P*=0.757) had no statistical significance.

Association of rs1024323 locus (*GRK4* A142V) with hypertension

We analyzed nine studies with 1,973 cases and 1,946 controls dealt with the association between rs1024323 and hypertension. The dominant model was determined according to the principle of genetic model selection (Table 6, Figure 3). The summary results indicated that there was no association between rs1024323 locus and the occurrence of hypertension. The pooled OR using random effects model was 1.190 (95% CI =0.785–1.805, *P*=0.413). Subgroup analysis for ethnicity indicated that the locus was associated with hypertension in Caucasians (OR =1.826, 95% CI =1.215–2.745, *P*=0.004) but not in East Asians and Africans (Table 7). Moreover, the association between rs1024323 locus and hypertension was observed when subgroup analysis for source of controls
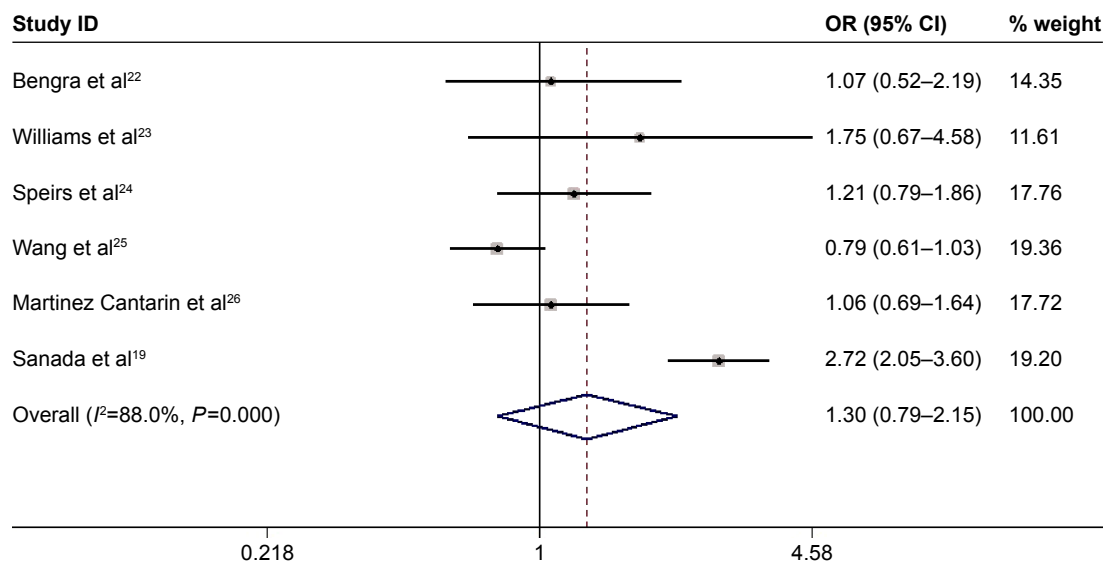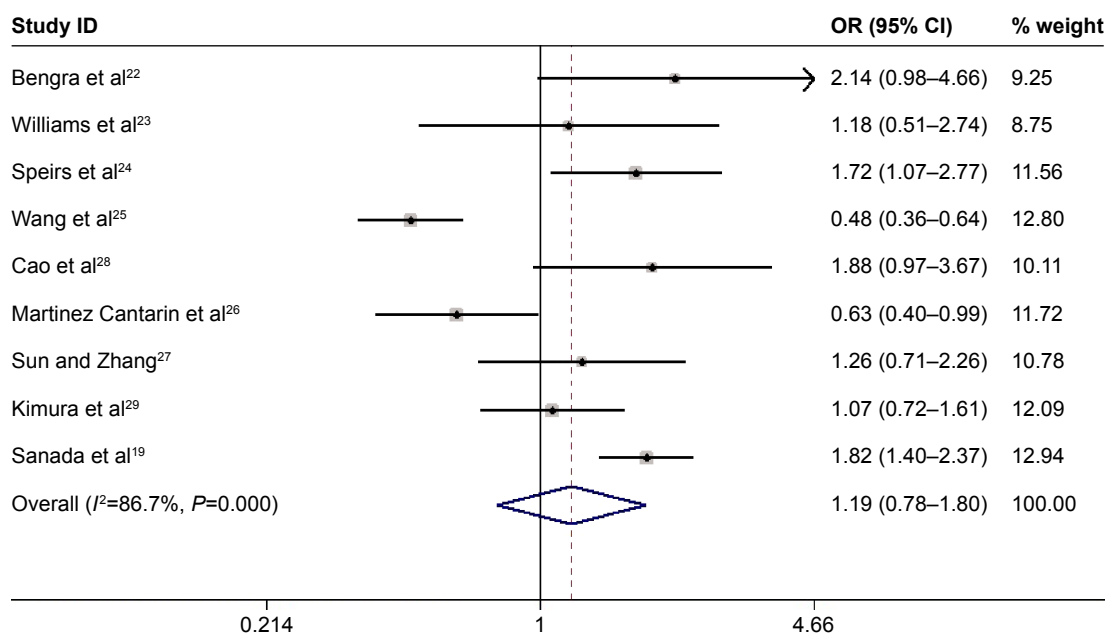
Figure 2 Forest plot of the association between *GRK4* rs2960306 (*GRK4* R65L) locus and hypertension in dominant model (CT + TT vs CC).
Note: Weights are from random effects analysis.
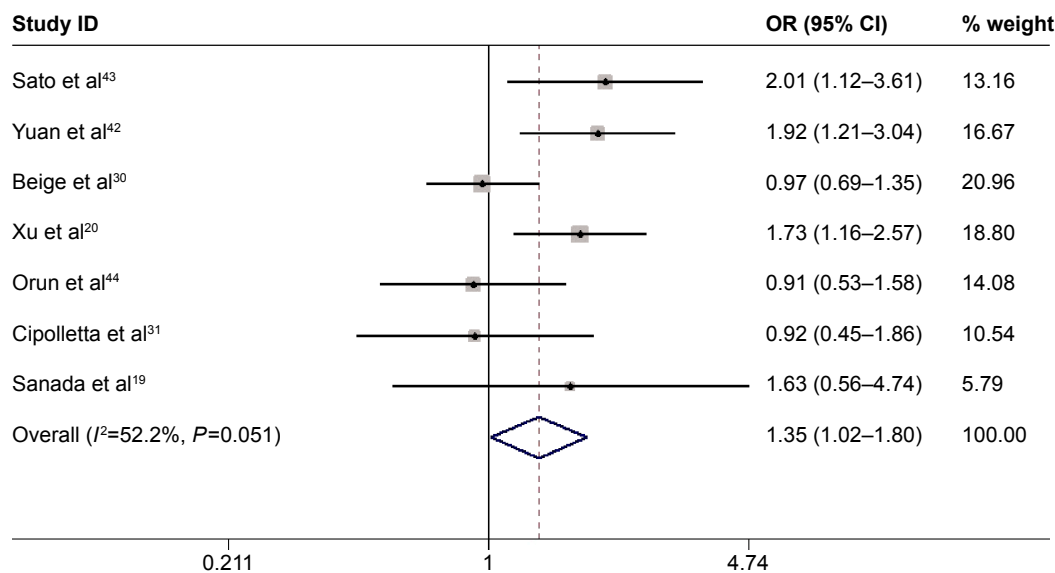**Abbreviations:** OR, odds ratio; CI, confidence interval.

