

Association of arg16gly and gln27glu, β 2-adrenergic receptor gene polymorphism with asthma. A systematic review and meta-analysis of case control studies

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Abstract

The study was planned to assimilate quantitatively the available evidences on association of Arg16Gly and Glu27Gln with asthma and to produce more precise results. All case-control studies conducted on adults were searched on Medline, Embase, PubMed, Wiley online library according to Preferred Reporting Items for Systematic Review and Meta-Analysis guidelines. The strength of association was measured by odds ratios with 95% confidence interval. A total of 17 case-control studies were included in the meta-analysis and there was no significant association of asthma with Arg16Gly (odds ratio = 1.19; 95% confidence interval = 0.75-1.50, $p=0.459$) and Glu27Gln of ADRb2 polymorphism (odds ratio=0.87, 95% confidence interval =0.44-1.71, $p=0.683$). Moreover, neither Gly16 allele (odds ratio = 0.98; 95% confidence interval = 0.70-1.38, $p=0.867$) nor Glu27 allele (odds ratio = 0.67, 95% confidence interval = 0.38-1.19, $p=0.169$) contributed to asthma susceptibility. There was also no significant association between haplotypes of both single nucleotide polymorphisms and asthma ($p>0.05$). Data indicated that adrenergic receptor b2 did not contribute markedly to susceptibility to asthma ($p>0.05$).

Keywords: Asthma, Polymorphism, Meta-analysis, β 2-AR gene, Genotypes, Haplotypes, Genetic, Allele.

Introduction

Asthma is a heterogeneous disease, usually characterised by chronic airway inflammation. It is defined by history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation.¹ Globally around 300 million people suffer from asthma resulting in increased burden on families, and health care systems.² In Pakistan, 20% of adults and

30% of children experience two or more severe asthmatic attacks per year.³ One of the most important contributing factors to differential response to asthma is genetic variability among the patients.⁴ Therefore, many studies are now focusing on genetic aspect of asthma. It is therefore important to find out the gene(s) responsible for the disease susceptibility, severity and response to medications.

One of the most extensively studied asthma susceptible gene is adrenergic receptor b2 (ADRB2).⁵ ADRB2 is a small intron-less gene located on 5q31, a region that is genetically linked to asthma and related phenotypes. ADRB2 is considered to be the key gene present on airway cells (hyperactive in asthma).⁶ β 2 adrenoceptor agonists are major class in the treatment of asthma. Functional polymorphisms in ADRB2 may influence disease susceptibility and response to the treatment.⁷ ADRB2 gene contains the most common variant at position 16 that codes for glycine (Gly) or arginine (Arg) and at position 27 that codes for Glutamine (Gln) or glutamic acid (Glu).⁸⁻¹⁰ The Gly16 phenotype results in enhanced receptor downregulation in human airway smooth muscles.¹¹ Increased airway responsiveness to endogenous catecholamine had been reported in individuals with Gln27 polymorphism, resulting in increased airway sensitivity to pro-inflammatory stimuli, leading to extent of long-term airway inflammation.¹¹ Functional genomics studies have deemed these polymorphisms to affect the signal transduction and cellular trafficking functions in invitro models. However, several case-control studies conducted to investigate the association of ADRB2 polymorphism with asthma fail to reach a consensus. Such inconsistencies may be attributed to sample size, among other factors. Latest meta-analysis conducted on the association between asthma and ADRB2 polymorphism was published in 2014, which took into account many Chinese studies, among others,¹² and failed to find a positive association between ADRB2 single nucleotide polymorphisms (SNPs) and asthma phenotype. We hypothesised that since functionally these SNPs change the protein code and are

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likely to be exhibit differential binding affinity to the endogenous catecholamines, a meta-analysis to include latest literature review and further analysis on allelic and haplotype will show us association between these SNPs and asthma. The current study was planned to assimilate quantitatively the available evidences on association of Arg16Gly and Glu27Gln with asthma and to produce more precise results.

