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Original Article

ACE I/D and 5-HTT STin2 VNTR polymorphisms in migraine: a meta-analysis

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Abstract: Both angiotensin-converting enzyme (ACE) and serotonin (5-HT) are known to be implicated in the pathogenesis of migraine, but data on the association between polymorphisms in the ACE gene and serotonin transporter (5-HTT) are inconsistent. The objective of this meta-analysis was to investigate the association between the ACE I/D and the 5-HTT STin variable-number tandem repeat (VNTR) polymorphisms and migraine. Relevant studies were identified using the PubMed and EMBASE databases. We used odds ratios (ORs) with 95% confidence intervals (CIs) to evaluate the effect of the ACE I/D and 5-HTT STin VNTR polymorphisms on migraine in a random-effects or fixed-effects model. We also performed subgroup analyses by ethnicity and migraine status. Nineteen studies were included in this meta-analysis (twelve on the ACE I/D polymorphism and seven on the 5-HTT ST in VNTR polymorphism). The overall results indicated a statistically significant association between the ACE I/D polymorphism and increased susceptibility to migraine with aura (MA) in heterozygote models (DI vs. II: OR = 1.16, 95% CI = 1.02-1.31), P = 0.02; I^2 = 17%) and dominant models (DD + DI vs. II: OR = 1.14, 95% CI = 1.02-1.28, P = 0.02; I^2 = 44%) and without aura (MO) in homozygote models (DD vs. II: OR = 1.13, 95% CI = 1.01-1.27, P = 0.03; I^2 = 11%), heterozygote models (DI vs. II: OR = 1.13, 95% CI = 1.02-1.24, P = 0.02; I^2 = 10%), and dominant models (DD + DI vs. II: OR = 1.12, 95% CI = 1.02-1.23, P = 0.02; I^2 = 17%). Subgroup analyses revealed that there is no increased migraine susceptibility observed among Asians. The STin2.12/12 genotype significantly increased the risk of developing MO (OR = 1.31, 95% CI = 1.03-1.66, P = 0.03; I^2 = 31%). Thus, this meta-analysis demonstrates that the ACE D-carrier genotypes are associated with increased risk of migraine with and without aura, although not among Asian populations. The 5-HTT STin2.12/12 genotype is a risk factor for MO.

Keywords: Migraine, SLC6A4, STin2, ACE I/D polymorphism, meta-analysis

Introduction

Migraine is a common, debilitating disorder characterized by recurrent headache attacks that typically last for 4-72 h and is often associated with nausea, vomiting, and/or photophobia and phonophobia [1]. It affects three times as many women as men, with a prevalence of 12% in the worldwide population [2]. Migraine is classified into two subtypes: migraine with aura (MA) and migraine without aura (MO). The former migraine attack follows the typical aura consisting of visual, sensory, and/or speech symptoms [1].

The pathogenesis of migraine is not fully understood. However, the trigeminovascular theory [3] is now widely accepted, and vascular mechanisms are obviously implicated [4]. Angiotensin-converting enzyme (ACE), dipeptidyl carboxypeptidase expressed in vascular endothelial cells, plays an important role in the vascular oxidative stress response [5]. That oxidative stress mediates the process of endothelial dysfunction partly explains the pathological vascular mechanism of migraine [5]. And ACE inhibitors play an improtant role in the proplylatic treatment of migraine [6]. Recent evidence also suggests that the serotonergic (serotonin, 5-HT) system affects migraine pathophysiology through changes in 5-HT metabolism and the processing of centralized 5-HT-mediated responses [7]. It is believed that low 5-HT levels activate the trigeminovascular nociceptive pathway, which leads to migraine attack. Selective 5-HT (5-HT1B/1D) receptor agonists can prove effective in migraine treatment [7]. 5-HT can be inactivated by presynaptic reup-

take mediated by a serotonin transporter protein [8]. Therefore, both ACE and serotonin transporters are implicated in migraine attack.

The ACE I/D polymorphism, located in intron 16, presents as an insertion (I) or a deletion (D) of the 287-bp Alu repeat sequence. This leads to three genotypes: D/D, I/D, and I/I. This polymorphism is correlated with serum ACE levels [9]. The human serotonin transporter is encoded by a single gene (SLC6A4, the solute carrier member 6, family 4) located on chromosome 17q11.1-17q12 [10]. VNTR, a polymorphism in intron 2 of the SLC6A4 gene, is a variable-number tandem repeat of a 17-bp sequence that has several alleles: STin2.7, STin2.9, STin2.10, STin2.11, and STin2.12 [11]. The biological function of this polymorphism is not well understood. However, the two major alleles of this polymorphism, STin2.10 (360 bp, 10 copies) and STin2.12 (390 bp, 12 copies), are related to transcriptional regulators [12]. A number of researchers have investigated the association between the ACE I/D or the STin2 VNTR polymorphism and migraine. Nevertheless, there is no consensus concerning the relationship. To conduct a more comprehensive analysis of the effects of the ACE I/D polymorphism and the STin2 VNTR polymorphism on migraine, we accumulated all available data to perform a meta-analysis.

Methods

Publication search

We searched for all studies related to the association between migraine and either ACE I/D or SLC6A4 STin2 polymorphisms using PubMed and EMBASE (updated to July 10, 2014). The following key words (text words and MESH terms) were used in our search strategies: angiotensin-converting enzyme (ace or angiotensin-converting enzyme or peptidyl-dipeptidase a or dipeptidyl carboxypeptidase) or serotonin transporter (serotonin or serotonin transporter or SLC6A4 or serotonin plasma membrane transporter proteins), combined with terms for genetic variations (gene or genetic variation or polymorphism or genetic polymorphism) and terms for headache and migraine (headache or headache disorders or migraine or migraine disorders). Only papers written in English were screened.

