Investigating possible effects of aryl hydrocarbon receptor G1661A polymorphism on asthma severity in adults

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Aryl hydrocarbon Receptor (AhR) is a ligand-activated transcription factor with an important role in lung health. The association of AhR polymorphisms with asthma severity has not been yet investigated. We analyzed the association of G1661A, the most prevalent polymorphism of AhR, with the asthma stages in a population-based study including 555 asthmatics (Intermittent: 93, Mild: 240, Moderate: 158, and Severe: 64). The SNP was genotyped using allele-specific PCR. Obtained data were analyzed using the Generalized-Ordered Logit Estimates. Genotypes GA (OR: 0.53, CI: 0.32-0.90, P=0.019) and AA (OR: 0.22, CI: 0.06-0.76, P=0.017) were associated with decreased risk of Severe, Moderate, Mild *vs.* Intermittent stage; and Severe, Moderate, *vs.* Mild, Intermittent stages respectively. However, Genotype GA (OR: 1.90, CI: 1.05-3.44, P=0.033), dominant model GA+AA (OR: 2.04, CI: 1.17-3.57, P=0.012), and allele A (OR: 1.68, CI: 1.06-2.66, P=0.027) were associated with increased risk of Severe stage *vs.* Moderate, Mild, Intermittent stages. Also, male sex and higher age were associated with an increased odds ratio for severe asthma. Furthermore, significant associations with asthma stages were found for the interactions of the SNP and sex, smoking, and alcohol consumption. In conclusion, we revealed that the mutant allele of AhR-G1661A may interact with independent variables and act as a protective factor against lower stages of asthma but it may increase the risk of severe asthma.

Keywords: AhR gene, Lung diseases, Genetic association, rs2066853, Polymorphism

As a common chronic inflammatory disease asthma is characterized by reversible airway obstruction and bronchial hyperresponsiveness, which affects an estimated 300 million people worldwide[1,2]. It causes temporary and permanent disabilities, impairment in work productivity, and even death, which results in a huge amount of economic and social burden[3]. Despite the availability of precise diagnostic approaches and effective therapies, asthma often remains misdiagnosed[2]. Although researchers from different disciplines try to find reliable treating methods for asthma it is still considered as poorly controlled or uncontrolled disease condition[2,4,5].

Multiple factors have been introduced as contributors to asthma development including genetic predispositions, immunological aberrations, and involvement of noxious environmental factors[6]. Surprisingly, there is a 600 million years old

ubiquitously-expressed and evolutionarily-conserved regulatory protein, Aryl hydrocarbon Receptor (AhR), which connects the molecular pathways of those contributors[7]. AhR has an important role in different types of human diseases and malignancies[7,8]. As a ligand-activated transcription factor, AhR regulates mucosal barrier function and immune responsiveness in the lungs through modulation of gene expression and influencing cell-cell adhesion, mucin production, and cytokine expression[9]. Also, upregulation of pro-inflammatory cytokines response to environmental contaminants like Benzo(a)pyrene in the lung is an AhR-dependent proccess[10]. However, it has also an important role in the protection of the lung against chemical hazards and maintenance of its physiological homeostasis and health[9]. It has been postulated that understanding the AhR pathway may suggest some potential therapeutic options to prevent the progression of pulmonary diseases[11].

Considering the importance of genetic factors in susceptibility to asthma, recently the significance of genetic analysis in the disease diagnosis has gained

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increased attention[12]. As a type of genetic factor, single-nucleotide polymorphisms (SNPs) are involved in susceptibility to a variety of diseases and studies have led to the identification of several SNPs with significant associations with asthma pathogenesis and development[13-17].

