SYSTEMATIC REVIEW AND META-ANALYSIS OF ASSOCIATION OF β2-ADRENERGIC RECEPTOR GENE POLYMORPHISMS WITH NOCTURNAL ASTHMA

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ABSTRACT

Objective: To integrate the quantitatively existing evidences on nocturnal asthma and their association with β2-adrenergic receptor (Arg16Gly and Glu27Gln) polymorphism.

Methodology: The whole procedure for systematic review and meta-analysis was conducted following PRISMA guidelines. The following databases Ovid, PubMed, Wiley online library, Springer link and the Web of Knowledge were searched for relevant articles. Random effect meta-analysis was conducted. Heterogeneity was evaluated by I2 statistic. The funnel plots and the Eager tests were used to assess the small study biases and potential publications.

Results: This systematic review and meta-analysis comprised a total of 9 studies. The brief statement estimates suggested heterozygous Arg/Gly (OR =2.32; 95% CI =1.53-3.54; P value = <0.001), homozygous Gly (OR =4.78; 95% CI =2.60-8.77, P-value =<0.001) and Gly allele (OR =1.71, 95% CI =1.14-2.56, P-value =0.009) was significantly associated with nocturnal asthma. While Glu27Gln polymorphism did not showed significant association with nocturnal asthma both in genotype (OR =1.28; 95% CI =0.63-2.59; P-value =0.496) and allelic (OR =1.65; 95% CI =0.64-4.27; P-value =0.301) analysis.

Conclusion: Heterozygous Arg/Gly, homozygous Gly/Gly and Gly Allele were found to be significantly associated with nocturnal asthma.

Key Words: Nocturnal, Asthma, Genotype, Allele, Polymorphism, Meta-analysis

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INTRODUCTION

The caliber of the human airways is not a constant. It may increase during day and decreased at night. Evidence shows that this circadian fluctuation in the caliber of both upper and lower airways is amplified in disease state, such as asthma. The question always in mind is why most of the in-hospital sudden death and ventilator arrest of asthmatics occur at night[1]? Nocturnal asthma (a unique subset of patient with asthma) is of particular interest because patient with this disease show that their caliber of airways decreases and causes peak dyspnea and wheezing between 2 and 6 AM[2]. A decrease in FEV1 (forced expiratory volume in 1 second) of atleast 15% at late night differentiates nocturnal asthma from general asthma. Some patients experience this variability in lung function upto 20% between bed and awakening times[3]. The major complaint regarding nocturnal asthma is difficulty in sleeping due to exacerbation at night that leads to poor quality of sleep[4,5]. Nocturnal exacerbation of asthma is common and clinically important asthma phenotypes that affects more than two third of the patients[6,7]. In reality, asthma causes more deaths at night time than day[8]. Thus nocturnal asthma is a hazardous and lethal expression and indicator of asthma that alarm and warrants recognition and treatment[9,10].