Selection criteria and data extraction

Studies for the meta-analysis were selected using the following criteria: 1) Authors must have investigated the association between ACE I/D and/or STin2 VNTR polymorphisms by using a population-based or hospital-based case-control study, with either a cross-sectional or cohort design; 2) Migraine must have been diagnosed according to the criteria of the International Headache Society (IHS); and 3) Studies must have provided either genotype data or allele frequencies, or sufficient data to calculate them [13]. When the same or overlapping populations were included in several publications, only the most recent or complete report was selected. When pertinent data were not included, we attempted to collect the necessary data directly from the authors.

The following information was extracted from eligible publications: the first author, publication year, country and race of the study population, control source and study design (population-based (PB) and hospital-based (HB)), study size, migraine status (all migraine, MA or MO), and allele and genotype frequencies. Ethnic subgroups were categorized as Caucasian or Asian. We calculated genotype frequencies where possible when they were not given in the original articles. Two investigators (Xinying Guan and Yuhan Yan) extracted data independently using these selection criteria and resolved any disagreements by consensus.

Statistical analysis

The odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to assess the strength of the association between the ACE I/D or the SLC6A4 STin2 VNTR polymorphisms and migraine. Compared to STin2.12 and STin2.10, other alleles of the STin2 VNTR (STin2.7, STin2.9, and STin2.11) are rare, and STin2.12 has a significantly stronger effect on transcriptional activity than STin2.10 [10]. We therefore grouped STin2.7, STin2.9, STin10, and STin2.11 together as 'other alleles'. The pooled ORs with 95% CIs were calculated in allele contrast and assuming one of three genetic models: the co-dominant model (homozygote model and heterozygote model), the dominant model, or the recessive model [14, 15].

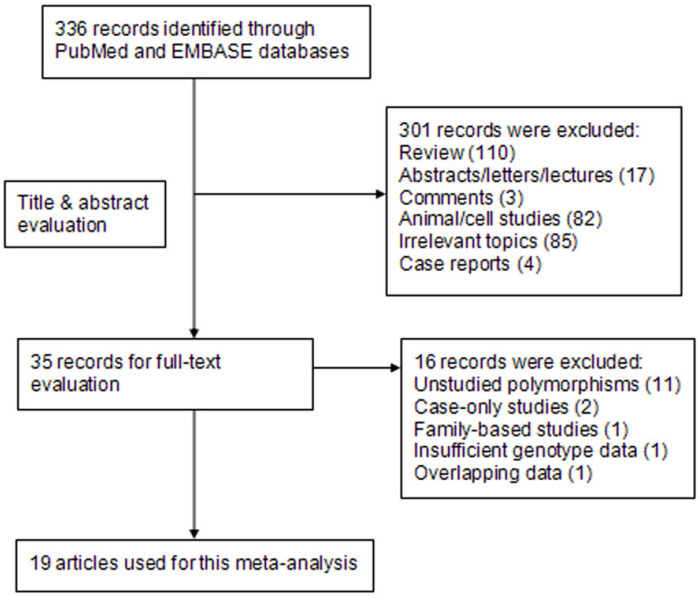

Figure 1. Flowchart of search procedure.

The heterogeneity assumption was calculated using the Chi-square (x^2) test based on the Q statistic. Quantification of heterogeneity was evaluated by the I^2 test (I^2 values of 25%: low, 50%: medium, 75%: high heterogeneity) [16]. If the I^2 values were not over 50%, suggesting a lack of heterogeneity among studies, the pooled ORs of all studies were calculated using the fixed-effect model (the Mantel-Haenszel method) [17]. Otherwise, we used the random-effect model (the DerSimonian and Laird method) [18]. The significance of the pooled ORs was evaluated by the Z-test.

We also used the goodness-of-fit x^2 test to evaluate Hardy-Weinberg equilibrium (HWE) for control subjects in each study. Subgroup analyses were stratified by ethnicity and migraine status. The potential publication bias was estimated by Begg's funnel plots and the Egger's linear regression test [19]. Sensitivity analysis was conducted by excluding a single study subsequently or by removing studies that deviated from HWE in controls and then by recalculating the ORs and 95% CIs to evaluate the stability of the results [15]. Statistical analysis was performed by Review Manager 5.2 (Oxford, England) and STATA 11.0 (STATA Corporation, College Station, TX, USA), and a $P<0.05$ was considered to be statistically significant with two-sided *P*-values.

Results

Study characteristics

We reviewed a total of 336 publications identified using keyword searches in the PubMed and EMBASE databases. After screening the title and abstract, we excluded 301 articles. For the remaining 35 studies, we evaluated the full articles, and nineteen studies were selected for this meta-analysis [20-38]. The procedure for identifying studies is shown in **Figure 1**.

The main characteristics of the 19 studies are summarized in **Table 1** based on the ACE I/D and the STin2 VNTR polymorphisms. Twelve studies [20-31], which involved 22,793 controls and 7,088 cases, investigated the association between the ACE I/D polymorphism and migraine. Seven studies [32-38], containing 1,166 controls and 897 cases, focused on the effect of the STin2 VNTR polymorphism on migraine. In studies of the ACE I/D polymorphism, eight were performed using Caucasian populations [20, 21, 24-26, 28, 29, 31], and four were performed using Asian populations [22, 23, 27, 30]. Nine studies included the results of MA [23-31], and nine others included the results of MO [20, 23-28, 30, 31]. In one study, the control group deviated from HWE in the controls [28]. In studies of the Stin2 VNTR polymorphism, five were performed using Caucasian populations [32-35, 37], and two were performed using Asian populations [36, 38]. Five studies included the results for MA [32, 33, 35, 37, 38], and five others included the results for MO [32, 33, 36-38]. The genotype frequency distributions of the STin2 VNTR polymorphism in control groups that deviated from HWE was equal to two in seven [33, 34].