Although exonic and intronic polymorphisms in the AhR pathway could greatly affect its function, however, the association of only a limited number of genetic variants in this pathway with human diseases has been studied yet[18-21]. Association of AhR polymorphisms with lung cancer and association of the polymorphisms in AhR regulated genes such as Glutathione-S-transferase and CYP1A1 with asthma risk has been investigated previously[22,23]. However, as far as we know SNPs in AhR have not been studied for the association with asthma development yet. Considering the special role of AhR in lung inflammation and metabolism of environmental toxicants, which both are major contributors to asthma development, analyzing the association of AhR genetic variations with asthma may provide valuable knowledge. Another important fact, which makes AhR polymorphisms significant targets for genetic studies on asthma is the emerging role of pharmacogenetics in asthma treatment and the central role of the AhR pathway in this discipline[24,25]. The most frequently studied polymorphism of the AhR gene is a "guanine to adenine" transition at position 1661 of AhR cDNA (G1661A), which results in Arg to Lys substitution at position 554 in the amino acid sequence of the protein (R554K) with possible effects on its interactions[26]. In this study, we evaluated AhR-G1661A SNP for the association with asthma severity in a sample population of Iranian-Azerbaijanis.

Materials and Methods

Study subjects and inclusion criteria

Asthmatic cases were selected from attended patients to the two offices of lung and respiratory disease specialists in Tabriz, Azarbaijan, Iran, between Sept 2017 and Nov 2019. Patients with chronic inflammatory diseases other than asthma, atopic diseases, tuberculosis, cancer, immune diseases, chronic obstructive pulmonary disease, allergy, and the use of hormones or immunosuppressive drugs were excluded. After the primary confirmation of asthma disease, the detailed clinical data and spirometry reports of included cases were reviewed and interpreted by two lung disease specialists independently for identifying the asthma stages based on the guideline of the National Institutes of Health[27]. Asthmatic participants were categorized into four classes including 64 (11.53%) severe, 158 (28.47%) moderate, 240 (43.24%) mild, and 93 (16.76%) intermittent asthma cases. Data of independent variables including age, sex, smoking, and alcohol consumption of the participants also were considered (Table 1). The study protocol was approved by the research ethics committee of the Tabriz University of Medical Sciences (IR.TBZMED.REC.1399.700) and all contributors gave written informed consent.

Blood sample collection, gDNA isolation, and Genotyping

Venous blood samples were collected from cases into sterile tubes containing sodium citrate. Total gDNA was isolated from blood samples by DNA extraction kit (Kalazist, Iran) following manufacturer instruction. PCR allele-specific amplification approach was considered for identifying genotypes of participants following previous descriptions with some minor modifications[28,29]. Figure 1 depicts the details of the genotyping method. PCR reactions were carried out in 20 uL volume of 2X Taq Master Mix

Characteristics	Intermittent (n=93)	Mild (n=240)	Moderate (n=158)	Sever (n=64)	P value	Total (n=555)
Age mean ± SD	39.45 ± 9.79	42.70 ± 11.95	43.84 ± 12.55	47.44 ± 13.37	0.001*	43.03 ± 12.13
Sex					0.013**	
Male	33 (35.48%)	90 (37.50%)	75 (47.47%)	36 (56.25%)		234 (42.16%)
Female	60 (64.52%)	150 (62.50%)	83 (52.53%)	28 (43.75%)		321 (57.84%)
Smoking					0.205**	
Yes	5 (5.38%)	20 (8.33%)	18 (11.39%)	9 (14.06%)		52 (9.37%)
No	88 (94.62%)	220 (91.67%)	140 (88.61%)	55 (85.94%)		503 (90.63%)
Alcohol consumption					0.604**	
Yes	8 (8.60%)	12 (5.00%)	11 (6.96.00%)	4 (6.25.00%)		35 (6.31%)
No	85 (91.40%)	228 (95.00%)	147 (93.04.00%)	60 (93.75.00%)		520 (93.69%)

Table 1 — Frequencies of independent variables in the included asthmatics

*ANOVA test. ** Fisher's exact test

Internal control
Forward primer: 5′-GGGCACGAAGGCTCATCATT-3′   Size
Reverse primer: 5′-GGCCCCTCCATCGTCCACCG-3′   493 bp