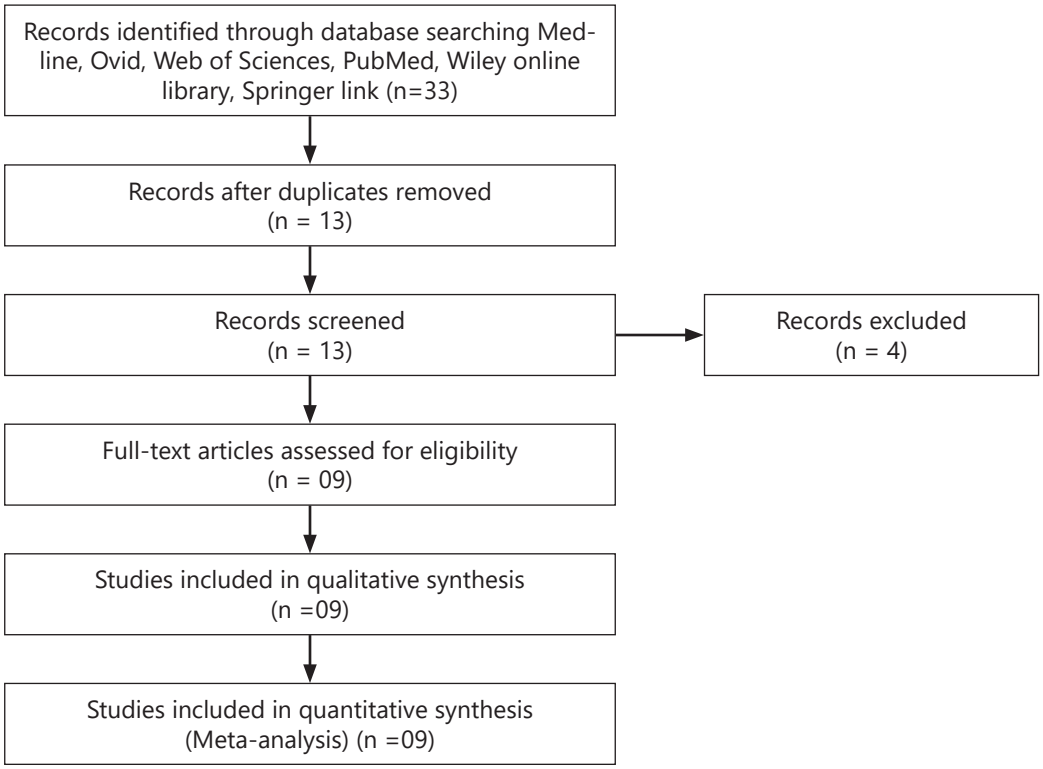
Asthma is heterogeneous in its responsiveness to common pharmacological therapies. Between 70 to 80% of patients with asthma has a variable response to common anti-asthma therapies, whether beneficial or detrimental[11,12]. The β2-adrenergic receptor agonists e.g. beta agonists are the first line and most commonly prescribed drugs among anti-asthma therapy for man-

agement of asthma and other pulmonary disorders[13]. Inhaled β2-adrenergic agonists are the main stay of treatment of acute asthma but response is highly variable and difficult to predict[14]. There are several factors e.g. age, disease severity and environmental exposure that have predisposed the response of β2-adrenergic agonists[14,15], but these don't explain a complete mechanism for drug resistance. The potential mechanism of resistance to β2 agonist is believed to be due to polymorphism in β2AR gene (human genome organization nomenclature [HUGO] named ADRβ2), which is most widely studied candidate gene[16-18]. β2-adrenergic receptor is the product of 1242-base intronless gene, located on long arm of chromosome 5q31-32[19, 20]. ADRβ2 was first sequenced approximately 20 years ago. ADRβ2 is a small, gene with one exon that encodes for a 413-amino acid, G-protein coupled receptor, the β2-adrenergic receptor[21, 22]. At least 9 single nucleotide polymorphisms (SNP's) have been known in the exonic gene region[23]. The single nucleic acid substitutions in gene at position 46 and 79 leads to substitution of glycine (G) to arginine (A) at position 16 and at aminoacid 27 it substitute glutamate for glutamine. These two polymorphisms at position 16 & 27 are found in high allelic frequency in general population[24]. These polymorphisms have been found to alter the receptor function by site-directed mutagenesis and recombinant expression studies, including substitutions of glycine for arginine at amino acid position 16 (Arg16/Gly16), glutamic acid for glutamine at position 27 (Gln27/Glu27)[25, 26]. Some studies suggest that the Gly16 allele showed enhanced downregulation of β2-AR, whereas the Glu27 allele was relatively resistant to downregulation of β2-AR during exposure to β2-AR agonists[26]. Children with homozygous Gln27 developed significantly greater bronchodilator desensitization to β2-AR agonist than those with homozygous Glu27[27]. Due to significance β2 - adrenergic receptor a lot of efforts have been done to find out whether these polymorphism in may β2-adrenergic receptor may mark susceptibility to nocturnal asthma. We conducted meta-analysis with more studies on ADRβ2 polymorphism and nocturnal asthma in order to approximate the level to which results of different studies coincide or not and to attain at some common assessment's for strength of proposed association.