Association between the ACE I/D polymorphism and all migraine, MA, and MO

The main results of this meta-analysis, heterogeneity, and publication bias tests, are shown in **Table 2**. We found a positive association between the ACE I/D polymorphism and all migraine in the allele contrast model (D vs. I: OR = 1.29, 95% CI = 1.02-1.64, P = 0.04), but

Table 1. Main characteristics of studies involved in this meta-analysis arranged by ACE I/D polymorphism and STin2 VNTR polymorphism

ACE I/D polymorphism							
First author (year)	Country	Ethnicity	Study Design	Gender	Study Size		HWE[1] (P)
					Control	Case	
Paterna (2000)	Italy	Caucasian	NS, case-control	mixed	201	302; MO[2]: 302	0.312
Cakmak (2003)	Turkey	Caucasian	NS, case-control	female/male	231	200	0.093
Lin (2005)	Taiwan	Asian	HB, case-control	female/male	200	240	0.156
Kowa (2005)	Japan	Asian	NS, case-control	mixed	248	176; MA[3]: 54, MO: 122	0.950
Lea (2005)	Australia	Caucasian	NS, case-control	mixed	244	250; MA: 151, MO: 99	0.811
Kara (2007)	Turkey	Caucasian	NS, case-control	mixed	210	180; MA: 59, MO: 109	0.121
Tronvik (2008)	Norway	Caucasian	HB, case-control	mixed	403	347; MA: 155, MO: 187	0.782
Joshi (2009)	India	Asian	HB, case-control	female/male	150	150; MA: 67, MO: 83	0.052
Schiruks (2010)	US	Caucasian	PB, cross-section	female	20424	4537; MA: 1270, MO: 1916	0.000
Horasanlı (2012)	Turkey	Caucasian	HB, case-control	mixed	22	53; MA: 53	0.869
An XK (2013)	China	Asian	HB, case-control	mixed	137	151; MA: 21, MO: 130	0.063
Palmirotta (2014)	Italy	Caucasian	HB, case-control	mixed	323	502; MA: 108, MO: 256	0.564
STin2 VNTR polymorphism							
First author (year)	Country	Ethnicity	Study Design	Gender	Study Size		HWE (P)
					Control	Case	
Ogilvie (1998)	Denmark	Caucasian	PB, case-control	mixed	133	267; MA: 94, MO: 173	0.326
Lea (2000)	Australia	Caucasian	PB, case-control	mixed	141	148; MA: 81, MO: 67	0.000
Yilmaz (2001)	Turkey	Caucasian	NS, case-control	mixed	79	37	0.010
Racchi (2004)	Italy	Caucasian	HB, case-control	mixed	32	44; MA: 44	0.480
Park (2006)	Korea	Asian	HB, case-control	female	100	97; MO: 97	0.677
Szilagyi (2006)	Hungary	Caucasian	PB, case-control	mixed	464	87; MA: 38, MO: 49	0.227
Guanjan (2010)	India	Asian	HB, case-control	mixed	217	217; MA: 84, MO: 133	0.208

[1]HWE: Hardy-Weinberg equilibrium. [2]MO: migraine without aura. [3]MA: migraine with aura.

not in any other genotype models. In the subgroup analysis of ethnicity, we found that the OR of the D allele was 1.46 (95% CI = 1.04-2.05; P = 0.03) in populations of Caucasian descent. However, we did not find an association among populations of Asian descent under any of the genetic comparisons.

We identified the overall effect of the ACE I/D polymorphism on increased risk of MA in allele contrast (D *vs.* I: OR = 1.45, 95% CI = 1.03-2.04, P = 0.03), the heterozygote model (DI *vs.* II: OR = 1.16, 95% CI = 1.02-1.31, P = 0.02), and the dominant model (DD + DI *vs.* II: OR = 1.14, 95% CI = 1.02-1.28, P = 0.02). After excluding studies involving Asian populations, the results for the allele contrast (D *vs.* I: OR = 1.59, 95% CI = 1.02-2.47, P = 0.04) and the heterozygote model (DI *vs.* II: OR = 1.14, 95% CI = 1.01-1.30, P = 0.04) suggested a significant role for the ACE I/D polymorphism in the development of MA among Caucasian populations. The ACE I/D polymorphism did not significantly affect MA development among Asian populations in any of the genetic models (**Table 2** and **Figure 2**).

Analysis of the nine pooled studies on the association between the ACE I/D polymorphism and MO suggested that both the DD and DI genotypes serve as minor risk factors for MO in the general population and in populations of Caucasian descent, but not in populations of Asian descent. Compared to the II genotype, the OR of the DD and DI genotypes using pooled studies was 1.13 (95% CI = 1.01-1.27, P = 0.03) and 1.13 (95% CI = 1.02-1.24, P = 0.02), respectively, for MO in the general populations, and was 1.14 (95% CI = 1.01-1.28, P = 0.03) and 1.14 (95% CI = 1.02-1.27, P = 0.02), respectively, in populations of Caucasian descent. Using the dominant model, we estimated the negative association between the II genotype and MO susceptibility among general populations (DD + DI *vs.* II: OR = 1.12, 95% CI = 1.02-1.23, P = 0.02) and Caucasian populations (OR = 1.14, 95% CI = 1.03-1.26, P = 0.01) (**Table 2**).