AHR SNP G1661A
Forward primer: 5′-ACTCTCTCAATCCTAGTTCC-3′
Reverse primer: 5′-TTTCATTCTGCATGTGTC-3′   G allele   333 bp
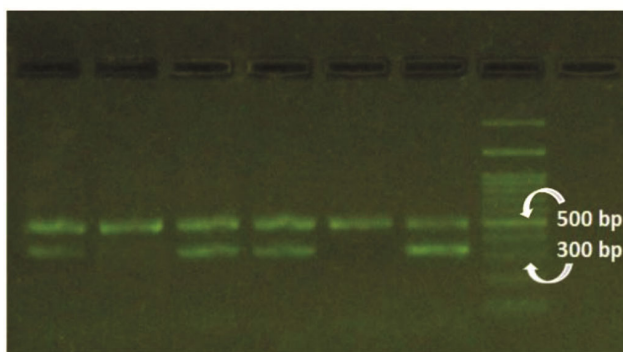Reverse primer: 5′-TTTCATTCTGCATGTGTT-3′   A allele   333 bp

Fig. 1 — The AhR-G1661A genotyping by allele-specific PCR method[28,29]. We considered two reaction tubes for each participant. Both tubes must amplify a 493-bp segment of the human actin gene as an internal control and each of the tubes contains a forward primer and the corresponding reverse primer of G1661A polymorphism of AhR which amplifies a 333-bp band or does not amplify any band according to the participant's genotype

RED (Ampliqon, Denmark), 10 pmol.L^{-1} of each primer (Takapouzist Co., Iran), and 50ng of genomic DNA as a template, overloaded with a drop of mineral oil. The touchdown PCR procedure was chosen for our purpose. The program began with an initial denaturation at 95°C for 5 min, and the first 15 cycles were programmed as follows; denaturation at 95°C for 30 sec, annealing at 62°C for 40 sec which drops 0.5°C in each cycle, and elongation at 72°C for 30 sec. For the following 22 cycles, parameters remained the same but the annealing temperature was considered 52°C for 40 sec. The program ended with elongation at 72°C for 5 min. All the PCR runs had negative (no DNA) control. All amplifications were performed in a Thermal Cycler (Peqlab PCR cycler Primus 96 advanced, Erlangen, Germany) and PCR products were subjected to electrophoresis (Peqlab horizontal electrophoresis, Erlangen, Germany) on a 2% agarose gel containing DNA safe stain (Sina-Clone®, Iran) and visualized by UV illumination. As depicted in Figure 1, the gel electrophoresis profile of allele-specific PCR product

discriminated the AhR-G1661A genotypes by the presence of 493 bp band, and presence or absence of 333 bp band on the gel (Fig. 1). The genotypes were identified without previous knowledge of the case or control status of the DNA sample. Blank control without template DNA and also positive and negative controls were included in each set of PCR tests. By failing any of these controls, the PCR was repeated for the batch of samples. Finally, 5% of the identified samples were randomly selected from cases and controls and were genotyped by different persons to examine the reproducibility of the genotyping procedure.

Statistical analysis

Independent variables including sex, smoking, alcohol consumption, and age were compared between the stages using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA) and analysis of variance (ANOVA) and Fisher's exact tests. For analyzing the association of AhR-G1661A SNP with asthma severity stages generalized ordered logit model was used. This method previously has been used in the study of SNP association with different stages of a disease condition[30]. All models were fitted using the statistical package the Stata software version 16 (College Station, TX: StataCorp LLC), and the Stata add- GOLOGIT2 was used extensively. The GOLOGIT2 program for generalized ordered logit models can fit three special cases of the generalized model: the proportional odds/parallel-lines model, the partial proportional odds model, and the logistic regression model[31]. In this analysis, we compared frequencies of the A allele, GA and AA genotypes, and GA+AA model between different stages of asthma disease: 1 (Intermittent), 2 (Mild), 3 (Moderate), and 4 (Severe) asthma. Also, the association of independent variables with the stages and interactions of the SNP with independent variables in different stages of asthma were analyzed (Table 5). This statistical modeling approach treats the scale as comprising ordinal categories. These models permit estimation of relationships between genotypes and asthma severity categories taking into account the ordinal nature of the outcome. They also permit adjustments for key independent variables such as age, sex, alcohol consumption, and smoking status. The main phenotype analyzed was a five-level ordinal categorical asthma stage 1, 2, 3, and 4. The SNP was a binary variable and measured independent variables included age, sex and smoking, and alcohol consumption categorized as