METHODOLOGY

We performed systematic review of published literature in accordance with the preferred reporting items for systematic review and meta-analysis (PRISMA) guidelines (http://www.prisma-statement.org) as shown in figure 1. The relevant search terms used for genotype, allele and haplotypes frequency were "beta2* or β2 AND prevalence AND gene". For Association between nocturnal asthma and gene polymorphism the search term used was: "Nocturnal Asthma* AND Polymorph*

Figure 1: PRISMA flow chart



or mutation or variant AND beta2* or β2 or ADRβ2 or Adrenergic receptor β2". Two independent reviewers used the search terms and removed the duplicate. The search was started from 1st November 2014 to 1st February 2015. The total search was done in 3 months. The electronic search was limited to studies conducted only on humans and translated to or written in English language. The studies who had evaluated the association between nocturnal asthma and ADRβ2 polymorphism estimated at codon 16 (Arg16Gly) and at codon 27(Glu27Gln) or one of these were included in this meta-analysis. For each study data were extracted by two independent reviewers and agreement were made on all items, author, year of publication, journal, country of origin, ethnic background, phenotypes assessed, study design and whither the results of genotypes, distribution of alleles and haplotypes validated. Any disagreement on items was reviewed by third author.

We conducted a random-effects meta-analysis of the association between ADRβ2 polymorphism in asthmatic patients compared to the non-nocturnal asthmatics and healthy control. To assess the degree of heterogeneity x^2 (Chi-square) statistics were calculated as shown in table 2. All statistical analysis was performed using SATA version 13 (Stata corps, college station Texas). The basic characteristics of the case-control studies examining the association between ADRβ2 polymorphism and nocturnal asthma is shown in table 1.

RESULTS

We included those studies that provided genotype and allelic data for polymorphisms of Arg16Gly and Glu27Gln of subjects with nocturnal asthma. Studies

Table 1: Basic characteristics of case-control studies examining the association between ADRβ2 polymorphism and nocturnal asthma

First Author	Year of Publication	Country	Gender	Age of Cases	Sample Size	Genotyping Method	Definition of Cases
Kaisheng Yin(28)	2006	China	Male-Female	34.2±1.7	119	PCR-Allele specific	Five consecutive bed time-to-morning peak flow decrements of ≥20%
RA Karam(29)	2013	Egypt	Male-Female	10.3±2.4	200	PCR-Allele Specific	Documented fall in PEFR of 20% or more on at least 4 of 7 nights of testing at home.
Abdullah M. Al-Rubaih(30)	2010	Saudi-Arabia	Male	11.4±4.6	130	PCR-RFLP	Participants should be well known nocturnal asthmatics
Jamal Turki(31)	1994	U.S.A.	Male-Female	33.9±1.3	45	PCR-Allele Specific	Five consecutive bed time-to-morning peak flow decrements of ≥20%
Ming-Yung Lee(26)	2010	Taiwan	Male-Female	9.9±3.3	51	PCR-RFLP	Documented fall in PEFR of 20% or more on at least 4 of 7 nights of testing at home.
Gao jinming(32)	2002	China	Male-Female	38.67±13.83	221	PCR-Allele specific	Patient having family history of asthma
Shachor(33)	2003	Israil	Male-Female	29	119	PCR-ARMS	Nocturnal asthma attacks at least four nights a week over a period of at least 6 consecutive months.
Santillan(34)	2003	Mexico	Male-Female	42±14	907	PCR-RFLP	History of nocturnal asthma defined as history of awakening caused by asthma at least once per week for 12 consecutive months.
Luming(35)	2004	China	Not defined	Not defined	49	PCR-Sequencing	All of the subjects were diagnosed with asthma in line with Chinese medical association.

