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Association of IREB2 Gene rs2568494 Polymorphism with Risk of Chronic Obstructive Pulmonary Disease: A Meta-Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: It is reported that the iron-responsive element-binding protein 2 (IREB2) gene rs2568494 polymorphism might be associated with COPD risk. The purpose of this meta-analysis was to collect all eligible studies to review the association between IREB2 gene rs2568494 polymorphism and susceptibility to COPD.


Material/Methods: We carried out a comprehensive document search of electronic databases of PubMed, MEDLIN, Web of Science, and included 4 eligible studies that examined the association between IREB2 rs2568494 polymorphism and COPD susceptibility. We performed a meta-analysis of these studies based on IREB2 rs2568494 genotypes.

Results: After meta-analysis with fixed or random effects, no significant associations were found under the heterozygote model (GG/GA; OR=0.908, 95%CI: 0.790–1.043; $P=0.172$), homozygote model (GG/AA; OR=0.880, 95%CI: 0.497–1.557; $P=0.661$), dominant model (GG/AA+GA; OR=0.941, 95%CI: 0.748–1.182; $P=0.599$), or allelic model (G/A; OR=0.953, 95%CI: 0.770–1.179; $P=0.655$). However, we found a significant correlation under the recessive model (AA/GA+GG; OR=1.384, 95%CI: 1.092–1.755; $P=0.007$).

Conclusions: The current results revealed that there was significant association between IREB2 gene rs2568494 polymorphism with susceptibility to COPD; the presence of allelic A might a genetic factor conferring susceptibility to COPD.

MeSH Keywords: **Iron Regulatory Protein 2 • Meta-Analysis • Polymorphism, Single Nucleotide • Pulmonary Disease, Chronic Obstructive**

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Background

Chronic obstructive pulmonary disease (COPD) is a genetically complex human disease resulting in persistent airflow limitation [1]; it became the third leading causes of mortality in the United States in 2010 [2]. Although cigarette smoking is widely recognized as a major environmental risk factor for the development of COPD [3], non-smokers can also develop the disease even without the influence of passive smoking [4]. Therefore, it is well accepted that multiple susceptibility genes and various environmental factors might contribute to individual susceptibility to COPD [5–7].

Over the past few years, many candidate genes have been investigated to identify the association with susceptibility of COPD. However, alpha 1-antitrypsin deficiency is to date the only proven genetic risk factor for the development of COPD [8,9]. It is found only in 1–2% of individuals with COPD [4,8–10]. Therefore, more studies with the identification of novel genes associated with the risk of COPD should be done. Recently, a genome-wide association study (GWAS) has demonstrated a statistically significant relationship between COPD risk and some single-nucleotide polymorphisms (SNPs) in several genes, including rs2568494 at the iron-responsive element-binding protein 2 (IREB2) gene, rs2568494 at the family with sequence similarity 13 member A (FAM13A) gene, and rs3821104 at the X-ray repair cross-complementing protein 5 (XRCC5) gene [11–15]. Subsequently, many case-control studies [4,5,10,16–18] were conducted to determine the association between these variants and COPD.

The IREB2 gene is located on chromosome 15q25 and shows a strong association with COPD [12]. Several genes, including members of the nicotinic acetylcholine receptors-CHRNA5, CHRNA3 and CHRNB4 genes, are also mapped on this region and are near the IREB2 locus. GWAS studies have also reported these genes might be linked to COPD [19,20], but the strong levels of linkage disequilibrium make it difficult to distinguish the effects of the CHRNA locus and IREB2. However, a previous report has shown the alteration of IREB2 expression in lung tissue from COPD patients compared to controls [21], providing evidence for IREB2 acting as a COPD susceptibility gene.

The SNP rs2568494 at IREB2 gene in several case-control studies [4,5,10,16] was identified the association with COPD susceptibility. However, the findings in these studies were inconsistent; some found a relationship but others did not. The evidence of the association of rs2568494 polymorphism and COPD remains controversial. Hence, our goal of this meta-analysis was to collect all eligible studies to determine the associations of IREB2 gene rs2568494 polymorphism with COPD.