Materials and Methods

This systematic review of published literature was conducted from November 2014 to January 2015 in accordance with the Preferred Reporting Items for

Systematic Review and Meta-Analysis (PRISMA) guidelines¹³ (<http://www.prisma-statement.org/>) Institute of Basic Medical sciences, Khyber Medical University, Peshawar. The relevant search terms used for genotype, allele and haplotype frequency were "beta2* or β2 AND prevalence AND gene". For association between asthma and gene polymorphism the search terms used were: "Asthma* AND Polymorph* OR mutation OR variant AND beta2* OR β2 OR ADRβ2 OR Adrenergic receptor β2". Two independent reviewers used the search term and removed the duplicate. The electronic search was limited to studies conducted only on humans and translated to or written in English language. We performed an exhaustive

Table: Characteristics of case-control studies examining the association between ADR22 polymorphism and asthma.

Author	Year	Country	Gender	Mean Age of Cases (S.D)	Sample Size	Genotyping Method	Definition of Case
Litonjua, AA ²⁵	2004	USA	Male	63.1±7.7	382	PCR-RFLP	Physician diagnosed asthma (Smoker)
Litonjua, AA ²⁵	2004	USA	Male	63.1±7.7	171	PCR-RFLP	Physician diagnosed asthma (Non-Smoker)
Al-Rubaish, AM ²⁶	2011	Saudi Arabia	Male	10.4(4.6)	158	PCR-RFLP	Physician diagnosed asthma
Yin, K ²⁷	2006	China	Male/Female	34.2±1.7	97	Allele specific-PCR	Physician diagnosed nocturnal asthma
Yin, K ²⁷	2006	China	Male/Female	36±2.0	94	Allele specific-PCR	Physician diagnosed non-nocturnal asthma
Karam, RA ¹¹	2013	Egypt	Male/Female	10±2.4	200	Allele specific-PCR	History of chest tightness and wheezing from previous 12 months
Santillan, AA ²⁸	2003	Mexico	Female	42±14	907	PCR-RFLP	Physician diagnosed asthma, wheezing.
Wang, Z ²⁹	2001	China	Male/Female	30.6±16.2	264	Allele specific-PCR	Physician diagnosed asthma
Al-Rubaish, AM ³⁰	2011	Saudi Arabia	Male	11.4±4.6	136	PCR-RFLP	Physician diagnosed Nocturnal asthma
Holloway, JW ³¹	2000	New Zealand	Female	30.1±0.9	185	PCR-RFLP	Physician diagnosed severe asthma with at least one admission in hospital
Holloway, JW ³¹	2000	New Zealand	Female	31.4±1.2	154	PCR-RFLP	Physician diagnosed mild asthma having no admission in hospital
Birbian, N ³²	2012	India	Male/Female	38.1±16	824	PCR-RFLP	Physician diagnosed asthma having SOB, wheeze and cough
Lee, YL ³³	2009	Taiwan	Children	12.0±1.6	398	PCR-RFLP	Asthmatics having wheeze in chest and free of cold/flu
Petrovic-Stanojevic, N ³⁴	2014	Serbia	Male	44±4.8	272	Sanger sequencing	Physician diagnosed asthma (age >18 years)
Salama, MS ³⁵	2011	Egypt	Children	40(2-12)	39	PCR-RFLP	Physician diagnosed asthma having history of asthma.
De Paiva, A ³⁶	2014	Brazil	Children	10.38±2.93	229	ARMS-PCR	Allergic asthma according to GINA guidelines
El Akkary IM ³⁷	2012	Egypt	Female and Male	35±9	120	PCR-RFLP	Previous diagnosed asthma according to American thoracic society guidelines
Larocca, N ³⁸	2013	Venezuela	Female	44.2±15.2	205	PCR-RFLP	Asthma diagnosed according to GINA guidelines
Isaza, C ³⁹	2011	Columbia	Children	11.6±5.4	236	RT-PCR	Physician diagnosed asthma
Ramphul, K ⁴⁰	2014	India	Children	3-12	382	PCR-RFLP	Physician diagnosed asthma
Lv, J ⁴⁰	2014	China	Children	3-12	382	PCR-RFLP	Physician diagnosed asthma

PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism

ARMS-PCR: Amplification-refractory mutation system-polymerase chain reaction

SOB: Shortness of breath

GINA: Global Initiative for Asthma.

searches of five electronic databases. Furthermore, only case-control studies which had evaluated the association between asthma and ADR β 2 polymorphism at codon 16(Arg16Gly) and codon 27(Glu27Gln) or one of these were included in this meta-analysis.