Table 2: Pooled estimate of the odds ratio for the association between ADRβ2 gene polymorphism and nocturnal asthma reference to non-nocturnal and healthy control

Gene Polymorphism	Pooled estimates		Heterogeneity	
	OR (95% CI)	P value	I2 (%)	P value
Arginine Genotype				
Homozygous Arg/Arg	1			
Heterozygous	2.32(1.53-3.54)	<0.001	0.0	0.472
Homozygous Gly/Gly	4.78(2.60-8.77)	<0.001	23.0	0.246
Glutamic acid Genotype				
Heterozygous	1.01(0.57-1.77)	0.980	0.0	0.594
Homozygous Glu/Glu	1.28(0.63-2.59)	0.496	66.9	0.004
Homozygous Gln/Gln	Reference			
Arginine Allele				
Homozygous Arg/Arg	Reference			
Homozygous Gly/Gly	1.71(1.14-2.56)	0.009	58.3	0.048
Glutamic acid Allele				
Homozygous Glu/Glu	1.65(0.64-4.27)	0.301	86.8	0.00
Homozygous Gln/Gln	Reference			

having incomplete genotype and allelic data and asthma definitions not according to GINA guidelines were excluded from the study. The electronic search identified 33 publication by using five different databases PubMed, Ovid, Web of Sciences, Wiley online library and Springer link. When combined and 20 publications excluded due to duplication, we got 13 publications. After reviewing the abstract of these articles, we found 4 publications were not fulfilling our criteria. Full texts of remaining 9 articles were assessed for eligibility. All of them were according to our inclusion criteria and were preceded for Qualitative synthesis. All studies included providing complete information regarding statistical analysis, polymorphism, study design and association. Ultimately all 9 articles met the inclusion criteria. The selected studies are shown in table 1. Three studies Lee MY et al, Gao JM et al and Schachor et al provide data only on Genotype distribution. Off these 09 studies one article by Santillan et al provided data on allele frequency and not on genotype distribution.

There was significant association between nocturnal asthma and Arg/Gly 16 polymorphism in genetic model of studied population (OR =2.32, 95% CI =1.53-3.54, P value =<0.001 (Figure 2.1). Genotype distribution of homozygous Gly polymorphism also showed significant association with nocturnal asthma having (OR =4.78, 95% CI=2.60-8.77 and P value =<0.001) (Figure 2.2). In case of allelic distribution Gly is significantly associated with nocturnal asthma (OR =1.71, 95% CI=1.14-2.56, and P value 0.009) (Figure 2.3). In case of genotypic distribution at Gln/Glu 27 we found no significant association of nocturnal asthma with heterozygous Gln/Glu polymorphism (OR =1.28, 95% CI =0.63-2.59, P value =0.496 (Figure 2.4). Homozygous Glu also found no-significant genotypic association (OR =1.01, 95% CI =0.57-1.77, P value =0.980) with nocturnal asthma (Figure 2.5). Non-significant association was found for allele of Glu with nocturnal asthma (OR=1.65, 95% CI =0.64-4.27, P value =0.301) (Figure 2.6).

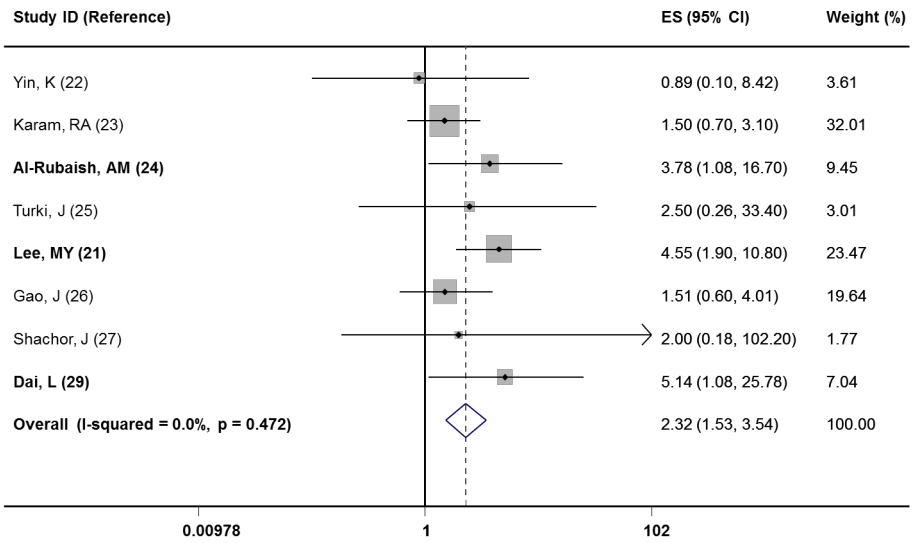
Potential publication bias was investigated using funnel plot and Eager's test. No evidence of publication bias was found in case of heterozygous Arg/Gly, homozygous Glu, and homozygous Gly.