Material and Methods

Publication search

The electronic databases of PubMed, MEDLIN, and Web of Science were searched for studies published before April 20, 2015, using the key words: “IREB2” or “rs2568494” or “polymorphism” and “COPD”. The search was restricted to studies conducted in humans. Concurrently, the references of reviews and retrieved articles were searched manually to find additional relevant articles. Only full-text articles published in English were included. When more than 1 publication included the same patient population, only the most recent or completes study was selected in this meta-analysis.

Inclusion and exclusion criteria

The following inclusion criteria were used for eligible studies: 1) a cohort or a case-control study; 2) the original study evaluated the association between IREB2 rs2568494 polymorphism and COPD susceptibility; 3) the study provided sufficient information on genetic frequency in COPD cases and controls for extraction; 4) COPD was diagnosed based on the definite clinical criteria; and 5) genotype distribution of control group was in accordance with Hardy-Weinberg equilibrium. The exclusion criteria were: 1) incomplete raw data; 2) repetitive studies (if studies had included overlapping populations, only the largest or most recent sample was selected); 3) animal studies; 4) non-English paper; and 5) studies inconsistent with Hardy-Weinberg equilibrium (HWE).

Quality score assessment

Quality of each study included was independently evaluated by the same 2 reviewers, based on the Newcastle-Ottawa scale (NOS) quality system [22]. In case of disagreement on the quality scores between the 2 reviewers, differences were resolved through discussion and consultation with a third reviewer. Genotype distribution of the controls in included studies was also assessed according to the HWE.

Data extraction

Data were independently extracted from the eligible studies by the 2 reviewers, according to the same inclusion criteria listed above. Disagreements were resolved through discussion. The extracted information from each study included: first author, country, year of publication, ethnicity, total number of subjects, smoking status of subjects including pack-years (smokers were those who were had at least 10 pack-years smoking history), and the distribution of genotype and allele.

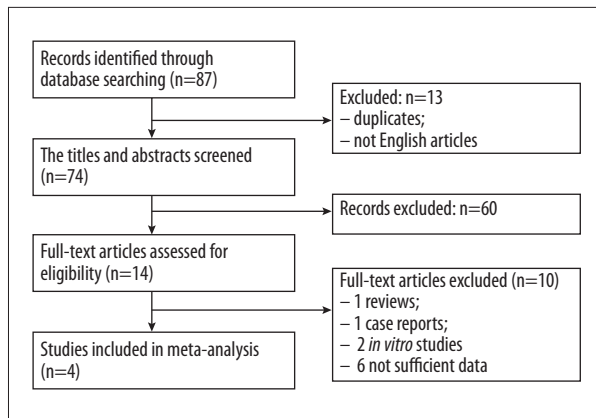


Figure 1. Flow diagram of study selection.

Statistical analysis

All statistical analyses were carried out using Stata statistical software. For each study, we detected whether the genotype distribution in controls was in accordance with the HWE using the χ^2 test. The combined odds ratios (ORs) with corresponding 95% CIs were determined for the heterozygote model (GG vs. GA), homozygote model (GG vs. AA), dominant model (GG vs. GA + AA), recessive model (AA vs. GA+GG), and allelic model (G vs. A).

Heterogeneity across all selected studies was assessed by the Q-test and the I^2 statistic (range, 0–100%) [23–25] and it was judged significant when $P < 0.1$ or $I^2 > 50\%$. A fixed-effects model was initially employed in the analysis when there was statistical heterogeneity ($P > 0.1$ or $I^2 < 50\%$). Otherwise, a random-effects model was used. Sensitivity analysis using the random-effects model was conducted to assess the stability of the crude results, after removing 1 study per time. Both the Begg's funnel plot and the Egger's linear regression test were used to detect publication bias [26,27]. A P value less than 0.05 was considered statistically significant.