For each study data was extracted by two independent reviewers and agreement was made on all items, including author, year of publication, journal, country of origin, ethnic background, phenotypes assessed, study design sample size, distribution of alleles, genotype and haplotypes (Table). All these attributes were validated by three different reviewers.

We conducted a random-effects meta-analysis of the association between ADR β 2 polymorphism in asthmatic patients compared to the healthy control. To assess the degree of heterogeneity I² statistics were calculated. All statistical analysis was performed using STATA version 13 (StataCorp, College Station, Texas, United States).

Results

The electronic search identified 490 publications (Figure-1). We used five different databases and when these were combined 200(40.81%) duplicates were found and excluded. The abstract of 290(59.18%) articles were reviewed. Of them, 180(62.0%) articles could not meet the inclusion criteria and were excluded, while 110(37.9%) articles were deemed relevant and full texts of these articles were reviewed. Out of these, 80(72.7%) publications were excluded on the account of having study design other than case-control; language used other than English and some of them were review articles. Among the remaining 30(27.3%) articles, 17(56.7%) were selected providing complete data regarding association, polymorphism, study design and statistics applied. They were published between 2004 and 2014. Besides, 3(17.6%) of these studies were conducted each in China and Egypt, 2(11.8%) were from India, while 1(5.9%) of each study was from the United States, New Zealand, Serbia, Columbia, Venezuela, Brazil and Mexico.

There was no significant association between Arg16Gly and asthma in any genetic model (odds ratio [OR] = 1.19; 95% confidence interval [CI] = 0.75-1.50, p=0.459) (Figure-

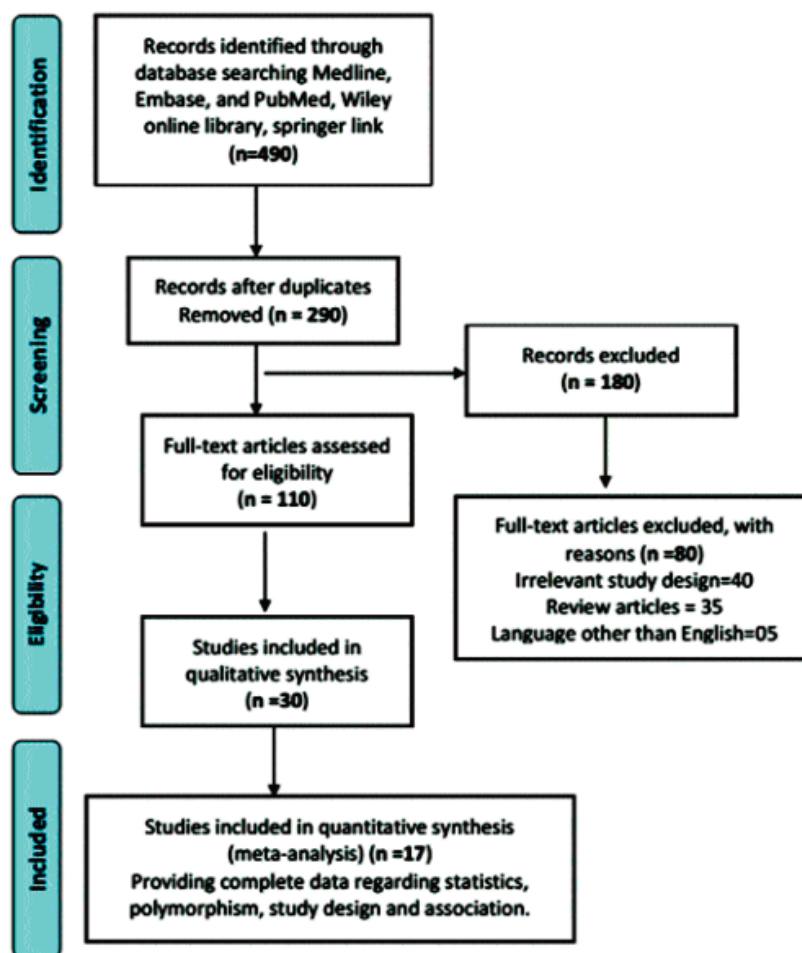


Figure-1: The electronic search identified 490 publications.