DISCUSSION

Nocturnal asthmatics are sole subgroup of asthmatics who experience worsening symptoms and airflow obstruction at night. The basic knowledge of this phenotype is still unknown, but β2-adrenergic receptors are believed to decrease overnight in nocturnal asthmatics as compared to healthy and non-nocturnal asthmatics. Two common ADRB2 gene polymorphisms and their association with nocturnal asthma have been studied for their possible association with asthma-related phenotypes, however conflicting data exist regarding their clinical importance and effect in different populations.

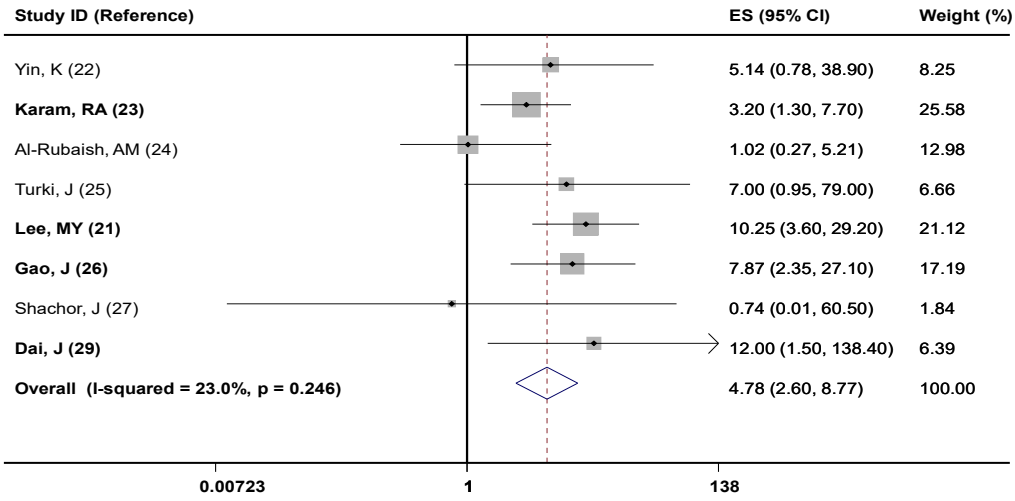
Two different ADRβ2 polymorphisms (Arg16Gly and Gln27Glu) and their genotypes and allele observed to make the conclusive findings. Based on data from 9 studies, this meta-analysis showed that heterozygous Arg/Gly (OR =2.32, 95% CI=1.53-3.54, P value =<0.001) was significantly associated with nocturnal asthma. Homozygous Gly were also found significantly related to