Results

Study characteristics

Finally, a total of 4 articles [4,5,10,16] were selected in this meta-analysis involving 1513 COPD cases and 1480 smoking controls. Figure 1 displays the detailed flow diagram of the study search process. Table 1 lists the main characteristics of the selected studies and Tables 2 and 3 show demographics of patients included, respectively. There was no study in which genotypic distribution in controls was not in agreement with HWE.

Meta-analysis results

Figure 2 presents the results on the association between the IREB2 rs2568494 polymorphism and COPD risk. The detailed results based on all pooled included studies showed genotypic AA carriers might have a higher risk for COPD. After meta-analysis with fixed or random effects, no significant associations were found under the heterozygote model (GG/GA; OR=0.908, 95%CI: 0.790–1.043; $P=0.172$), homozygote model (GG/AA; OR=0.880, 95%CI: 0.497–1.557; $P=0.661$), dominant model (GG/AA+GA; OR=0.941, 95%CI: 0.748–1.182; $P=0.599$), and allelic model (G/A; OR=0.953, 95%CI: 0.770–1.179; $P=0.655$), respectively. However, we found a significant correlation under the recessive model (AA/GA+GG; OR=1.384, 95%CI: 1.092–1.755; $P=0.007$).

Sensitivity analysis

Sensitivity analysis was performed to assess the stability of the crude results. The results showed that no single study influenced the stability of the crude results because the corresponding pooled ORs were not materially altered.

Publication bias

Begg's funnel plot and Egger's test were used to evaluate publication bias. Begg's funnel plot did not present asymmetry

Table 1. Major characteristics of the studies included in the meta-analysis.

Study [Ref.]	Year	Ethnicity	Study design	Sample size		HWE	Quality score
				Cases	Smoking controls		
Arja et al. [8]	2014	Indian	Case-control	236	146	Yes	8
Zhou et al. [4]	2012	Chinese	Case-control	488	687	Yes	8
Guo et al. [14]	2011	Chinese	Case-control	275	434	Yes	7
Chappell et al. [5]	2011	Caucasian	Cohort	1002	900	Yes	8

HWE – Hardy-Weinberg equilibrium.

Table 2. Demographics of subjects included in this meta-analysis.

Study	Age		Gender		BMI		Pack years		FEV ₁ (%)		FEV ₁ /FVC	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Arja et al. [8]	63.2	61.1	236/0	146/0	19.8	22.01	43.0	48.2	36.8	71.7	55.4	86.0
Zhou et al. [4]	62.7	60.9	N/A	N/A	22.5	22.4	33.8	29.2	64.2	102.4	53.6	79.3
Guo et al. [14]	60.9	61.2	192/83	307/127	N/A	N/A	32.8	30.8	52.8	91.5	48.2	90.3
Chappell et al. [5]	65.9	60.8	700/302	575/325	N/A	N/A	48.8	38.6	43.1	95.3	47.5	77.8

BMI – body mass index; FEV₁ – forced expiratory volume in 1s; FVC – forced vital capacity; N/A – not applicable.

Table 3. Genotype and allele counts for rs2568494 polymorphism at IREB2 gene in COPD patients and controls.

Study	G		A		GG		GA		AA	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Arja et al. [8]	366	219	106	73	142	82	82	55	12	9
Zhou et al. [4]	850	1190	126	182	368	518	114	154	6	14
Guo et al. [14]	484	761	66	107	215	334	54	93	6	7
Chappell et al. [5]	1187	1178	817	622	351	380	485	418	166	102

(Figure 3) and no publication bias was confirmed by Egger’s test (p=0.137).

Discussions

To the best of our knowledge, this is the first meta-analysis of genetic studies on the association of IREB2 rs2568494 polymorphism with susceptibility to COPD. In the current meta-analysis (based on 1513 cases and 1480 control subjects from 4 eligible studies), we demonstrated that there might be significant association between the IREB2-rs2568494 polymorphism and COPD risk in the overall populations. We found that homozygotes AA of rs2568494 polymorphism were a high risk factor of developing COPD and there was a trend of higher risk in T allele variant carriers. These findings revealed that the presence of allelic A might be a genetic factor conferring susceptibility to COPD.