never smoked or drink as the reference category, and currently smoking and drinking. For an ordinal outcome with five categories, the model essentially consists of four standard logistic regression models fitted simultaneously, with the four categories collapsed into two in each logistic model and with the effect size (measured as an OR) estimated for three specific comparisons. In each of these comparisons the lower (less asthma severity) combined category is the reference group, so that an OR > 1 for a variable means that the odds of being in a higher (more severe asthma) category are increased for those with a greater value for that variable. Comparison A estimated the OR for 4, 3, 2 *vs*. 1, comparison B estimated the OR for 4, 3, *vs*. 2, 1, comparison C estimated the OR for 4, *vs*. 3, 2, 1. The comparison associations were expressed in unadjusted odds ratios and adjusted (OR_{adj}) for age, sex, smoking, and alcohol consumption with the corresponding 95% CI. A P value <0.05 was considered statistically significant. All data will be provided upon asking from the correspondent.

Results

Results of the ANOVA test showed that means of age between the stages are significantly different (Table 1). Also, the distribution of male and female sexes was significantly different between the stages (Table 1). However, there wasn't a significant difference in smoking and alcohol consumption between the stages (Table 1).

Table 2 presented the frequencies of genotypes and alleles in different stages of asthma. The generalized ordered logit model of association of AhR-G1661A with asthma severity discovered odds ratios (ORs) for comparisons A, B, and C (Table 3). GA and AA genotypes in comparison A, and B were associated with significantly decreased OR_{adj} respectively.

Therefore, genotype GA may protect the carriers from being in severity stages 4, 3, 2 *vs*. stage 1, and genotype AA may protect carriers from being in 4, 3 stages *vs*. stages 2, 1. However, for the most severe stage, 4, the genotype GA, GA+AA dominant model, and the A allele were associated significantly with increased OR_{adj} versus lower stages. The SNP may act as a decreasing factor for risk of lower stages of asthma, however, it may act as a risk factor for severe asthma (Table 3).

The generalized ordered logit model of association of age with asthma severity revealed that OR_{adj} of age were significantly higher than 1 in comparisons A, B, and C. This implied that the increase of age increases the probability of being in the more severe group of disease significantly (Table 4). Also, in comparisons B and C the OR_{adj} of male *vs*. female sex was significantly increased. Therefore, being male increased the probability of occurring in a higher severity class of asthma.

The outcome of those analyses that resulted in significant OR_{adj} for interactions between SNP and independent variables in asthma stages are presented in (Table 5). There were significant associations between SNP-variable interactions with asthma stages. Table 5 shows that female sex interaction with SNP was associated with decreased OR_{adj} asthma stages. In comparison A, OR_{adj} for GA genotype was 0.53 (Table 3) and for female sex was 0.67 (Table 4), which decreased to 0.33 for their interaction (Table 5). Also, the significantly decreased OR_{adj} for G and A alleles interaction with female sex may indicate that sex is an important factor in the SNP effect. Although the interaction of no-smoking condition and SNP was associated with decreased OR_{adj} in comparisons A and B, the OR_{adj} was increased for the severer stage in comparison C for the dominant model and no-smoking condition could just lower OR_{adj} slightly from 2.04 for

Table 2 — Frequencies of rs2066853 genotypes and alleles in asthma stages

Genotype/Allele	Intermittent		Mild		Moderate		Severe		Total	
Population	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent
Genotypes										
GG	62	66.7	189	78.8	127	80.4	43	67.2	421	75.9
GA	26	28.0	37	15.4	28	17.7	21	32.8	112	20.2
AA	5	5.4	14	5.8	3	1.9	0	0.0	22	4.0
Total	93	100	240	100	158	100	64	100	555	100
Alleles										
G	154	82.8	412	85.8	272	86.1	99	77.3	937	84.4
A	32	17.2	68	14.2	44	13.9	29	22.7	173	15.6
Total	186	100	480	100	316	100	128	100	1110	100

Table 3 — Generalized ordered logit estimates on the association of rs2066853 with asthma severity.