2). The same was the case for homozygous Arg and Gly with (OR = 1.28; 95% CI=0.57-2.88 p=0.551) and (OR = 1.04; 95% CI = 0.63-1.73, p=0.867) (Figures-3,4). No significant association with asthma was also found in case of allelic frequency of Arg and Gly with (OR = 0.87; 95% CI = 0.57-1.43, p=0.586) and (OR = 0.98; 95% CI = 0.70-1.38, p=0.931) (Figures-5,6).

For Glu27Gln, no evidence was found for association of both homozygous and heterozygous Glutamine and Glutamic acid with asthma. For Glu27Gln the (OR=0.87, 95% CI = 0.44-1.71, p= 0.683) and for homozygous Glu the (OR = 0.66; 95% CI = 0.44-1.06, p=0.170). For homozygous Gln (OR = 0.96; 95% CI = 0.27-3.44, p=0.949) (Figures-7-9). In case of Gln and Glu allele (OR = 1.03; 95% CI = 0.62-1.69, p=0.922) and (OR = 0.67, 95% CI = 0.38-1.19, p=0.169) (Figures-10,11).

Haplotypes of other studies were excluded due to insufficient data. Here, 3 linkages were found between

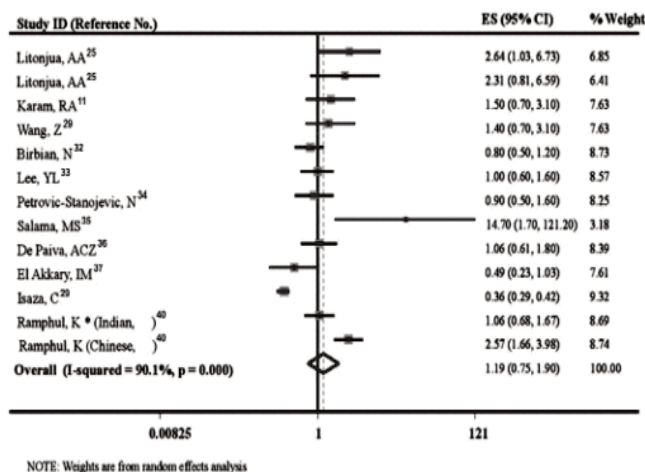


Figure-2: Forest plot of the association between Arg16Gly and asthma.

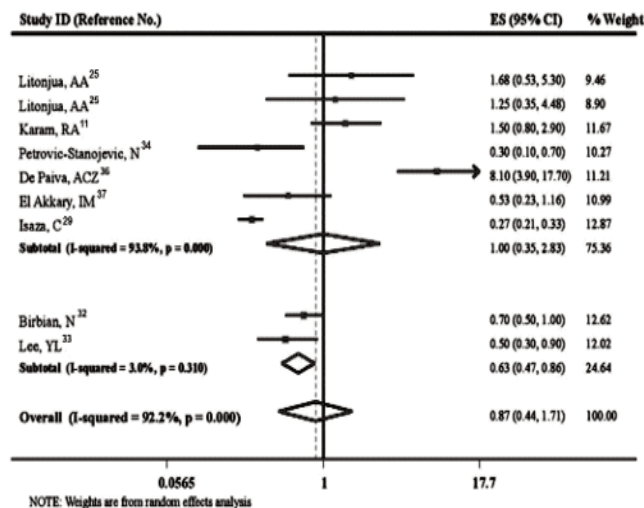


Figure-5: Forest plot of the association between Glu27Gln and asthma.