was conducted (hospital-based subgroup: OR =1.546, 95% CI =1.092–2.190, P=0.014). Significant heterogeneity was observed, thus a meta-regression was conducted subsequently to explore the heterogeneity sources. However, the results of meta-regression indicated that publication date (P=0.913), ethnicity (P=0.640), source of controls (P=0.234), age of the control group (P=0.442), age of the case group (P=0.917), and sex index (P=0.674) had no statistical significance.

Association of rs4532 locus with hypertension

We analyzed seven studies with 2,083 cases and 1,383 controls dealt with the association between rs4532 and hypertension. The dominant model was determined according to the principle of genetic model selection (Table 6, Figure 4). The summary results indicated that there was an association between rs4532 locus and the occurrence of hypertension. The pooled OR using random effects model was 1.353

Figure 3 Forest plot of the association between *GRK4* rs1024323 (*GRK4* A142V) locus and hypertension in dominant model (CT + TT vs CC).
Note: Weights are from random effects analysis.
Abbreviations: OR, odds ratio; CI, confidence interval.

Study ID		OR (95% CI)	% weight
Sato et al[43]		2.01 (1.12–3.61)	13.16
Yuan et al[42]		1.92 (1.21–3.04)	16.67
Beige et al[30]		0.97 (0.69–1.35)	20.96
Xu et al[20]		1.73 (1.16–2.57)	18.80
Orun et al[44]		0.91 (0.53–1.58)	14.08
Cipolletta et al[31]		0.92 (0.45–1.86)	10.54
Sanada et al[19]		1.63 (0.56–4.74)	5.79
Overall (I^2=52.2%, P=0.051)		1.35 (1.02–1.80)	100.00

0.211   1   4.74

Figure 4 Forest plot of the association between *DRD1* rs4532 locus and hypertension in dominant model (GA + GG vs AA).
Note: Weights are from random effects analysis.
Abbreviations: OR, odds ratio; CI, confidence interval.