Figure 2.1: Heterozygous Arg-Gly



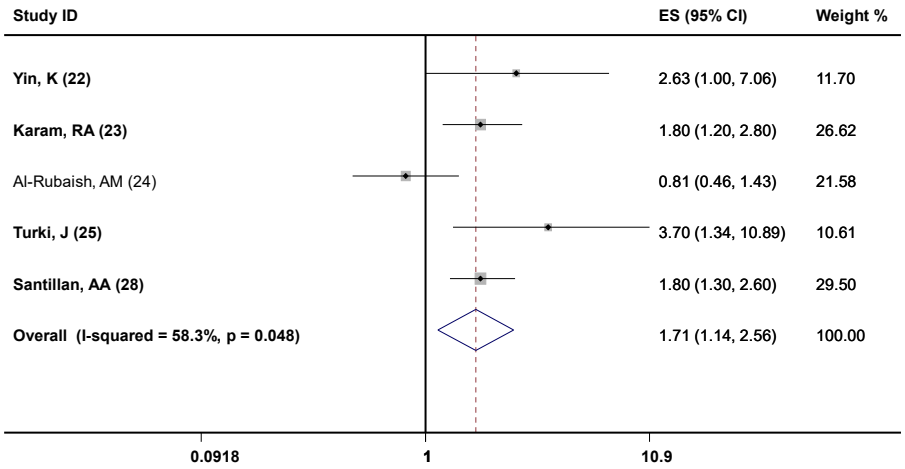
Note: Weights are from random effects analysis

Figure 2.2: Homozygous Gly



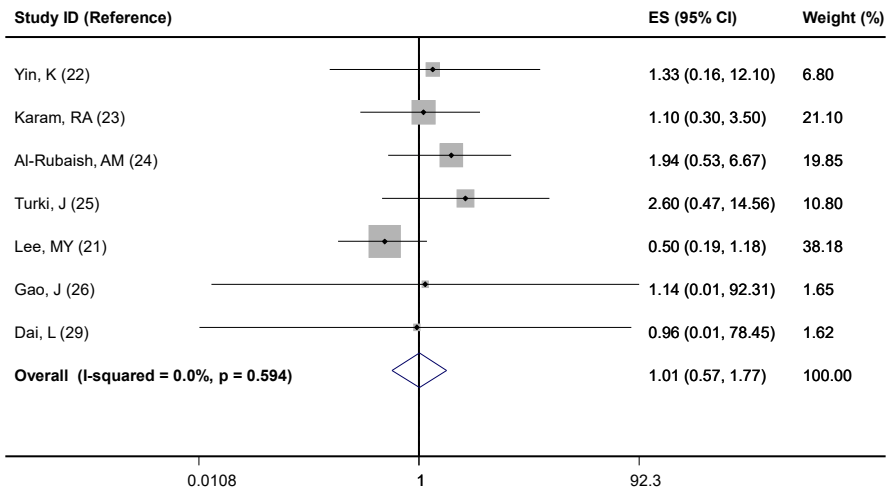
Note: Weights are from random effects analysis

Figure 2.3: GLY allele



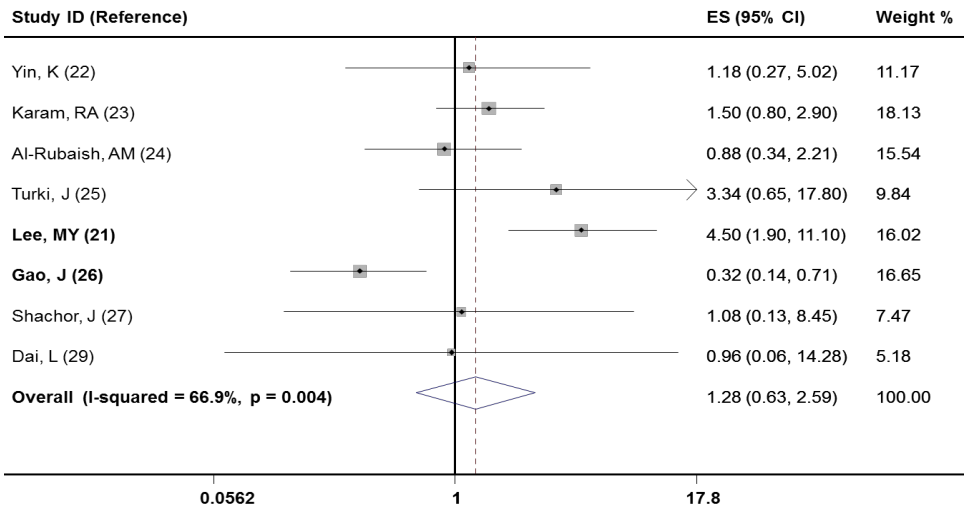
Note: Weights are from random effects analysis