It is well known that multiple factors, including genetic and environmental factors, might have complicated roles in the development of COPD [28]. Over the past decades, genome-wide association studies (GWAS) have become an important tool for identification of potential genes and loci associated with COPD susceptibility [29–32]. The IREB2 gene is located on chromosome 15q25, which is a particularly compelling region for detecting the genetic components of COPD [33]. IREB2 reportedly

had an influence on the regulation of cellular iron metabolism, together with IREB1 [10]. With encoding an iron-binding protein, the IREB2 gene plays a role in maintaining human cellular iron metabolism. It was reported that iron homeostasis and free iron concentration might have important effects in mediating oxidative stress and iron could therefore be involving in local damage by this mechanism [4,34,35]. Some studies have reported that increased expression levels of IREB2 m-RNA could be detected in the lung tissues of smokers and COPD patients [21]. DeMeo et al. [21] also found increased IREB2 protein in human lung tissues via comparison of COPD patients with controls. Therefore, the association between IREB2 gene and COPD risk should be investigated.

DeMeo et al. [21] investigated several SNPs at IREB2 gene and reported significant associations in both a COPD case-control studies and family-based studies. Arja et al. [10] also found that IREB2 gene rs2568494 polymorphism was associated with COPD susceptibility. They demonstrated haplotypes of carrying major alleles of IREB2 carrying major alleles of rs2568494 (G) had a negative effect on lung function, whereas those of major alleles of rs2568494 (A) showed positive association with lung function. However, a recent case-control study in a Chinese Han population by Guo et al. [16] reported no association of IREB2 rs2568494 with COPD risk. Zhou [4] also failed to find these 2 SNPs in IREB2 associated with COPD in non-smoking subjects. Therefore, a systematic review and meta-analysis is

