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Genetic polymorphism in Angiotensin-Converting Enzyme (ACE) and Asthma risk: A comparative meta-analysis based on Four gene model strategy

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Article

ABSTRACT

Recent research indicates that angiotensin-converting enzyme (ACE) gene activity contributes to the etiology of asthma disease. Over the years, various studies on the ACE gene polymorphism have been conducted, but the results have been inconsistent. We conducted a meta-analysis to assess the relationship between the ACE gene polymorphism (I/D) and asthma risk using the conventional meta-analysis method and four-gene model strategy proposed by Horita and Kaneko. A systematic search of PubMed, Medline, Embase, etc. was conducted to retrieve all studies pertaining to ACE gene polymorphism. Data were extracted from all the eligible studies, and pooled Odds ratio (OR) and 95% CI were calculated to determine the strength of an association between ACE gene polymorphism and asthma risk. Using software such as SPSS, Review Manager 5.4, and JASP (Jeffrey's Amazing Statistics Program), the data were statistically analyzed. In our analysis, we contrasted a number of gene polymorphism models. In accordance with standard meta-analysis procedures, we selected the dominant genetic model to be the random effect model in our meta-analyses. Using this model, we found that DD homozygotes have an increased risk of asthma disease compared to DI heterozygotes and II homozygotes (OR=1.61, CI=1.28-2.03, I2=68%, p<0.00001). According to ethnicity-based stratified analyses, Asians are more susceptible to asthma than Europeans. Moreover, age-based analysis revealed that minors are more susceptible to asthma than adults. Our research aimed to compare conventional meta-analysis practices to the four-gene model strategy as it reduces type 1 error. We have chosen the dominant genetic model as the random effect (RE) model under the conventional meta-analysis technique and observed the significant association between ACE gene polymorphism and asthma disease. Although, while performing the four-gene model strategy, the over-dominant model was selected as the best possible model for the meta-analysis under which no significant association was observed.

Keywords: Angiotensin-converting enzyme, polymorphism, Susceptibility, Asthma, Genetic model

INTRODUCTION

Asthma is a chronic inflammatory disease of the upper respiratory tract. It is characterized by airway hyper-responsiveness, which results in recurrent episodes of wheezing, coughing, sneezing, chest stiffening, and bronchoconstriction.[1] The aetiology of the disease is influenced by genetic determinants and environmental conditions because the response to treatment depends on an individual's genetic conditions. In contrast, the appearance and prevalence of the disease are influenced by environmental conditions. In recent years, numerous genes have been implicated in the etiology of asthma, including the angiotensin-converting enzyme (ACE) gene. The renin-angiotensin-aldosterone system (RAAS) regulates the glomerular filtration rate (GFR) and systemic blood volume. Renin converts angiotensinogen to angiotensin I, which is then converted to the vasoconstrictor angiotensin II by the ACE. Additionally, ACE degrades inflammatory substances such as Bradykinin and substance P, thereby functioning as anti-inflammatory molecules.[2] These enzymes are predominantly found in the lungs and kidneys. The gene encoding ACE is highly polymorphic due to the insertion/deletion (I/D) of a 287 bp segment in intron 16;[3] it resides on chromosome 17q3 and is composed of 26 exons and 25 introns. The polymorphism of the ACE gene frequently results in variable serum ACE levels, which may increase asthma susceptibility.

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Various studies have determined the association between ACE gene polymorphism and asthma susceptibility over the years, but the results have been controversial and equivocal, possibly due to the small sample size. In this context, three meta-analyses[4–6] have been conducted to date, but they have contained specific errors. Determining the association between the ACE gene polymorphism and asthma required us to conduct a conventional meta-analysis with updated data in order to reach a definitive conclusion. In addition, we attempted to determine the optimal genetic model for our meta-analysis using the four-gene model strategy proposed by Horita and Kaneko.[7]

MATERIALS AND METHODS

Publication search

We searched PubMed, Medline, and Embase for previous studies demonstrating correlations between ACE gene polymorphism and asthmatic disease. "ACE gene polymorphism", "ACE gene polymorphism and asthma disease", "ACE gene loci", and "asthmatic genes" were the search terms. All eligible studies published between 1997 and 2021 were extracted from the relevant databases. In addition, bibliography and cross-references were investigated to locate additional eligible studies.

Selection of studies

Numerous investigations from PubMed and other databases demonstrated the association between ACE gene polymorphism and asthma. For our meta-analyses, the inclusion criteria were (a) studies should be published in the English language; (b) It should be a case-control study; (c) studies should have provided genotypes for the estimation of the Odds ratio and 95% CI: (d) genotype for the control groups should be in agreement with the Hardy-Weinberg equilibrium (HWE); (e) studies available only with PubMed, Medline and Embase databases. The criteria for exclusion were (a) studies without control groups. Studies published in languages besides English. (c) Editorials, letters, systematic reviews, and meta-analyses that have been published. An outline of selected studies has been shown in Figure 1.