Association between the 5-HTT STin2 VNTR polymorphism and all migraine, MA, and MO

As shown in **Table 3**, there was no significant association between the 5-HTT STin2 VNTR

Table 2. Quantitative analysis of the association between the ACE I/D polymorphism and migraine

	Variable	Studies (n)	Fixed-effects Model		Random-effects Model		Heterogeneity (P value, I^2)	Publication bias (P)	
			OR (95% CI)	P	OR (95% CI)	P		Begg	Egger
Allele Contrast									
D vs. I	All	12	—		1.29 (1.02-1.64)	0.04	0.00; 93%	0.537	0.010
	Caucasian	8	—		1.46 (1.04-2.05)	0.03	0.00; 95%	—	—
	Asian	4	—		1.04 (0.82-1.33)	0.74	0.05; 61%	—	—
	MA	9	—		1.45 (1.03-2.04)	0.03	0.00; 91%	0.754	0.673
	Caucasian	6	—		1.59 (1.02-2.47)	0.04	0.00; 94%	—	—
	Asian	3	—		1.22 (0.69-2.15)	0.49	0.02; 75%	—	—
	MO	9	—		1.30 (0.97-1.74)	0.08	0.00; 93%	0.602	0.943
	Caucasian	6	—		1.48 (0.99-2.22)	0.06	0.00; 95%	—	—
	Asian	3	1.00 (0.82-1.23)	0.97	—		29%	—	—
Homozygote Model									
DD vs. II	All	12	1.05 (0.97-1.14)	0.19	—		0.02; 50%	0.436	0.120
	Caucasian	8	—		1.21(0.96-1.52)	0.10	0.05; 51%	—	—
	Asian	4	1.35 (0.94-1.92)	0.10	—		0.16; 46%	—	—
	MA	9	—		1.34 (0.96-1.87)	0.09	0.01; 59%	0.536	0.309
	Caucasian	6	1.08 (0.94-1.24)	0.29	—		0.25; 24%	—	—
	Asian	3	—		1.74 (0.57-5.34)	0.33	0.00; 83%	—	—
	MO	9	1.13 (1.01-1.27)	0.03	—		0.34; 11%	0.251	0.160
	Caucasian	6	1.14 (1.01-1.28)	0.03	—		0.26; 23%	—	—
	Asian	3	1.01 (0.66-1.56)	0.95	—		0.32; 13%	—	—
Heterozygote Model									
DI vs. II	All	12	1.07 (1.00-1.15)	0.05	—		0.08; 39%	0.640	0.718
	Caucasian	8	—		1.21 (0.97-1.51)	0.09	0.04; 53%	—	—
	Asian	4	1.03 (0.82-1.29)	0.80	—		0.37; 4%	—	—
	MA	9	1.16 (1.02-1.31)	0.02	—		0.30; 17%	0.536	0.793
	Caucasian	6	1.14 (1.01-1.30)	0.04	—		0.22; 29%	—	—
	Asian	3	1.30 (0.85-1.98)	0.22	—		0.33; 11%	—	—
	MO	9	1.13 (1.02-1.24)	0.02	—		0.36; 10%	0.602	0.781
	Caucasian	6	1.14 (1.02-1.27)	0.02	—		0.20; 32%	—	—
	Asian	3	1.03 (0.77-1.39)	0.83	—		0.55; 0%	—	—
Dominant Model									
DD+DI vs. II	All	12	1.06 (1.00-1.14)	0.07	—		0.03; 49%	0.755	0.687
	Caucasian	8	—		1.22 (0.98-1.51)	0.08	0.03; 56%	—	—
	Asian	4	1.05 (0.85-1.30)	0.65	—		0.13; 47%	—	—
	MA	9	1.14 (1.02-1.28)	0.02	—		0.07; 44%	0.536	0.652
	Caucasian	6	1.12 (0.99-1.26)	0.07	—		0.16; 37%	—	—
	Asian	3	—		1.30 (0.67-2.54)	0.44	0.07; 61%	—	—
	MO	9	1.12 (1.02-1.23)	0.02	—		0.29; 17%	1	0.434
	Caucasian	6	1.14 (1.03-1.26)	0.01	—		0.21; 30%	—	—
	Asian	3	1.03 (0.77-1.36)	0.86	—		0.36; 3%	—	—
Recessive Model									
DD vs. DI+II	All	12	1.01 (0.95-1.07)	0.78	—		0.15; 30%	0.016	0.194
	Caucasian	8	1.00 (0.94-1.07)	0.91	—		0.18; 31%	—	—
	Asian	4	1.12 (0.84-1.49)	0.44	—		0.16; 42%	—	—
	MA	9	1.02 (0.91-1.13)	0.75	—		0.06; 47%	0.118	0.270
	Caucasian	6	0.99 (0.89-1.11)	0.91	—		0.50; 0%	—	—
	Asian	3	—		1.60 (0.66-3.90)	0.30	0.08; 60%	—	—
	MO	9	1.05 (0.96-1.14)	0.25	—		0.56; 0%	0.035	0.218
	Caucasian	6	1.05 (0.96-1.15)	0.29	—		0.39; 5%	—	—
	Asian	3	1.00 (0.67-1.48)	0.99	—		0.48; 0%	—	—