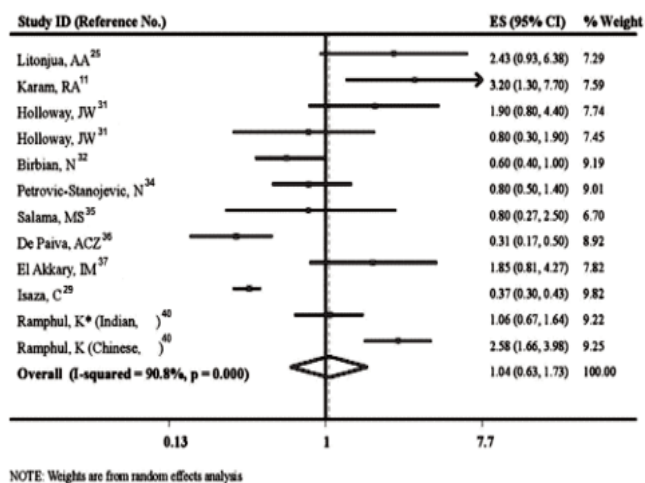


Figure-3: Forest plot of the association between Gly16Gly and asthma.

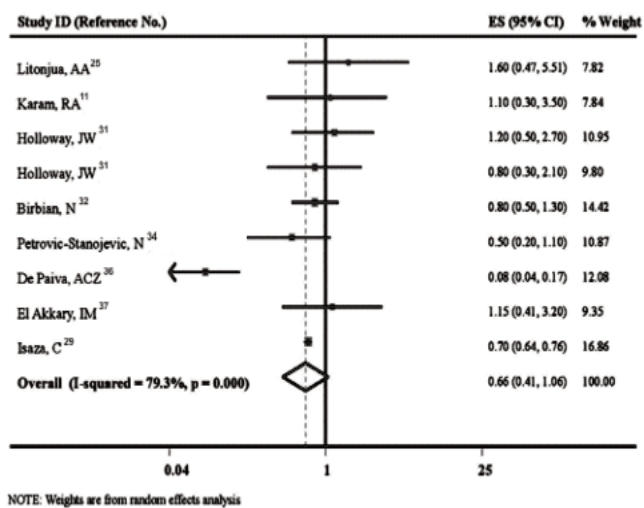


Figure-6: Forest plot of the association between Glu27Glu and asthma.

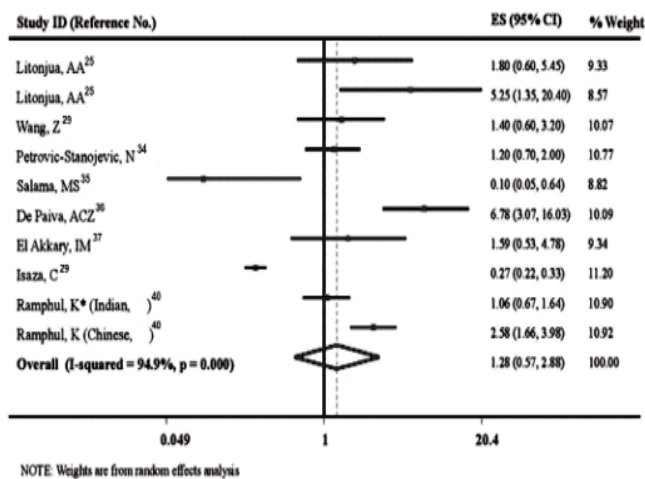


Figure-4: Forest plot of the association between Arg16Arg and asthma.

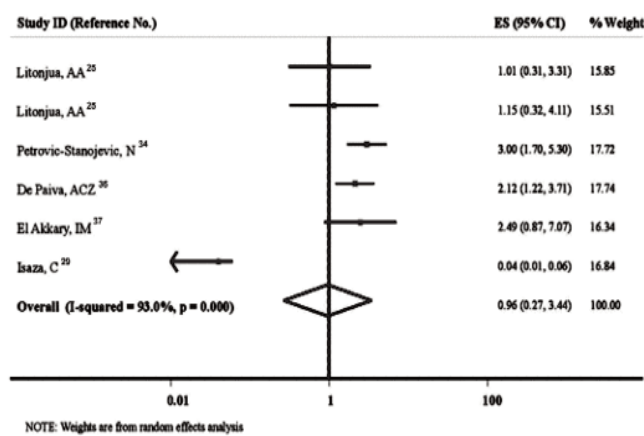


Figure-7: Forest plot of the association between Gln27Gln and asthma.