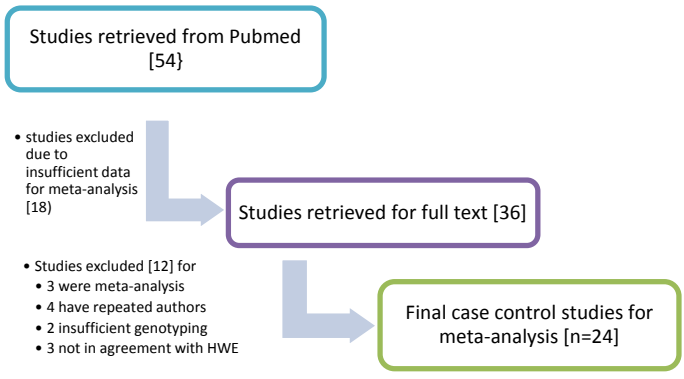

Figure 1: Flowchart illustrating the selection of studies for the meta-analysis

Data analysis

The Hardy Weinberg equilibrium (HWE) test was used to compare the observed and expected genotype frequencies of each control group in each study. By calculating the Odds Ratio (OR)

Table 1: Attributes of case-control studies included in meta-analyses

Author	Year	ethnicity	Age	Asthma definition	Sample size	Genotyping method
Guo[8]	2006	Asian	Children	Not described	52/72	PCR
Lu[9]	2004	Asian	Adults	Chinese Asthma diagnosis criteria	18/15	PCR
Song[10]	2001	Asian	Children	Chinese asthma diagnosis criteria	108/56	PCR
Gao[11]	2000	Asian	Not described	Doctor diagnosis	50/50	PCR
Qin[12]	2000	Asian	Children	Chinese asthma diagnosis criteria	52/40	PCR
Lue[13]	2006	Asian	Children	ATS criteria	105/102	PCR
Lee[14]	2000	Asian	Adults	ATS criteria	310/121	PCR
Eryuksel[15]	2009	Asian	Adults	ATS criteria	97/96	PCR
Nakhama[16]	1999	Asian	Adults	ATS criteria	119/208	PCR
Tomita[17]	1998	Asian	Adults	ATS criteria	71/142	PCR
Gao[18]	1998	Asian	Not described	Not described	300/100	Not mentioned
Winchester[2]	2000	Asian	children	Questionnaire	6/275	PCR
Isa Abdi[19]	2011	Asian	Not described	Not described	62/212	PCR
Elshafai [20]	2011	Asian	Adults	GINA	30/30	PCR
E bora[21]	2013	Asian	Children	GINA	102/99	PCR
Saba[22]	2016	Asian	Adults	Doctor diagnosis	333/521	PCR
Zheng[23]	2012	Asian	Children	Doctor diagnosis	198/110	PCR
Chagani[24]	1999	European	Not described	Doctor diagnosis	231/43	PCR
Gao[18]	1998	European	Not described	Not described	150/150	Not mentioned
Winchester [2]	2000	European	children	Questionnaire	20/416	PCR
Benessiano [25]	1997	European	Adults	ATS criteria	100/100	PCR
Holla[26]	1999	European	Adults	ATS criteria, questionnaire	161/141	PCR
Yildiz[27]	2004	European	Adults	ATS criteria	42/46	PCR
Lee[28]	2009	European	Adults	Questionnaire	610/8428	PCR

HWE: Hardy Weinberg Equilibrium, ATS: American Thoracic society, GINA: Global Initiative of Asthma guidelines

with 95% confidence intervals (CI), the strength of association between ACE gene polymorphism and asthma was estimated. A value of OR>1 indicates an association between disease and gene polymorphism, whereas a value of OR1 indicates no association between disease and gene polymorphism. The significance of the OR value was determined using the Z test, with a significance level of $p<0.5$. We calculated the combined OR for four distinct genetic models: dominant (DD vs. DI+II), recessive (II vs. DD+DI), over-dominant (DI vs. DD+II), and co-dominant (DD vs. II). In addition, we have computed the OR value for the allelic model (D versus I). Based on ethnicity and age categories, subgroup analysis was conducted. The heterogeneity between the studies was determined using a Chi-square-based Q test, where $p<0.5$ was considered statistically significant. The I^2 statistical test was also conducted to estimate heterogeneity that indicates percentage variability within the study. I^2 values range from 0% to 100%, with greater than 50% heterogeneity implicating the random effect model and less than 50% implicating the fixed effect model. In addition, publication bias was examined using the Begg funnel plot and Egger regression test.[29] SPPSS v.25 was used to conduct statistical analysis. At $p<0.05$, the results were considered significant.