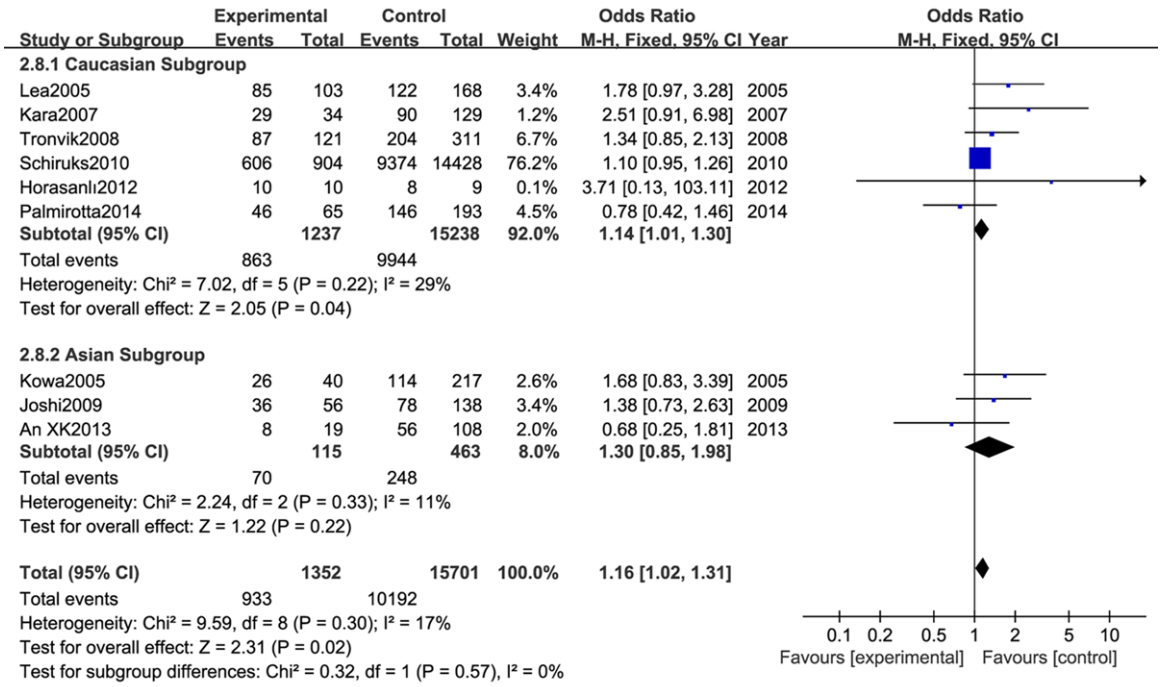

Figure 2. Forest plot of MA risk associated with the ACE I/D polymorphism (for DI vs. II), stratified by ethnicity.

polymorphism and all migraine in the total populations (for the allele contrast model, 12 *vs.* other: OR = 1.09, 95% CI = 0.84-1.40, P = 0.53; co-dominant model 12/12 *vs.* other/other: OR = 1.00, 95% CI = 0.74-1.33, P = 0.98; 12/other *vs.* other/other: OR = 0.90, 95% CI = 0.67-1.21, P = 0.49; dominant model 12/12 + 12/other *vs.* other/other: OR = 0.94, 95% CI = 0.72-1.23, P = 0.64; recessive model 12/12 *vs.* other/other + 12/other: OR = 1.14, 95% CI = 0.80-1.62, P = 0.46). In the stratified analysis of ethnicity, neither populations of Asian descent nor populations of Caucasian descent indicated an association between all migraine and the STin2 VNTR polymorphism.

In the allele contrast and recessive models, analysis of the STin2.12 allele and the 12/12 genotype suggests an increased risk trend for MA. However, the results were not statistically significant. In the subgroup analysis of ethnicity, similar results as above were observed in Caucasian populations (**Table 3**).

Similar to the results shown in the analysis of all migraine, MO risk was not correlated with the STin2 VNTR polymorphism in either the co-dominant model or the dominant model, while the STin2.12 allele indicated an increased risk trend for MO in the allele contrast that was not statistically significant. Compared to the carriers of the (12/other + other/other) genotypes, however, the 12/12 genotype carriers indicated a significantly higher disease risk (OR = 1.31, 95% CI = 1.03-1.66, P = 0.03, I^2 = 31%) for MO (**Figure 3**). There was no association between the STin2 VNTR polymorphism and MO when the data were stratified based on ethnicity (**Table 3**).

Publication bias

We performed Begg's funnel plots and an Egger's test to assess the publication bias across all studies. In the analyses of the association between the ACE I/D polymorphism and migraine, we did not detect asymmetry in the shapes of the Begg's funnel plots using the allele contrast and most genotype models (**Table 2** and **Figure 4**). However, asymmetry was detected using the recessive model (all migraine: P = 0.016, MO: P = 0.035). Statistical results from the Egger's test did not indicate a publication bias in the recessive model but did suggest bias in the allele contrast model for all migraine (P = 0.010). Similarly, the Begg's funnel plots did not indicate publication bias in the association between the 5-HTT STin2 VNTR

Table 3. Quantitative analysis of the association between the 5-HTT STin2 VNTR polymorphism and migraine