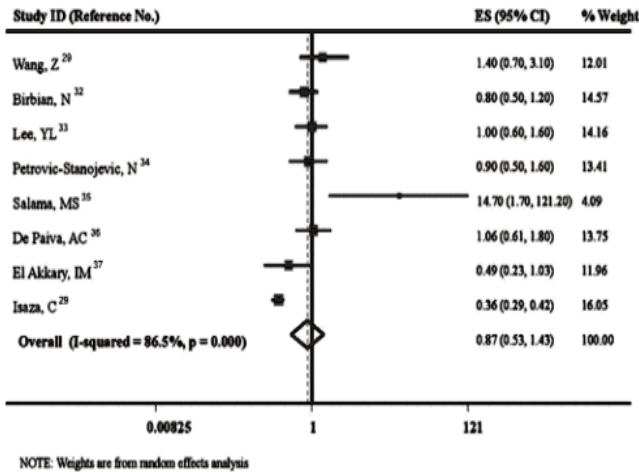


Figure-8: Forest plot of the association between Arg16 Alleles and asthma.

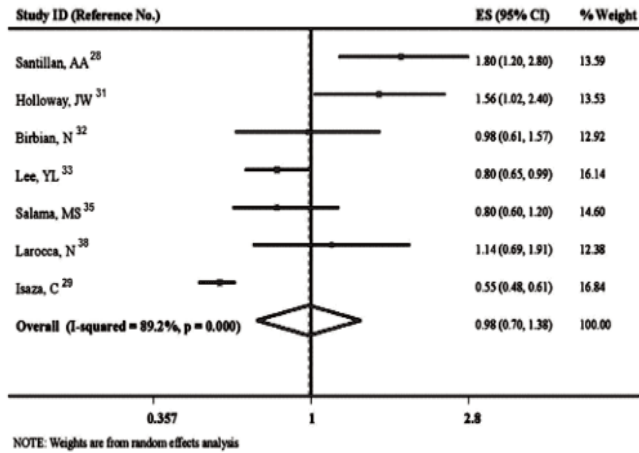


Figure-9: Forest plot of the association between Gly16 Alleles and asthma.

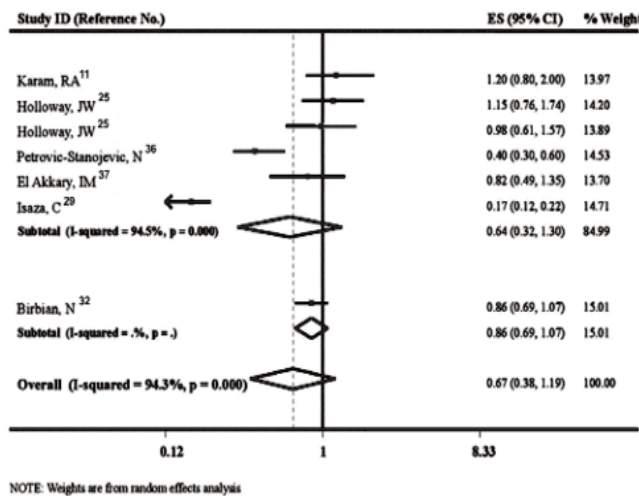


Figure-10: Forest plot of the association between Glu27 Alleles and asthma.

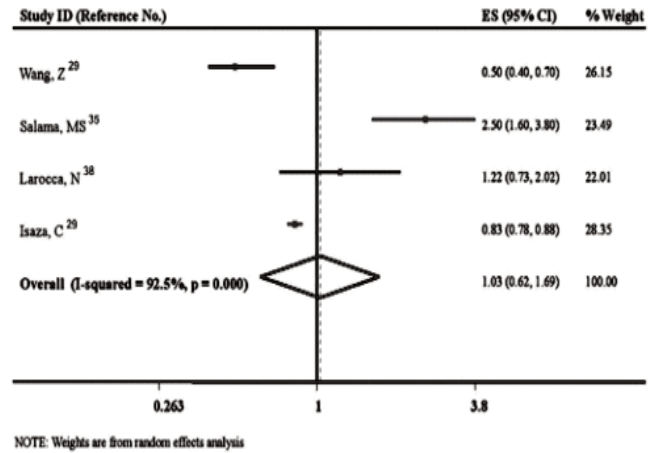


Figure-11: Forest plot of the association between Gln27 Alleles and asthma.