RESULTS

Characteristics of studies

A comprehensive search was performed using the PubMed, Medline and Embase databases. In the preliminary stage of our meta-analyses, few irrelevant studies were excluded as they failed to provide any statistical outcome on ACE gene polymorphism and its association with asthma disease. Out of the remaining studies, 3 earlier conducted meta-analyses were also excluded. Furthermore, two studies with insufficient control group data,[30,31] two studies with control group genotype violating the Hardy-Weinberg equilibrium,[32,33] and one study lacking a control group[34] were also excluded. After applying all the inclusion and exclusion criteria, in total, 24 studies were considered eligible for further analysis (Table 1). Based on ethnicity, control groups from 15 studies had Asian descendants, 5 had European descendants, and control groups from 2 studies had both Asian and European descendants. Based on age groups,[32] studies have been performed on the Adults,[14-18,20-24,34] while, 7 studies were performed involving the children[2,3,19,25-28] and remaining studies have not mentioned the Age groups.[29,30,32,33 19]

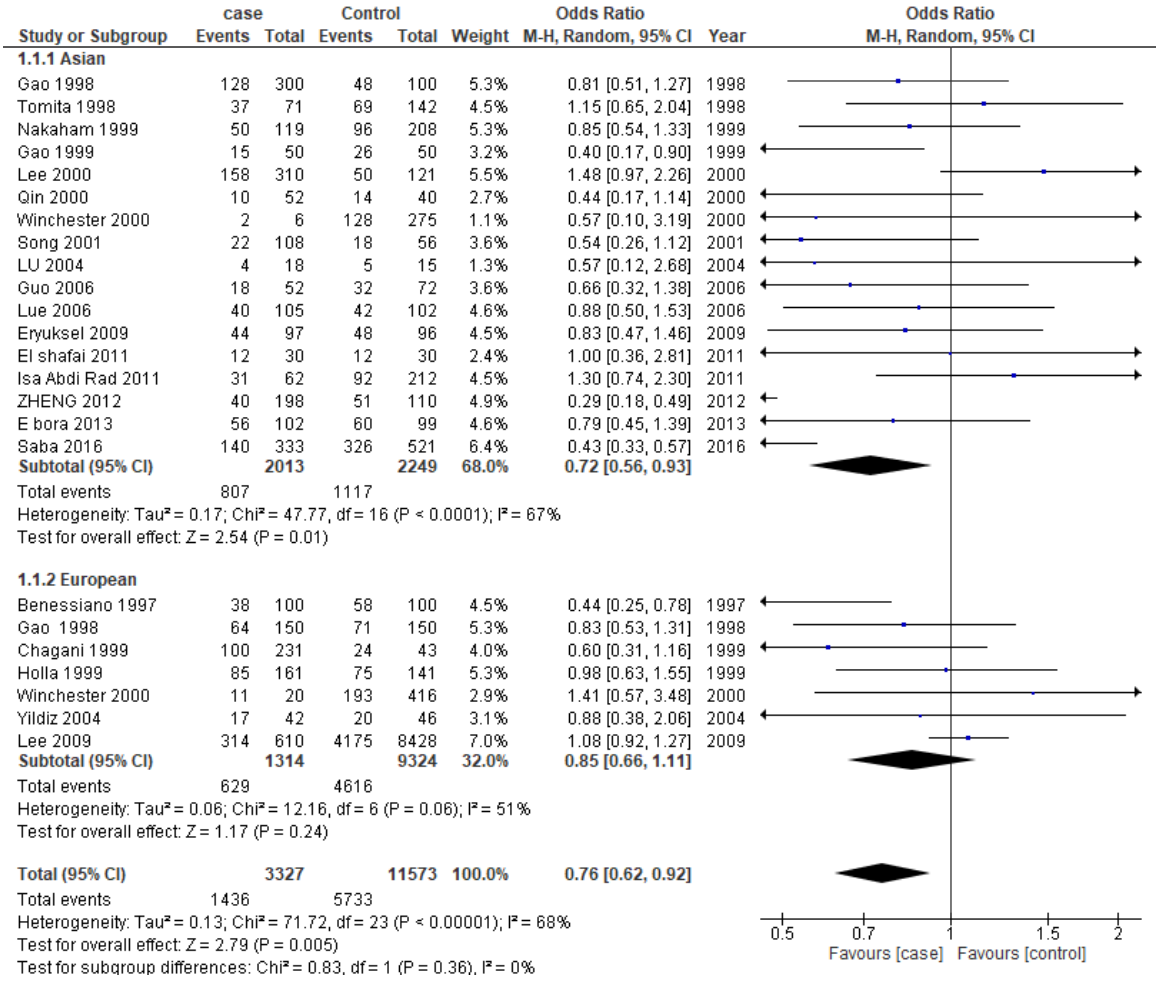

Figure 2: Forest plot of over-dominant under the Four-gene model strategy

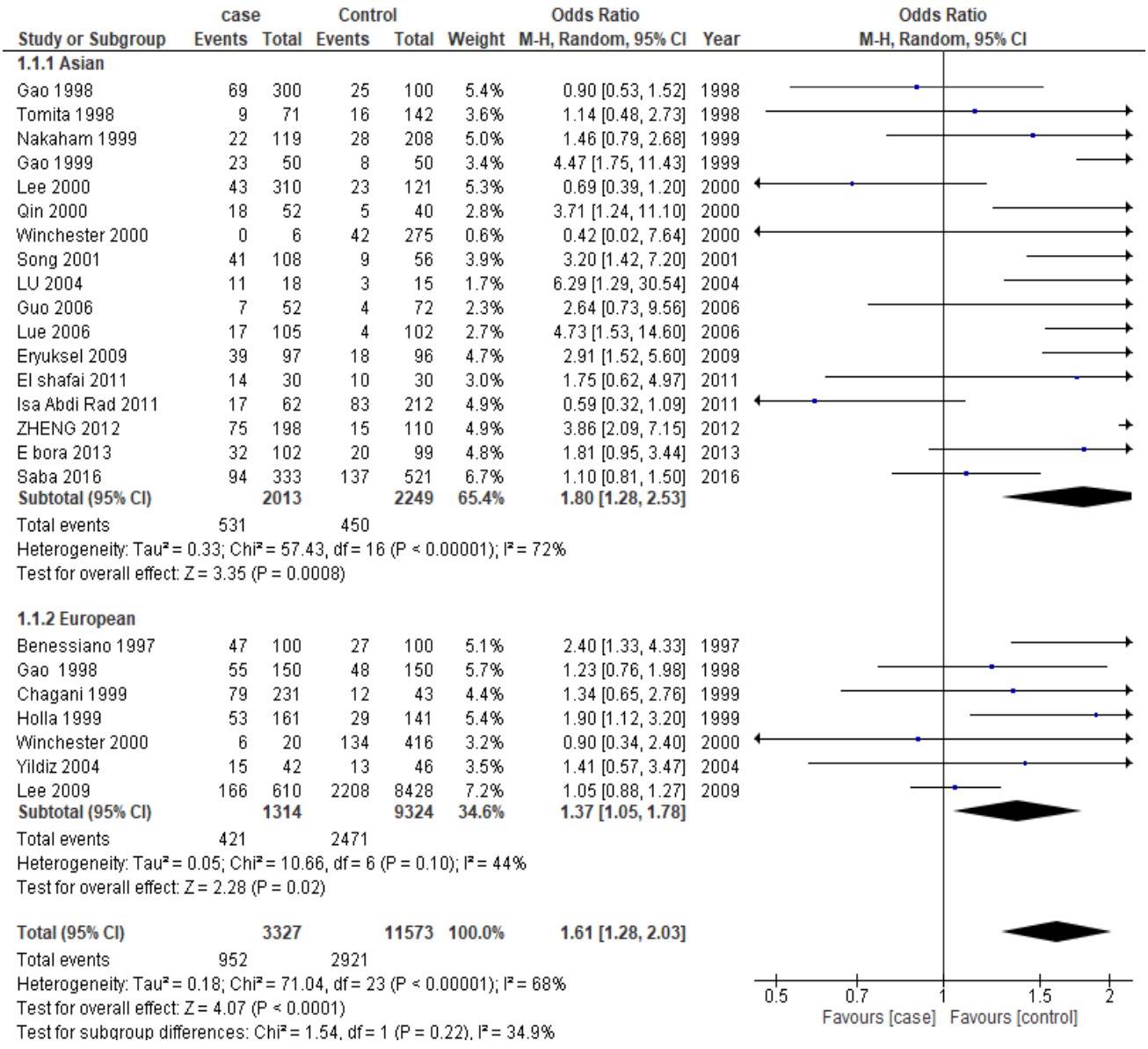