Comparison A (4, 3, 2 *vs*. 1)

Genotype/Allele	Unadjusted OR (95% CI)	P	OR$_{adj}$ (95% CI)	P*
GG	Ref.		Ref.	
GA	0.57 (0.34-0.96)	0.033	0.53 (0.32-0.90)	0.019
AA	0.59 (0.21-1.65)	0.312	0.55 (0.19-1.58)	0.267
GA+AA	0.76 (0.47-1.24)	0.247	0.71 (0.43-1.17)	0.174
G	Ref.		Ref.	
A	0.87 (0.57-1.32)	0.505	0.81 (0.53-1.24)	0.332

Comparison B (4, 3 *vs*. 2,1)

GG	Ref.		Ref.	
GA	1.15 (0.75-1.75)	0.520	1.09 (0.71-1.68)	0.689
AA	0.23 (0.07-0.80)	0.021	0.22 (0.06-0.76)	0.017
GA+AA	1.23 (0.83-1.80)	0.302	1.19 (0.80-1.77)	0.381
G	Ref.		Ref.	
A	1.11 (0.80-1.55)	0.521	1.09 (0.78-1.52)	0.631

Comparison C (4 *vs*. 3, 2, 1)

GG	Ref.		Ref.	
GA	2.03 (1.15-3.59)	0.015	1.90 (1.05-3.44)	0.033
AA	-	-	-	-
GA+AA	2.05 (1.19-3.53)	0.01	2.04 (1.17-3.57)	0.012
G	Ref.		Ref.	
A	1.70 (1.09-2.67)	0.020	1.68 (1.06-2.66)	0.027

*OR (95% CI) adjusted for age, sex, smoking, and alcohol consumption.Intermittent asthma (1), Mild asthma (2), Moderate asthma (3), and Severe asthma (4)

Table 4 — Generalized ordered logit estimates on the association of independent variables with asthma severity

Comparison A (4, 3, 2 *vs*. 1)

Variable	Unadjusted OR (95% CI)	P	OR$_{adj}$ (95% CI) *	P
Sex (Male *vs*. Female)	1.40 (0.88-2.22)	0.154	1.50 (0.90-2.50)	0.123
Sex (Female *vs*. Male)	0.71 (0.45-1.13)		0.67 (0.40-1.12)	
Age	1.03 (1.01-1.05)	0.002	1.03 (1.01-1.05)	0.004
Smoking (Yes *vs*. No)	1.99 (0.77-5.16)	0.155	2.24 (0.76-6.61)	0.143
Drinking (Yes *vs*. No)	0.66 (0.29-1.50)	0.321	0.41 (0.15-1.08)	0.071

Comparison B (4, 3 *vs*. 2, 1)

Sex (Male *vs*. Female)	1.71 (1.21-2.41)	0.002	1.66 (1.15-2.40)	0.007
Sex (Female *vs*. Male)	0.59 (0.41-0.83)		0.60 (0.42-0.87)	
Age	1.02 (1.01-1.04)	0.004	1.02 (1.00-1.03)	0.009
Smoking (Yes *vs*. No)	1.71 (0.96-3.02)	0.068	1.31 (0.70-2.43)	0.396
Drinking (Yes *vs*. No)	1.13 (0.57-2.27)	0.722	0.84 (0.40-1.79)	0.658

Comparison C (4 *vs*. 3, 2, 1)

Sex (Male *vs*. Female)	1.90 (1.12-3.22)	0.016	1.80 (1.03-3.14)	0.04
Sex (Female *vs*. Male)	0.53 (0.31-0.89)		0.56 (0.32-0.97)	
Age	1.03 (1.01-1.05)	0.002	1.03 (1.01-1.05)	0.007
Smoking (Yes *vs*. No)	1.70 (0.79-3.69)	0.175	1.19 (0.53-2.69)	0.67
Drinking (Yes *vs*. No)	0.99 (0.34-2.90)	0.984	0.79 (0.26-2.42)	0.682

* OR (95% CI) adjusted for the other variables. Intermittent asthma (1), Mild asthma (2), Moderate asthma (3), and Severe asthma (4)