	Variable	Studies (n)	Fixed-effects Model		Random-effects Model		Heterogeneity (P value, I^2)	publication bias (P)	
			OR (95% CI)	P	OR(95% CI)	P		Begg	Egger
Allele Contrast									
12* vs. other #	All	7	—		1.09 (0.84-1.40)	0.53	0.01; 63%	0.764	0.582
	Caucasian	5	—		1.05 (0.78-1.42)	0.74	0.03; 63%	—	—
	Asian	2	—		1.34 (0.54-3.28)	0.53	0.02; 81%	—	—
	MA	5	1.09 (0.90-1.33)	0.36	—		0.25; 26%	0.462	0.209
	Caucasian	4	1.21 (0.96-1.51)	0.10	—		0.44; 0%	—	—
	Asian	1	0.83 (0.57-1.21)	0.33	—		—	—	—
	MO	5	1.14 (0.95-1.36)	0.15	—		0.2; 33%	1	0.695
	Caucasian	3	1.16 (0.93-1.45)	0.20	—		0.49; 0%	—	—
	Asian	2	—		1.37 (0.59-3.20)	0.46	0.04; 77%	—	—
Homozygote Model									
12/12 vs. other/other	All	7	1.00 (0.74-1.33)	0.98	—		0.10; 43%	0.368	0.725
	Caucasian	5	—		1.12 (0.64-1.94)	0.69	0.07; 54%	—	—
	Asian	2	0.71 (0.40-1.25)	0.23	—		0.74; 0%	—	—
	MA	5	1.08 (0.74-1.58)	0.69	—		0.18; 36%	0.086	0.015
	Caucasian	4	1.29 (0.83-2.01)	0.26	—		0.27; 24%	—	—
	Asian	1	0.63 (0.30-1.33)	0.23	—		—	—	—
	MO	5	1.05 (0.72-1.51)	0.81	—		0.67; 0%	1	0.647
	Caucasian	3	1.23 (0.78-1.94)	0.37	—		0.70; 0%	—	—
	Asian	2	0.75 (0.40-1.43)	0.38	—		0.77; 0%	—	—
Heterozygote Model									
12/other vs. other/other	All	7	0.90 (0.67-1.21)	0.49	—		0.13; 40%	0.133	0.241
	Caucasian	5	1.04 (0.74-1.46)	0.82	—		0.12; 45%	—	—
	Asian	2	0.58 (0.32-1.05)	0.07	—		0.81; 0%	—	—
	MA	5	—		0.99 (0.51-1.93)	0.99	0.04; 61%	0.086	0.063
	Caucasian	4	—		1.20 (0.50-2.87)	0.68	0.03; 68%	—	—
	Asian	1	0.61 (0.28-1.34)	0.22	—		—	—	—
	MO	5	0.82 (0.57-1.19)	0.31	—		0.75; 0%	0.806	0.802
	Caucasian	3	0.97 (0.62-1.52)	0.90	—		0.95; 0%	—	—
	Asian	2	0.56 (0.29-1.10)	0.09	—		0.82; 0%	—	—
Dominant Model									
12/12 + 12/other vs. other /other	All	7	0.94 (0.72-1.23)	0.64	—		0.10; 43%	0.368	0.271
	Caucasian	5	—		1.08 (0.66-1.78)	0.76	0.07;53%	—	—
	Asian	2	0.66 (0.38-1.13)	0.13	—		0.78; 0%	—	—
	MA	5	—		1.07 (0.60-1.90)	0.82	0.06; 56%	0.806	0.149
	Caucasian	4	—		1.30 (0.64-2.64)	0.47	0.06; 59%	—	—
	Asian	1	0.62 (0.30-1.27)	0.19	—		—	—	—
	MO	5	0.94 (0.67-1.32)	0.71	—		0.79; 0%	1	0.550
	Caucasian	3	1.08 (0.72-1.64)	0.70	—		0.96; 0%	—	—
	Asian	2	—		0.68 (0.37-1.25)	0.21	0.79; 0%	—	—
Recessive Model									
12/12 vs. 12/other + other/other	All	7	—		1.14 (0.80-1.62)	0.46	0.01; 63%	0.368	0.234
	Caucasian	5	—		1.02 (0.65-1.61)	0.92	0.02; 66%	—	—
	Asian	2	—		1.52 (0.61-3.76)	0.37	0.04; 77%	—	—
	MA	5	1.21 (0.92-1.58)	0.17	—		0.31; 16%	0.806	0.137
	Caucasian	4	1.36 (0.99-1.86)	0.06	—		0.40; 0%	—	—
	Asian	1	0.90 (0.54-1.49)	0.68	—		—	—	—
	MO	5	1.31 (1.03-1.66)	0.03	—		0.21; 31%	1	0.875
	Caucasian	3	1.28 (0.94-1.75)	0.12	—		0.31; 16%	—	—
	Asian	2	—		1.58 (0.69-3.62)	0.28	0.06; 71%	—	—

*12: The STin2 VNTR STin2.12 allele. #Other: STin2.7, STin2.9, STin10 and STin2.11.

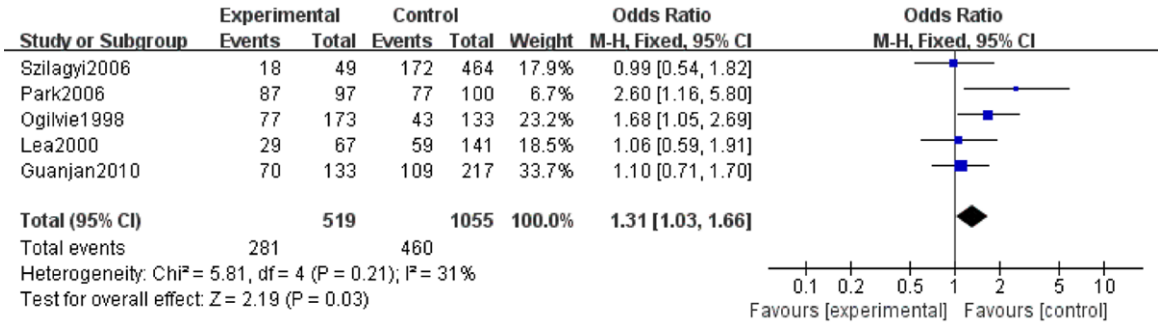

Figure 3. Forest plot of MO risk associated with the 5-HTT STin2 VNTR polymorphism (for 12/12 vs. 12/other + other/other).

polymorphism and migraine, while the Egger's test indicated publication bias in the homozygote model for MA (12/12 *vs.* other/other in MA: P = 0.015) (**Table 3**).