Figure 3: Forest plot for the dominant genetic model under random effect

Quantitative synthesis

The overall evaluation of the association between ACE gene polymorphism and asthma disease is given in Table 2. As mentioned earlier, we have analyzed various genetic models in our study. By the conventional meta-analyses practices, the random effect model was the dominant genetic model. In the dominant genetic model, a statistically significant association was observed (OR=1.61, CI=1.28-2.03, I^2 =68%, p< 0.00001) Figure 2. On the basis of four-gene model concept, the over-dominant model came out as the best possible model for the meta-analyses. However, statistically, no association was observed under the over-dominant genetic model (OR=0.76, CI=0.62-0.92, I^2 =68%) and asthma disease Figure 3.

Table 2: Summary analysis of the various genetic models used in the meta-analyses

Genetic model	Categories	Odds ratio (OR)	95% C	Z value	p value	I^2	p value
Dominant genetic model (DD VS DI+II)	All (24)	1.61	1.28-2.03	4.07	<0.0001	68 %	<0.00001
	Asians(17)	1.80	1.28-2.53	3.35	0.0008	72 %	<0.00001
	Europeans(7)	1.37	1.05-1.78	2.28	0.02	44 %	<0.10
	Adults(11)	1.45	1.11-1.90	2.69	0.07	62 %	0.003
	Children(7)	2.51	1.58-4.01	3.87	0.0001	43 %	0.10
	Age not mentioned(6)	1.31	0.80-2.14	1.06	0.29	67 %	0.009
Co-dominant	All(24)	1.58	1.17-2.15	2.95	0.003	72 %	<0.00001