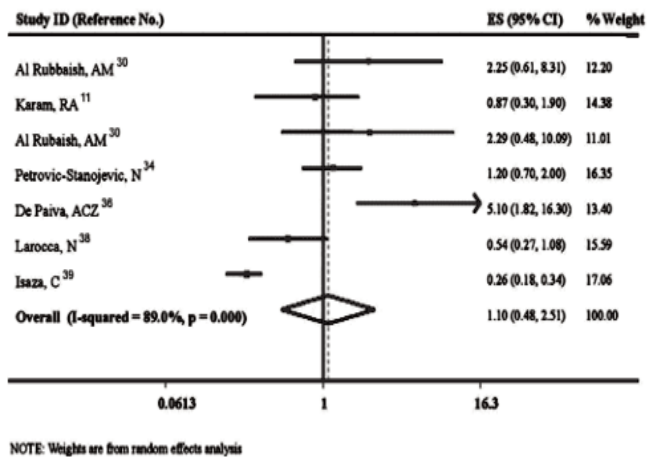


Figure-12: Forest plot of the association between Arg16Arg-gln27gln haplotypes and asthma.

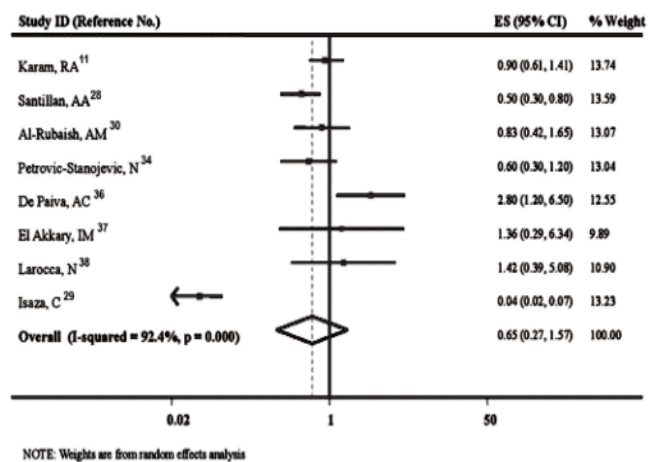


Figure-13: Forest plot of the association between Gly16Gly-Glu27Glu haplotypes and asthma.

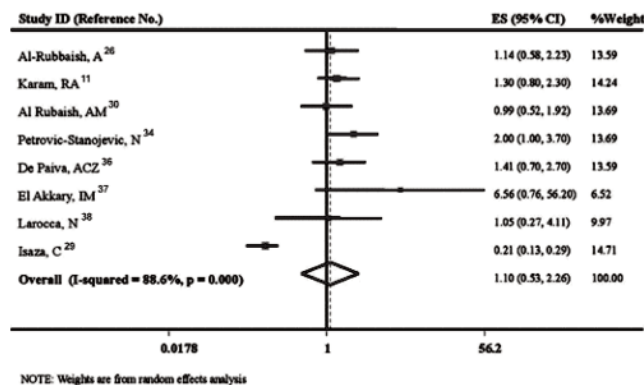


Figure-14: Forest plot of the association between Gly16Gly-Gln27Gln haplotypes and asthma.

Arg/Gly and Glu/Gln in most studies. No association of haplotypes found with asthma. These haplotypes were Arg16Arg-Gln27Gln (OR = 1.10, 95% CI = 0.48-2.51, $p=0.827$). Gly16Gly-Glu27Glu (OR = 0.65, 95% CI = 0.27-1.57, $p=0.341$) and for Gly16Gly-Gln27Gln OR = 1.10, 95% CI = 0.53-2.26, $p=0.801$ (Figures-12-14).