Sensitivity analyses

Sensitivity analyses were performed by removing sequentially single studies or by excluding studies that deviated from HWE in controls, to reflect the stability of the overall ORs.

For the analyses of the association between the ACE I/D polymorphism and all migraine, MA, and MO, the between-study heterogeneity was significantly high for the allele contrast model. Kara's study [25] was an important source of heterogeneity. After excluding Kara's study [25] using the largest OR value, there was a decrease in heterogeneity, and the positive association with all migraine and MA disappeared, while the association between the D allele and MO remained insignificant. When removing Schurks' study [27], which deviated from HWE in controls, the heterogeneity did not change, but the positive association of the allele contrast model in all migraine (D *vs.* I: OR = 1.34, 95% CI = 0.97-1.86, P = 0.07) and MA (D *vs.* I: OR = 1.54, 95% CI = 0.97-2.45, P = 0.07) disappeared. For sensitivity analyses using the other genotype model, the pooled ORs were unchanged or slightly changed. When removing Kara's study [25], the OR for MO in the DD *vs.* II model was 1.11 (0.99-1.24). Excluding Schurks' study [28], which deviated from HWE in controls, the ORs when using the DD *vs.* II genetic model were 1.21 (1.02-1.44) for all migraine and 1.25 (0.99-1.56) for MO. When using the DI vs. II genetic model and the DD + DI *vs.* II genetic model, the ORs were 1.18 (0.98-1.43) and 1.19 (0.99-1.42), respectively, for MO. In the subgroup analysis of ethnicity, the OR for all migraine in the DD *vs.* II model was 1.25 (1.02-1.54), and the OR for MA in the DD + DI *vs.* II genetic model was 1.35 (1.01-1.79) in Caucasian populations, which were only slightly changed. However, the results still indicated a statistically significant association between the ACE I/D polymorphism and MA in heterozygote models (DI *vs.* II: OR = 1.37, 95% CI = 1.02-1.85, P = 0.04; I^2 = 28%), together with MO in homozygote models (DD *vs.* II: OR = 1.35, 95% CI = 1.03-1.76, p = 0.03; I^2 = 16%), heterozygote models (DI *vs.* II: OR = 1.30, 95% CI = 1.01-1.66, P = 0.04; I^2 = 36%), and dominant models (DD + DI *vs.* II: OR = 1.31, 95% CI = 1.04-1.66, P = 0.02; I^2 = 31%) in Caucasian populations after removing Schurks' study [28], which deviated from HWE in controls.

Sensitivity analyses on the association between the 5-HTT STin2 VNTR polymorphism and all migraine did not alter our results, except when analyzing the effect for all migraine in the recessive model. The pooled ORs for all migraine were 1.27 (1.04-1.56) and 1.33 (1.06-1.67) in the recessive model after removing Yilmaz's study [33], with the largest OR value, and the study that deviated from HWE in controls, respectively.

Discussion

The present meta-analysis, which contained twelve published papers with 22,793 controls and 7,088 cases, found that D-carriers (DD + DI) were significantly associated with MA and MO in the dominant model. When compared with the II genotype, the DI genotype was more pronounced in MA and MO, while the DD genotype was more pronounced in MO. Taken together, our data suggest that there is statisti-

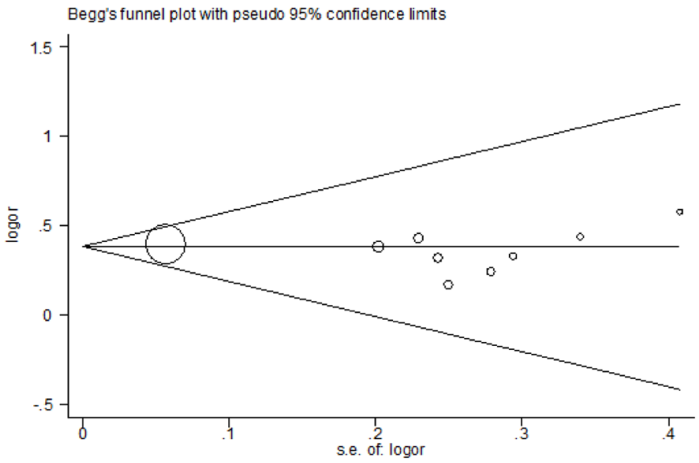

Figure 4. Funnel plot of publication bias on the association between ACE I/D polymorphism and MO (DD + DI vs. II: P = 0.434).

cally significant positive association between the ACE I/D polymorphism and MA susceptibility in the heterozygote and dominant models, and a positive association between the ACE I/D polymorphism and MO susceptibility in the homozygote, heterozygote, and dominant models.

Previous meta-analysis conducted by Schurks *et al.* (nine published papers were included) suggested that the II genotype is associated with a reduced risk for MA and MO in recessive model (II *vs*. DI + DD) and additive model (ID *vs*. DD) [39]. Moreover, the correlations were only identified in non-Caucasian populations [39]. The results of our meta-analysis further supports that the II genotype serve as a protective factor for MA and MO in Heterozygote model (DI *vs*. II) and dominant model (DD + DI *vs*. II), however, the effect of the ACE I/D polymorphism on increased risk of MA and MO in Homozygote model (DD *vs*. II) was only identified in our meta-analysis. Moreover, our results suggest the significant role for the ACE I/D polymorphism in the development of MA and MO is among Caucasian populations but not among non-Caucasian populations. The different results in the two meta-analyses might be due to more studies included and larger sample size in our meta-analysis.