Table 5 — Generalized ordered logit estimates on the genotypes/allele interactions with independent variables

Interactions	Severity stages	Unadjusted OR (95% CI)	P	OR$_{adj}$ (95% CI) *	P
Genotype#Sex					
GA#Female	Comparison A (4, 3, 2 *vs.* 1)	0.36 (0.17-0.77)	0.008	0.33 (0.15-0.72)	0.005
Allele#Sex					
G#Female	Comparison A (4, 3, 2 *vs.* 1)	0.67 (0.46-0.96)	0.029	0.61 (0.41-0.90)	0.014
G#Female	Comparison B (4, 3 *vs.* 2,1)	0.64 (0.49-0.83)	0.001	0.66 (0.50-0.87)	0.003
A#Female	Comparison B (4, 3 *vs.* 2,1)	0.53 (0.33-0.87)	0.011	0.55 (0.33-0.91)	0.019
G#Female	Comparison C (4 *vs.* 3, 2, 1)	0.52 (0.34-0.79)	0.002	0.56 (0.36-0.86)	0.009
Genotype#Smoking					
GA#NO	Comparison A (4, 3, 2 *vs.* 1)	0.62 (0.36-1.07)	0.085	0.57 (0.33-0.99)	0.045
AA#NO	Comparous B (4, 3 *vs.* 2,1)	0.17 (0.04-0.75)	0.02	0.16 (0.04-0.73)	0.018
GA+AA#NO	Comparison C (4 *vs.* 3, 2, 1)	1.99 (1.11-3.58)	0.021	1.98 (1.09-3.61)	0.026
Allele#Smoking					
G#YES	Comparison A (4, 3, 2 *vs.* 1)	2.36 (1.07-5.21)	0.034	2.58 (1.08-6.14)	0.033
Genotype#Alcohol					
GA#YES	Comparison A (4, 3, 2 *vs.* 1)	0.14 (0.04-0.54)	0.004	0.05 (0.01-0.30)	0.001
AA#NO	Comparison B (4, 3 *vs.* 2,1)	0.16 (0.04-0.69)	0.014	0.15 (0.03-0.67)	0.013
GA#NO	Comparison C (4 *vs.* 3, 2, 1)	2.14 (1.19-3.86)	0.011	1.98 (1.08-3.65)	0.028
GA+AA#YES	Comparison A (4, 3, 2 *vs.* 1)	0.30 (0.10-0.95)	0.041	0.08 (0.01-0.49)	0.006
GA+AA#NO	Comparison C (4 *vs.* 3, 2, 1)	2.08 (1.18-3.65)	0.011	2.04 (1.14-3.64)	0.016
Allele#Alcohol					
A#YES	Comparison A (4, 3, 2 *vs.* 1)	0.35 (0.12-1.06)	0.062	0.14 (0.04-0.52)	0.004
A#NO	Comparison C (4 *vs.* 3, 2, 1)	1.73 (1.09-2.76)	0.021	1.70 (1.06-2.74)	0.028

* OR (95% CI) adjusted for the other variables. Intermittent asthma (1), Mild asthma (2), Moderate asthma (3), and Severe asthma (4)

GA+AA (Table 3) to 1.98 (Table 5). Also, smoking interaction with the G allele showed an increased effect on OR$_{adj}$ in comparisons A. GA genotype and dominant model interactions with alcohol consumption were associated with decreased OR$_{adj}$ in comparisons A. However, the interaction of no-alcohol consumption condition with AA genotype in comparisons B was associated with decreased OR$_{adj}$. In comparison C, the interaction of the no-alcohol consumption condition with the dominant model was associated with increased OR$_{adj}$. Finally, the results for A allele interaction with alcohol consumption were decreased OR$_{adj}$ for Yes in comparisons A and increased OR$_{adj}$ for NO in comparisons C.

Discussion

Although environmental factors are important determinants of asthma, numerous studies have discovered that asthma has a strong genetic component and genetic factors are also important in its etiology[6,12]. Among these factors, polymorphisms

are important contributors, which affect human susceptibility to lung diseases as well as asthma. Discovering candidate SNPs provides opportunities to further identify individuals with a higher risk for asthma development and confirmed SNPs could help to the invention of new diagnostic and therapeutic approaches[12,17].