genetic model (DD VS II)	Asians(17)	1.72	1.09-2.73	2.31	0.02	79 %	<0.00001
	Europeans(7)	1.36	1.04-1.78	2.27	0.02	17 %	0.30
	Adults(11)	1.53	0.97-2.42	1.83	0.07	80 %	<0.00001
	Children(7)	2.60	1.79-3.80	4.97	<0.00001	0%	0.59
	Age not mentioned(6)	1.10	0.66-1.84	0.35	0.72	54 %	0.06
Over-dominant model (DI VS DD+II)	All(24)	0.76	0.62-0.92	2.79	0.005	68 %	<0.00001
	Asians(17)	0.72	0.56-0.93	2.54	0.01	67 %	<0.0001
	Europeans(7)	0.85	0.66-1.11	1.17	0.24	51 %	0.06
	Adults(11)	0.84	0.63-1.12	1.17	0.24	77 %	<0.00001
	Children(7)	0.62	0.40-0.95	2.18	0.03	58 %	0.03
	Age not mentioned(6)	0.77	0.59-1.02	1.83	0.07	25 %	
Recessive model (II VS DD+DI)	All (24)	0.90	0.72-1.13	0.89	0.38	71 %	<0.00001
	Asians(17)	0.91	0.66-1.26	0.56	0.57	76 %	<0.00001
	Europeans(7)	0.83	0.69-1.01	1.86	0.06	5%	0.39
	Adults(11)	0.80	0.53-1.21	1.05	0.29	85 %	<0.00001
	Children(7)	0.87	0.58-1.31	0.65	0.52	51 %	0.06
	Age not mention(6)	1.17	0.90-1.54	1.17	0.24	0%	0.71
	All (24)	1.24	1.06-1.44	2.74	0.006	46 %	0.008
	Asians(17)	1.26	1.01-1.57	2.06	0.04	56 %	0.003
	Europeans(7)	1.16	1.01-1.33	2.16	0.03	0%	0.44
	Adults(11)	1.18	0.95-1.46	1.48	0.14	56 %	0.01
	Children(7)	1.54	1.13-2.08	2.77	0.006	0%	0.64
	Age not mention(6)	1.01	0.78-1.30	0.05	0.96	16 %	0.31

Table 3: Regression test for Funnel plot of over-dominant model asymmetry ("Egger's test")

	z	p
sei	-0.746	0.456

Stratified subgroup analysis

We also conducted subgroup analysis based on age and ethnicity. Analysis by ethnicity using multiple genetic models reveals that populations with the ACE gene polymorphism are susceptible to an increased risk of asthma in both Europe and Asia (Table 2). Under the co-dominant model, Asian populations were found more prone to the asthmatic condition (OR=1.72, CI=1.09-2.73, p=<0.00001, I^2= 79%) as compared to the European ones (OR=1.36, CI=1.04-1.78, p=0.30, I^2= 17%). Under the dominant model, similar outcomes were also observed in the Asian (OR=1.80, CI=1.28-2.53, p<0.00001, I^2=72%) and European (OR=1.37, CI=1.05-1.78, p=0.10, I^2=44%) ethnic groups. Adults and children were susceptible to asthma disease according to both the dominant and co-dominant models, as determined by an analysis stratified by age group. Under the dominant genetic paradigm, however, children are more susceptible to the disease (OR=2.51, CI=1.58-4.01, p=0.10, I2= 43%) than adults (OR=1.45, CI=1.11-1.90, p=0.003, I2= 62%).

Four-gene model strategy

In addition to the traditional meta-analysis technique, we also conducted a meta-analysis using the four-gene model strategy (Horita and Kaneko, 2015 [7]). This strategy identifies the optimal genetic model for meta-analysis, particularly for case-control studies. Listed below are the results of the four gene model strategy.

The pooled OR1 between genotypes DD and DI was calculated (Figure 4). There was a statistically significant association between the two genotypes (OR = 1.62, CI = 1.24 to 2.11, p <0.00001, I^2 = 74%). Similarly, pooled OR2 was calculated between genotypes DI and II (Figure 5).; however, no association was statistically observed (OR=0.90, CI=0.70-1.15, p=0.00001, I^2= 71%).As suggested by Horita and Kaneko [7], the combined OR1 and OR2 values were plotted on a logarithmic scale OR1-OR2 plane (Figure 5). The observed values fell within the prevalent model region, indicating that it is the optimal model for our meta-analysis.Calculating the OR3 for the selected model was the third phase. In this instance, the over-dominant genetic model is selected; its OR3 value has been calculated as follows: (OR3= 0.76, CI=0.62-0.92, p=0.000001, I^2=68%). The fourth phase involved calculating OR1mod and OR2mod based on OR3. Horita and Kaneko give the relationship between OR1mod and OR2mod for each model. The relationship between the OR1mod and the OR2mod is as follows:

OR1mod=1 and OR2mod=OR3 indicate a recessive model.

OR1mod=OR2mod=OR3 for co-dominant model

OR1mod=OR3 while OR2mod = 1 for the dominant model

OR1mod=OR3 and OR2mod = 1/OR3 according to the dominant model.

The values for OR1mod and OR2mod are shown in Table 4.