(95% CI =1.016–1.802, P=0.038). Subgroup analysis for ethnicity indicated that the locus was associated with hypertension in East Asians (OR =1.833, 95% CI =1.415–2.376, P<0.001) but not in Caucasians (Table 7). Moreover, no association between rs4532 locus and hypertension was observed when subgroup analysis for source of controls was conducted. Significant heterogeneity was observed, thus a meta-regression was conducted subsequently to explore the heterogeneity sources. However, the results of meta-regression indicated that publication date (P=0.185), source of controls (P=0.911), age of the case group (P=0.240), and sex index (P=0.082) had no statistical significance except for ethnicity (P<0.001) and age of the control group (P=0.002).

Sensitivity analysis

Sensitivity analysis was carried out for each meta-analysis to address the influence of each study. Corresponding pooled ORs showed no significant change when one study was omitted at a time from each meta-analysis, implying that the results were stable and reliable.

Publication bias

A funnel plot was used to analyze the potential publication bias (Figure 5). Egger's test was performed to provide the statistical evidence for funnel plot symmetry and the result did not show any evidence of publication bias.

Discussion

The present meta-analysis included 15 articles containing 29 studies that investigated the association between *GRK4*

and *DRD1* gene polymorphism and the occurrence of hypertension. Overall, our meta-analytical results provided evidences that rs1024323 (*GRK4* A142V) and rs4532 loci were associated with hypertension in Caucasians and East Asians, respectively. Subgroup analysis stratified by ethnicity and source of controls further explored the distribution disequilibrium of cases and controls. Sensitivity analysis and the examination of publication bias strengthened the validity of the results.

Previous studies reported the association between the polymorphisms of *GRK4* gene and hypertension.[13–16] Additionally, one meta-analysis has been done to investigate the association of *GRK4* polymorphisms with hypertension, which suggested that rs1801058 (*GRK4* A486V) and rs2960306 (*GRK4* R65L) loci were associated with hypertension.[33] However, our results observed that rs1024323 and rs4532, rather than rs1801058 and rs2960306, were related to hypertension. To some extent, the current meta-analysis possessed several advantages over the previous study with respect to the following points. First, we included the recent published studies concerned with the association between *GRK4* polymorphism and the occurrence of hypertension, which could provide more credibility for the final results. Second, besides stratified analyses by ethnicity and source of controls, we further performed meta-regression to assess potential sources of heterogeneity and study stability. Finally, for rs4532 locus of *DRD1* gene, it was the first time that its association with the occurrence of hypertension was evaluated. To some degree, our study could provide a more precise assessment
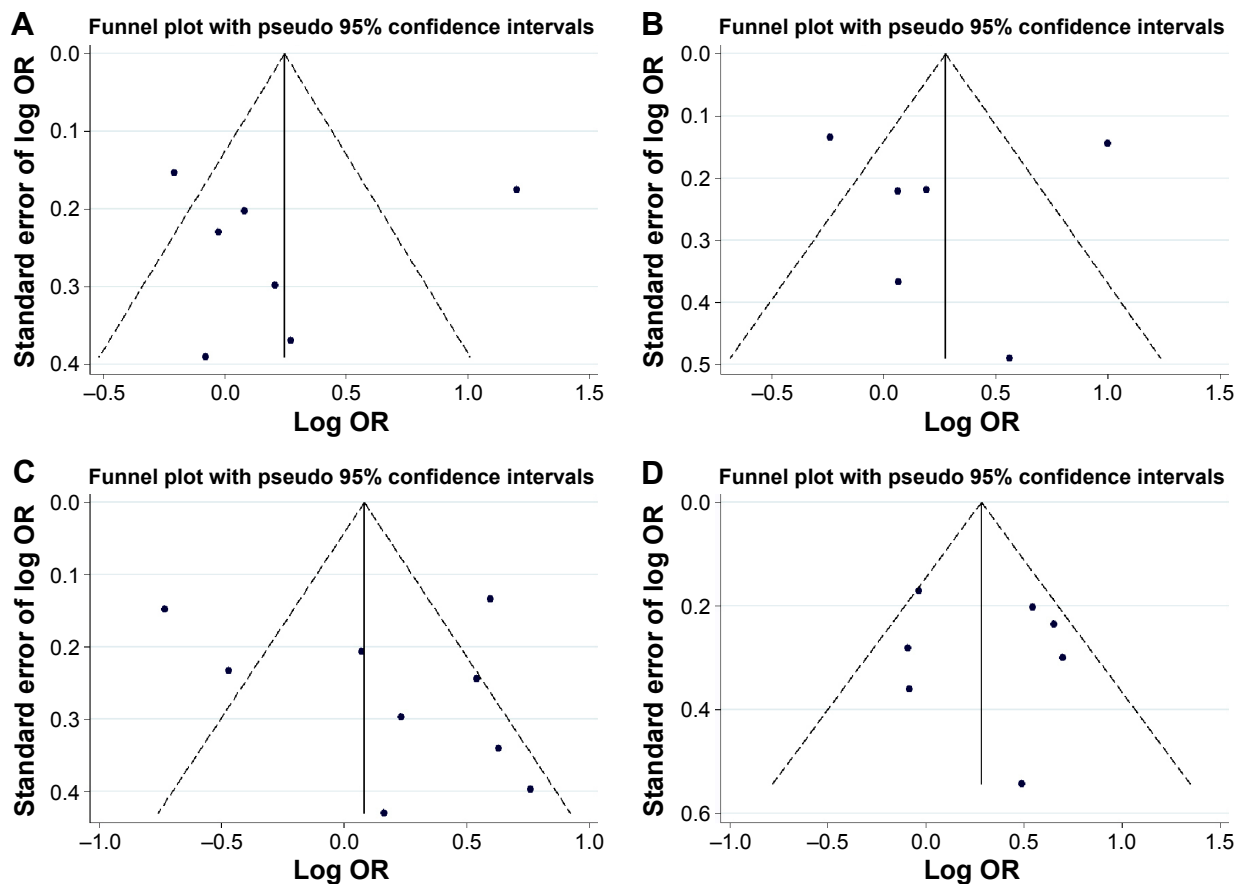
Figure 5 Funnel plot analysis on the detection of publication bias in the association between *GRK4* and *DRD1* gene polymorphisms and hypertension.
Notes: (**A**) For *GRK4* rs1801058 (*GRK4* A486V); (**B**) for *GRK4* rs2960306 (*GRK4* R65L); (**C**) for *GRK4* rs1024323 (*GRK4* A142V); (**D**) for *DRD1* rs4532.
Abbreviation: OR, odds ratio.