Figure 2.4: Heterozygous Glu-Gln



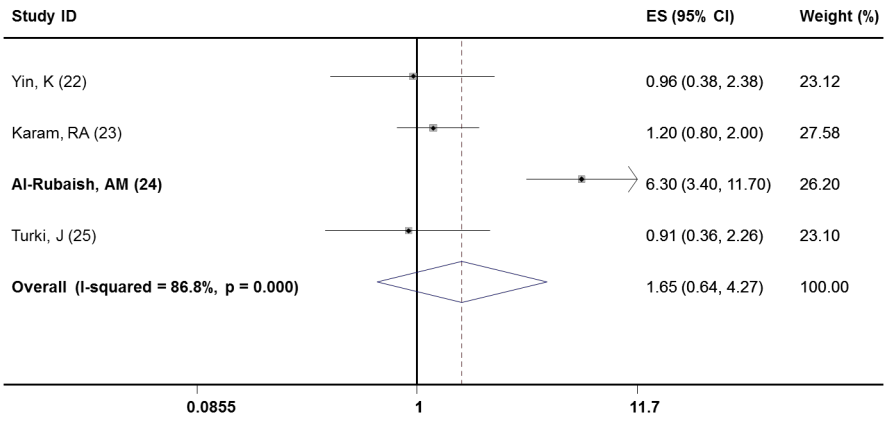
Note: Weights are from random effects analysis

Figure 2.5: Homozygous Glu



Note: Weights are from random effects analysis

Figure 2.6: GLU allele



Note: Weights are from random effects analysis

nocturnal asthma while non-significant association of both genotype and allele of Glu27Gln found with nocturnal asthma.

There has been one systematic review and meta-analysis on association between ADRβ2 polymorphism with nocturnal asthma in 2005 but that was mainly focused on Gly. Our study is also consistent with meta-analysis conducted by Contopoulos-Loannidis et al who suggested association of Arg/Gly polymorphism could possibly associated with nocturnal asthma. Most of the published literatures have found significant association of either genotype or allele of ADRβ2 gene polymorphism with nocturnal asthma, but some studies have recorded very poor or weak link. Our study was conducted in accordance with PRISMA guidelines and we searched five databases to ensure that we identified all relevant studies.

The research studies on orientation mutagenesis and recombinant expression revealed that Gly16 β2-AR could strengthen the down regulation effect predisposed by the β2-agonist, which aroused speculation that Gly16 might be related to the downregulation of β2 -AR in patients with nocturnal asthma[28]. It has also been found that Gln27 Polymorphism may lead to increased airway hyper-responsiveness to endogenous catecholamine, resulting in increased airway sensitivity to pro-inflammatory stimuli leading to extent of long term airway inflammation[29]. This meta-analysis had focused on heterozygous and homozygous genotypes and alleles of Arg and Gln SNP's, which was not discussed previously. We have also included latest studies in our meta-analysis to make conclusive results.

LIMITATION

Only 9 papers were available, could do further analysis as meta-influence, eta-cumulative, meta-regression, meta-bias due to lesser than 10 studies.

CONCLUSION

The current meta-analysis suggests that heterozygous Arg16Gly, homozygous Gly and Gly allele polymorphism is associated with risk of asthma. This systematic review and meta-analysis suggests that ADRβ2 gene is an important contributing factor of specific asthma phenotypes especially nocturnal asthma.

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CONTRIBUTORS

IK conceived the idea, planned the study, and drafted the manuscript. ZUH, NA, AS, MZA and SS Helped in drafting the manuscript and did statistical analysis. NA and RA critically revised the manuscript. All authors contributed significantly to the submitted manuscript.