It has been revealed that exposures to xenobiotics are associated with an increased risk of asthma and recently, the effects of SNPs in the components of xenobiotics metabolism pathway on asthma development and severity have attracted lots of research interest[32,33]. However, until now these studies were almost limited to the association of polymorphisms in biotransformation enzymes such as GSTP1, PON1, UGT1A6, EPHX1, and different types of CYP, and the main regulatory genes of xenobiotics metabolism pathway such as AhR, ARNT, and AhRR have not been considered[34–36].

AhR is an important ligand-activated transcription factor with particular involvement in asthma

immunopathophysiology and pulmonary health maintenance and diseases, which make it a very special target for genetic studies of asthma[9,11,37]. AhR plays a connecting role between the outside of the cells and inside molecular pathways with both beneficial and deleterious outcomes in asthma. Its activation in bronchial epithelial cells and dendritic cells prevents airway inflammation and blocks the generation of pro-inflammatory T cells respectively. At the cellular level, it shifts macrophages towards an anti-inflammatory M2 phenotype and suppresses Th2 differentiation, and controls T cell activation. However, in the deleterious mode of action, AhR increases allergic airway inflammation by activating lung epithelial cells and fibroblasts and dysregulates antigen-presenting cells and pathologic T cells in asthma[9,11,37].

Previously, some studies investigated the role of AhR polymorphisms in lung diseases other than asthma. For instance, Cauchi *et al*. found that AhR-G1661A plays a key role in the CYP1A1 inducibility and in the susceptibility to develop lung cancer[38]. Here, as the first study on the effect of AhR polymorphism on asthma, we showed that the AhR transition G1661A may act as a protective factor against lower stages of the disease, however, it may increase the risk of the severe stage of asthma. Previously, a comprehensive *in silico* analysis by Aftabi *et al*. aimed to find possible justifications for opposite and contradictory effects of AhR-G1661A SNP[26]. They reported that the SNP may affect the local secondary structure and alter solvent accessibility, hydropathy features, post-translational modification, and pattern of the binding residues in the acidic sub-domain of the AhR transactivation domain[26]. They suggested that the SNP may affect this domain interactions, especially with the TATA-binding protein, and influence target genes expression. However, the modular transactivation domain of AhR has a flexible structure and may intervene with the SNP effects and result in different outcomes in different conditions[26].

Another finding of this study was the increasing role of higher age on odds ratios of persistence asthma severity consistently with previous reports. For instance, in a cross-sectional study, Zein *et al*. reported that age has a greater effect than asthma duration on the risk of severe asthma in Americans[39]. Also, Mincheva *et al*. found that Italian patients with two or more signs of asthma were older[40]. Elderly patients with asthma have a higher rate of allergic sensitization, decreased lung function, and highest rates of morbidity and mortality from their disease than younger patients and underlying airway inflammation of asthma in this age group likely differs from younger patients, which might reflect structural changes in the lungs over time[39,41]. In contrast with other reports that found female sex associated with higher severity of asthma, our analysis showed that in our studied sample population it was the male sex that was associated with severer asthma[42,43]. It has been hypothesized that sex differences in asthma could be explained by hormonal factors[44]. Indeed, it has been shown that intrinsic factors such as sex can interact with genes and SNPs to modify asthma risk[45].

Conclusion

We studied the effect of the most frequent SNP of the main regulator gene of xenobiotics metabolism pathway, AhR, with asthma severity. We showed that the mutant allele of G1661A transition in the aryl hydrocarbon receptor is associated with the decreased risk of lower stages of asthma (Intermittent, Mild, and Moderate), however, it was associated with increased risk of severe asthma in comparison to the lower stages. Also, male sex and higher age were associated with the increased odds ratio of the asthma severity. Furthermore, interactions of the SNP with sex, smoking, and alcohol consumption showed significant associations with increased/decreased odd ratios of asthma severity stages.

Conflict of interest

All authors declare no conflict of interest.

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