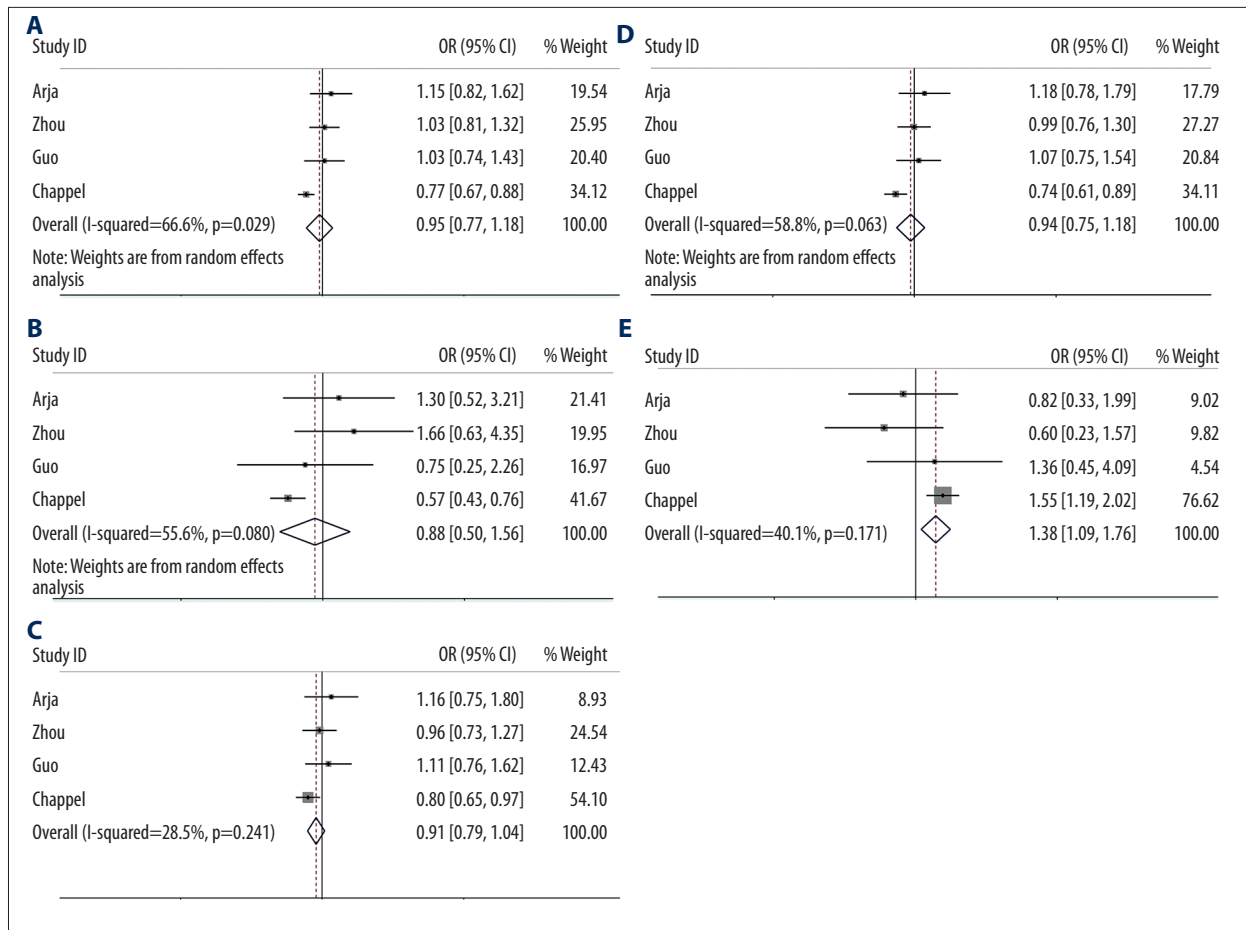


Figure 2. Forest plot for the association of IREB2 rs2568494 polymorphism with COPD risk; (A) Forest plot for allele model (G vs. A); (B) Forest plot for GG vs. AA; (C) Forest plot for GG vs. GA; (D) Forest plot for dominant model (GG vs. GA+AA); (E) Forest plot for the recessive model (AA vs. GA+GG).

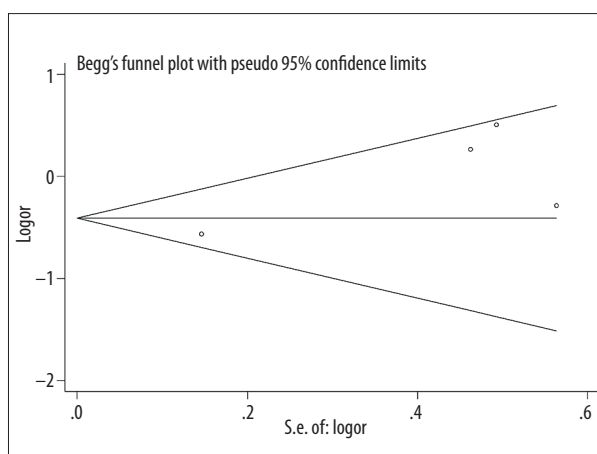


Figure 3. A funnel plot was used to assess publication bias.

needed to resolve this controversy. In this first meta-analysis, we found that minor allele (A) carriers might have an increased risk for COPD, without a publication bias, because the funnel

plot was acceptably symmetrical, and neither Egger's test nor Begg's test displayed publication bias.

In our meta-analysis, however, several limitations should not be ignored. Firstly, the sample size of our meta-analysis was relatively small, which might provide insufficient statistical power to evaluate the association of IREB2 gene with susceptibility to COPD. Secondly, only studies published in English were selected in this meta-analysis, and might result in bias of the results in this meta-analysis. Finally, only concentration on the IREB2 rs2568494 polymorphism without involving other genes or polymorphisms might require further study to investigate whether this polymorphism is integrated with other risk factors to increase the predictive power.

Conclusions

The current results revealed that there was a significant association between IREB2 gene rs2568494 polymorphism and

susceptibility to COPD; genotypic AA carriers might have a higher risk for COPD, suggesting that the presence of allelic A may be a genetic factor in susceptibility to COPD. Due to the limitations in this meta-analysis, further well-designed studies with larger sample sizes are necessary to confirm our findings.

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Statement

The authors declare that they have no competing interests.