The underlying mechanism of the ACE I/D polymorphism on migraine is still not well understood. It has been postulated that the difference in ACE activity and ACE level resulting from I/D polymorphism could lead to altered vascular oxidative stress and endothelial dysfunction [4, 5]. Previous functional studies have shown that ACE activity is significantly higher in MA and in MO than controls [40] And those with the ACE DD genotype had higher levels of von Willebrand factor activity, a marker of endothelial dysfunction, compared with the DI and II genotypes [41]. Additionally, expression studies have indicated that serum ACE levels are higher in subjects with the DD genotype than in those with the II genotype, and serum ACE may be at intermediate levels in subjects with the DI genotype [23, 25]. These data are consistent with what we found in our meta-analysis and may partly explain the finding that the II genotype plays a protective role in associating with MA and MO in the heterozygote (DI *vs*. II) and dominant models (DD + DI *vs*. II), while associating with MO in the homozygote (DD *vs*. II) model. However, it is difficult to explain why there is no association with MA in the homozygote model. Therefore, these results will require further confirmation in independent cohorts. On the other hand, ACE I/D polymorphism is thought to account for only half the variation in ACE activity [9] and tremendous other variation of the ACE gene may also influence ACE activity and serum ACE level.

Our meta-analysis of the association between the 5-HTT STin2 VNTR polymorphism and migraine was based on seven case-control studies with 897 migraine cases and 1,166 controls and produced interesting results. Although the general analyses of the STin2 VNTR polymorphism did not indicate a significant relationship with all migraine, in the stratified analyses of migraine status, the STin2.12 and STin2.12/12 genotypes showed an enhanced trend for MA and MO susceptibility. Most notably, the STin2.12/12 genotype significantly increased the risk of developing MO.

Previous studies have shown that circulating serotonin levels and metabolism change during onset of a migraine attack [7, 42]. Plasma 5-HT levels decrease [43], and platelets capacity for 5-HT reuptake, mediated by the serotonin transporter protein (5-HTT) [8], decreases during migraine attacks [44]. VNTR, a polymor-

phism of the SLC6A4 gene that encodes 5-HTT, has been indicated in a number of studies as having an association with migraine. Because it is difficult to draw a definitive and reliable conclusion from an independent study, several meta-analyses have been conducted. For example, Schurks *et al.* performed a meta-analysis of association based on five datasets (no Asians, 849 controls, 557 any migraine, 257 with MA, and 289 with MO) and found that the STin2.12/12 genotype is significantly associated with any migraine, especially among populations of European descent [45]. In a separate meta-analysis of four studies (no stratified analysis of ethnicity, 729 controls, 495 cases, 352 MA, 628 MO), Liu *et al*. concluded that the STin2.12 allele or 12/12 genotype was associated with a significantly increased risk factor for all migraine, MA and MO [46]. Our meta-analysis, based on data from seven studies, further supports the significant association of the STin2.12/12 genotype with MO, but not with MA or all migraines. The association between the STin2.12/12 genotype and MO disappeared after a stratified analysis of ethnicity.

The biological function of the STin2 VNTR polymorphism is not yet well understood. The VNTR polymorphism has been suggested to act as a transcriptional regulator with allele dependent, enhancer-like properties [47]. Expression studies indicated that both the STin2.12 and the STin2.10 alleles can increase transcriptional activity and that the effect of Stin2.12 is stronger [48]. Individuals who are homozygous for the STin2.12 allele appeared to have reduced 5-HT uptake in platelets compared to individuals who are heterozygous for the STin2.10/STin2.9 allele [47]. These data are consistent with our findings that the STin2.12/12 genotype is associated with MO, but they do not explain our findings for MA. Therefore, independent cohorts and further functional investigations are needed.

A number of limitations of our meta-analysis should also be considered. First, only seven studies (1,166 controls and 897 cases) on the 5-HTT STin2 VNTR polymorphism, and only 12 studies (22,793 controls and 7,088 cases) on the ACE I/D polymorphism were included in this meta-analysis. These sample sizes may not be large enough to allow a definitive conclusion. Second, migraine is a complex, multifactorial disorder that is susceptible to environmental factors [48, 49]. Therefore, the influence of other genes and environmental factors cannot be predicted. Moreover, many genetic variants work together to produce the migraine phenotype, which means that each of the genetic variants only confers a small to moderate change in risk for migraine [50]. Third, linkage disequilibrium (LD) and haplotype analyses were not performed in our meta-analysis. Fourth, selection bias may have occurred because this study only included articles that were written in English. Fifth, the moderate or high heterogeneity in this study underscores the need for caution when interpreting this type of data.

Despite the challenge of identifying migraine susceptibility genes, which is partly because migraine is a complex and multifactorial disorder, future large-scale, case-controlled, and population-based association studies on the role of these two genetic polymorphisms in migraine are necessary to validate the risks indicated in our meta-analysis. Future studies should include a large sample size and should analyze subgroups based on ethnicity, gender, and migraine status. These studies should also include methods for the standardization of the data sets. Further research is also needed to investigate the potential gene-gene and gene-environment interactions between the two gene polymorphisms and migraine.

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Disclosure of conflict of interest

None.

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