Calculate the OR1 and OR2 values for each study between the genotypes DD versus DI and DI versus II. The combined values of OR1 and OR2 were then calculated.On a logarithmic scale, the combined OR1 and OR2 values were plotted on the OR1-OR2 plane. Log-scale is divided symmetrically into four equal regions. We determined the optimal model (over-dominant) by plotting the values against it.The OR3 value is then calculated for the optimal model selected for each study. After that, the combined OR3 for the best-selected model is calculated.OR1 mod and OR2 mod are computed based on the total OR3.Data analyses for the Four-gene model strategy were as follows:

Table 4: Four gene model summary analysis

First step (OR1, OR2)	**Second step**	**Third step OR3**				**Fourth step OR1mod, OR2mod**			
		Re	Do	Ov	Cod	Re	Do	Ov	Cod
(1.62, 0.90)	OV	0.90 (0.72-1.13)	1.61 (1.28-2.03)	0.76 (0.62-0.92)	1.58 (1.15-2.17)	(1, 0.90)	(1.61, 1)	(0.76, 1.31)	(0.76, 0.76)

Re; Recessive model, Do; Dominant model, Ov; Over-dominant model, Cod; Co-dominant model

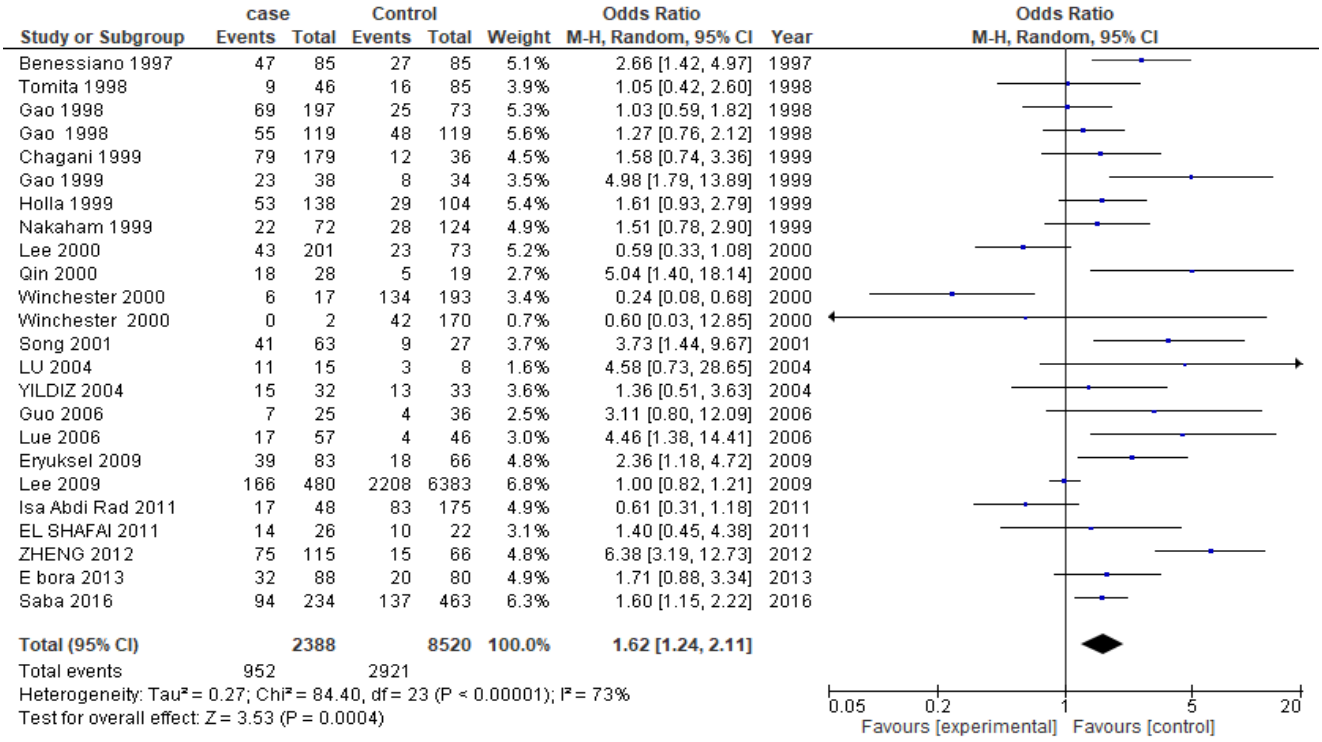

Figure 4: Calculation of the OR1 between the genotypes DD VS DI under the Four-gene model strategy