Discussion

The current meta-analysis is based on systematic review following the PRISMA guidelines. The strength of a systematic review is to include the articles which are not based on authors' choice but the number of articles retrieved from electronic databases. Therefore, it is dependent on the quality and sample size of studies included in the systematic review. A meta-analysis is a technique that pools the effect sizes of available scientific literature. A meta-analysis can be conducted on 2 or more articles. However, 10 is a good number to allow authors to conduct different tests in meta-analysis. The pooled analysis gives more precise results from individual studies due to higher sample size (combined sample of all included studies). In this study we included 17 studies with a total of 5,835 subjects (including children's, male and female). "

Pathology of asthma is marked by many cellular and molecular events, in which both genetics and environment play no small part. Of all the low-penetrance genes that are linked with asthma, either directly or indirectly, ADRb2 holds the promise of personalised therapy for asthma. Although the gene itself contains 49 sites with single nucleotide variation, Gly16Arg and Glu27Gln remain the most pertinent and studied single nucleotide polymorphisms of ADRb2. However, inconclusive and/or inconsistent findings of individual research endeavour investigating possible correlation between asthma and ADRb2 phenotypes has rendered

this area greyer than expected. Early pharmacogenetic studies in two cohorts of asthmatic children demonstrated that Arg16 homozygotes experience greater forced expiratory volume during the first second (FEV1) bronchodilation in response to one-time administration of albuterol compared with Gly16 homozygotes.¹⁴⁻¹⁶ Two retrospective pharmacogenetics studies of regular long-term albuterol therapy have also demonstrated that Arg16 homozygotes experience decline in peak expiratory flow rate (PEFR), whereas Gly16 homozygotes did not experience a change in PEFR during regular albuterol treatment.^{17,18} Similarly, Martinez et al. also found that children homozygous for Arg16 were 5.3 times more likely to respond to a single dose of albuterol than the children who were homozygous for Gly16.¹⁹

Recombinant cell studies and primary culture of human airway smooth muscle cells studies show that the Gly16 allele enhances agonist-promoted ADR β 2 down regulation while the Glu27 variant appear to play a protective role against it. The presence of Glu27 allele in Thai asthmatic patients is associated with a decreased asthma severity, higher % FEV1 values, less frequent hospitalisations and emergency visits, and decreased inhaled corticosteroid and a long-acting beta-agonist (ICS/LABA) usage.²⁰ Karam RA et al. noted that individuals with ADR β 2 Gln27 polymorphism may have increased airway hyper-responsiveness to endogenous catecholamine, resulting in increased airway sensitivity to proinflammatory stimuli leading to extent for long-term airway inflammation.¹¹

The purpose of this meta-analysis was to combine and analyse the results from different but related studies to analyse the data in bigger cohorts and to enhance the reliability. All the studies fulfilling our inclusion criteria were included. Publication bias was detected using funnel plot and Egger's test. Systematic review and random effect meta-analysis was performed using PRISMA guidelines. In this analysis, we combined and analysed relevant case-control studies and found no association between adrenergic β 2 receptor polymorphisms (Arg16Gly and Glu27Gln) with asthma in overall samples.

The findings of the current study are consistent with those of Mitigia and Contopoulos-Ioannidis^{21,22} where the odds ratios were non-significant using random effect both for Arg16Gly and Glu27Gln. Ammarin Thakkinian who found protective effect of heterozygous Gln/Glu for asthma among adults but found no significant association for Arg/Gly.²³ A meta-analysis conducted by Yaron Finkelstein found significant association between favourable therapeutic response of inhaled beta-agonists

and asthmatic children with Arg/Arg phenotypes at position 16 compared to Arg/Gly and Gly/Gly.²⁴ Si-Qiao Liang et al. found that none of ADR β 2 polymorphism reproducibly associated with risk of asthma among different ethnic populations.¹³ A total of five meta-analyses have been performed to date evaluating the association of ADR β 2 phenotypes with asthma. We included only case-control studies and also added certain studies that weren't analysed in the previous meta-analyses. Those individual studies having multiple data are divided into two by their definition of cases. The difference in the number of studies in each forest plot is due to the availability of data regarding that plot.

Some publications are not included because of insufficient statistical results that may bias the results. We could not analyse drug response because that was reported in fewer articles. Due to lesser number of articles we could not do sub group analysis. We did not use generalised additive model, which is considered more suitable for prediction, on account that the present study was a case-control review rather than a clinical trial.

Conclusions

Arg16Gly and Glu27Gln polymorphism did not bear any significant association with asthma. High-quality and well-designed studies with large sample size and various ethnicities should be conducted to confirm these results.

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Conflict of Interest: None.

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