of the association between *GRK4* gene and hypertension on account of the aforementioned points.

Obviously, it is reasonable that the participation degree of the hereditary factor is distinct among various ethnic populations.[46] Actually, the frequency distributions of many loci are also not the same in different ethnicities. Thus, it could well be explained that rs1024323 polymorphism was associated with hypertension in Caucasians but not in East Asians and Africans, and the association between rs4532 and hypertension was observed in East Asians but not in Caucasians. Nevertheless, the flowing and mixing of the current population will complicate the future studies and raise the rebalance of blood pressure. The variant of rs1024323 is the allelic variant GCC to GTC, which results in the amino acid substitution of alanine to valine in residue 142. This change is related to a constitutive increase in GRK4 activity in proximal tubular cells.[47] In addition, the experimental cell and animal models implicate abnormalities in dopamine receptor regulation due to receptor desensitization resulting from increased *GRK4* activity, then decreased *DRD* activity and increased *AT1* receptor activity.[48–50] For rs4532, located

in the 5′ untranslated region of *DRD1* gene, the variant modulates the expression and stability of mRNA and influences *DRD1* gene expression by modifying the interaction between DNA binding domain and the regulatory elements.[51]

Limitations

There are several potential limitations in the present study. First, we did not assess the influence of incongruity among different definitions of hypertension patients and normotensive controls, mainly due to lacking sufficient original data. Second, rs1024323 polymorphism was associated with hypertension in Caucasians. However, there were only two studies in a Caucasian population, so the sample size was not large enough to draw meaningful conclusions, considering that small samples with limited participants are usually accompanied by selection bias.[52]

Conclusion

In summary, our results suggest that rs1024323 of *GRK4* and rs4532 of *DRD1* loci were associated with hypertension in Caucasians and East Asians, respectively. Large

sample epidemiological studies, especially in different ethnic populations, need to confirm the findings of our meta-analysis and investigate the latent gene–gene and/or gene–environment interactions between the susceptibility gene and hypertension.

Disclosure

The authors report no conflicts of interest in this work.

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