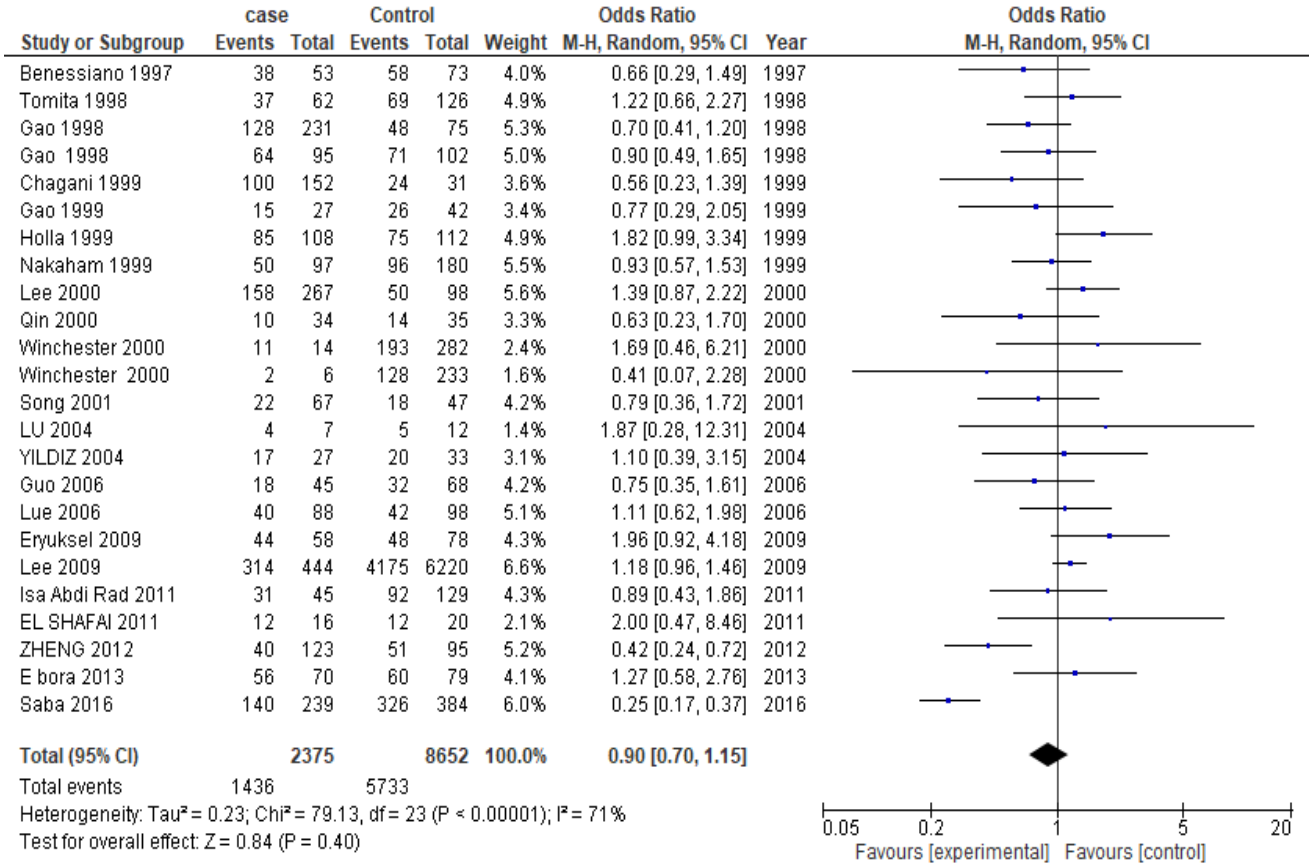

Figure 5: Calculation of the OR2 between the genotypes DI VS II under the Four-gene model strategy

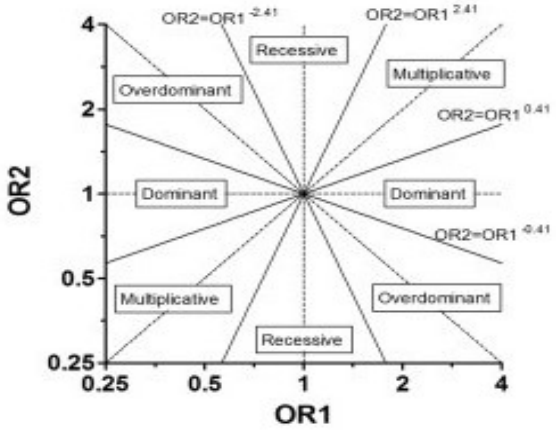

Figure 6: The log scale OR1-OR2 graph represented here is proposed by Horita and Kaneko (2015) under four gene model strategy. The OR1 (1.62) and OR2 (0.90) values is calculated between the genotypes and values is plotted on this log scale. Based on the this scale the over-dominant model is selected for our study under the four gene model strategy.

Publication bias

The publication bias was evaluated using the Funnel plot and Egger's regression asymmetry (Figure 7& Table 3)

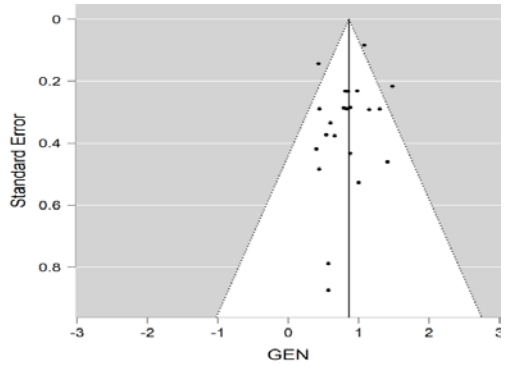

Figure 1: Funnel plot of over-dominant genetic model

DISCUSSION

It is well recognized that both genetic and environmental factors contribute to the etiology of asthma. Various studies have been conducted in recent years to identify the genes implicated in the etiology of asthma disease; the ACE is one of these genes. In order to investigate the association between ACE gene polymorphism (I/D) and asthma disease, we have conducted a meta-analysis considering 14592 subjects, of whom 3327 were cases and 11573 were controls. The meta-analyses[4–6] conducted in this regard showed inconsistent results. In the previous meta-analyses conducted by Zhang et al. in 2011,[4] the data pertaining to the Benessiano study[25] were incorrectly considered. In addition, the largest study conducted on the ACE gene polymorphism was excluded from the meta-analyses. Similar errors were also found in the meta-analyses conducted by Ding et al (2012).[5] Many independent studies have been conducted in the later years; also, not have been considered in that meta-analysis. Consequently, the outcomes of previous meta-analyses appeared inconsistent. We considered a few more additional studies than previously conducted meta-analyses; consequently, the results appear to be more consistent. In addition to the conventional meta-analysis method, we employed the four-gene model strategy proposed by Horita and Kaneko[7] to conduct the meta-analyses. In terms of type 1 error reduction, the four-gene model has advantages over traditional meta-analysis.

In our meta-analyses, we have found a statistically significant association of the ACE gene polymorphism (I/D) with asthma disease under the dominant gene model. Identical outcomes were observed in the co-dominant genetic paradigm. It has been observed that DD homozygotes are more susceptible to asthma than DI heterozygotes and II homozygotes. The allelic genetic model has also produced comparable results. The molecular mechanism of the ACE gene in the development of asthma is unknown. However, a conclusion can be drawn from these results. As stated previously, ACE is capable of degrading inflammatory substances such as Bradykinin and Substances p; as it can function as an anti-inflammatory molecule. Consequently, DD homozygous individuals will have a defective or non-functional ACE gene due to the presence of the deletion (D) allele in their genome. This results in a lower serum ACE level and an increased level of inflammatory molecules in the lungs, which leads to bronchoconstriction.

Additionally, as stratified analyses based on race and age were conducted. In accordance with the dominant and co-dominant genetic model, Asians were more susceptible to developing asthma than Europeans. This dissimilar characteristic may be attributable to the distinct environmental conditions and genetic backgrounds of the two ethnic groups. Until now, the majority of studies have focused on Asians and Europeans; additional research on the various ethnic groups is required for a more comprehensive interpretation. From our study, age groups based

stratified analyses indicate that children have increased susceptibility to Asthma as compared to adults. Genome-wide Association Studies (GWAS) performed to identify the distinct and shared genetically linked loci for childhood-onset and adult-onset asthma appear to be marginally consistent with our findings. The most significant loci associated with asthma were identified at 17q12-21 and are highly expressed in childhood-onset asthma compared to adult-onset asthma. Our ACE gene is present at locus 17q23, but it has been shown that loci other than 17q12-21 have limited power of expressibility in the genome; consequently, loci other than 17q12-21 are unknown in the GWAS.

In addition, in order to overcome the limitations of traditional meta-analysis, we have adopted the four-gene model strategy for our meta-analyses. In conventional procedures, researchers first calculate the OR values for the various genetic models and then select the model that yields the most conclusive findings. In contrast, in the four-gene model strategy, OR1 and OR2 values are calculated for the genotypes and then depicted on the log OR1-OR2 scale, which aids in determining the optimal model for the meta-analyses, and then OR3 is calculated for that model. This strategy is primarily considered to prevent selection biases in genetic models for meta-analyses. In our study, a log scale depiction of

OR1 and OR2 values indicated that the over-dominant model was the optimal model for our meta-analyses. In contrast to the models (dominant and co-dominant) that were chosen using the conventional meta-analysis technique, no statistically significant association was observed in the over-dominant model. This indicates that the DI heterozygotes are less likely to develop asthma than the DD homozygotes. It may be due to the presence of an insertion allele (I) in the genome of DI heterozygotes, which maintains the normal serum ACE level in the lungs and, consequently, lower levels of inflammatory molecules, thereby decreasing asthma disease susceptibility.

CONCLUSION

In conclusion, a meta-analysis to assess the association between the ACE gene polymorphism (I/D) and the risk of asthma have been performed. In addition, we compared the conventional meta-analysis strategy with the four-gene model approach. In accordance with previously conducted meta-analyses, conventional meta-analyses have concluded that ACE gene polymorphism is substantially associated with asthma conditions. Our ethnicity- and age-based subgroup analyses reveal that Asian populations are more susceptible to asthmatic conditions than European descendants, and that toddlers have a higher risk of asthma than adults. Nevertheless, outcome-based four-gene model strategies revealed a negative relationship between ACE gene polymorphism (I/D) and asthma risk. Therefore, we propose that additional research is required to validate our findings. Additionally, we suggest that in the future, more research should be conducted on various ethnic groups. So that we may have a comprehensive understanding of the association between asthma and ACE gene polymorphism.

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CONFLICT OF INTEREST STATEMENT

There is no Conflict of Interest for publication of this work.

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