The Relationship Between Arginase Genes Polymorphisms and Preschool Wheezing Phenotypes

pinar Gokmirza Ozdemir[1], Damla Eker[1], velat çelik[2], Burcin Beken[3], Hakan Gürkan[2], Necdet Süt[2], and Mehtap Yazicioglu[2]

[1]Trakya University
[2]Trakya University Faculty of Medicine
[3]Trakya Universitesi

August 14, 2020

Abstract

Background: The association between arginase I (ARG1) and arginase II (ARG2) genes and asthma has been reported in previous studies, but associations between polymorphisms in ARG genes and preschool wheezing (PSW) phenotypes are unknown. Objective: To examine the association between genetic variation in ARG1 and ARG2 genes and PSW phenotypes and to compare results with asthmatic patients. Methods: We enrolled 102 patients and 86 healthy controls. The patient group included three subgroups: episodic wheezing (EW) (n = 42, median age 41 months), multiple-trigger wheezing (MW) (n = 41, median age 39 months), and asthma (n = 19, median age 72 months). We genotyped six single nucleotide polymorphisms (SNPs) in ARG1 and six SNPs in ARG2. Eighteen haplotypes for ARG1 and 31 haplotypes for ARG2 were constituted, and the distributions of SNPs and haplotypes in patients and controls were analyzed. Results: The frequency of homozygote cytosine-cytosine genotype of the ARG1 rs2781667T>C SNP in the EW group was significantly lower than controls (p = 0.006) [OR (95% CI): 0.26 (0.10-0.66)], the MW group (p = 0.002) [OR (95% CI): 0.19 (0.06-0.52)], and asthmatics (p = 0.025) [OR (95% CI): (0.22 (0.06-0.75)]. ARG1 haplotype 4 was more frequent in the MW group, asthmatics, and controls than in the EW group (p < 0.0001) [OR (95% CI): 7.77 (2.54-23.7)], (p = 0.036) [OR (95 %CI): 4.31 (1.15-16.15)], and (p = 0.030) [OR (95% CI): 3.44 (1.20-10.0)]. Conclusion: Variations in ARG1 gene may be useful in discriminating PSW phenotypes.

MAIN TEXT

INTRODUCTION

Preschool wheeze (PSW) is a highly prevalent clinical problem[1] that presents within a wide spectrum of severity and is associated with considerable healthcare costs[2]. Asthma has its origins in early childhood; however, different patterns of childhood wheezing vary in their association with subsequent asthma, atopy, and bronchial hyper-responsiveness. Wheezing phenotypes used in epidemiological studies are frequently defined retrospectively and are therefore not usable in clinical practice[3]. In 2008, the European Respiratory Society's preschool study group divided wheezing phenotypes into episodic (viral) wheeze (EW) and multiple-trigger wheeze (MW)[4]. According to this classification, wheezing attacks triggered only by viral respiratory tract infections with no complaints between attacks are classified as EW. Wheezing attacks triggered by allergens, exercise, cold weather conditions, or smoke, in addition to viral respiratory tract infections, with complaints between attacks are classified as MW[4]. However, wheeze patterns in young children vary over time and with treatment, rendering the distinction between EW and MW unclear in many patients[5]. Therefore,

we need predictive markers that are useful for differentiating between wheezing phenotypes and predicting asthma development in children with PSW as a guide for clinicians in early treatment decision-making.

There has been increasing interest among researchers to find a relationship between different genes and wheezing phenotypes and persistence and the development of childhood asthma[6,7]. Arginase (*ARG*) genes have been investigated in this context. Arginase, an essential enzyme in the hepatic urea cycle, is involved in L-arginine homeostasis by catalyzing the reaction in which L-arginine is converted to L-ornithine and urea. Arginase is also expressed in nonhepatic tissues, including in the airways. In humans, two arginase isoenzymes have been identified, arginase 1 and arginase 2, and both are constitutively expressed in the airways. Arginase and nitric oxide synthases (NOS) compete for their common substrate, L-arginine. Increased arginase expression and low L-arginine levels have been found to be associated with asthma and airway remodeling[8-12]. After defining the important contributions of arginase in asthma, genetic studies have attempted to identify an association between genetic variation in *ARG* genes and susceptibility for asthma, atopic sensitization, and bronchodilator response (BDR) in different populations[13-21]. These studies have reported many associations between different single nucleotide polymorphisms (SNPs) in *ARG1* or *ARG2* genes and atopic sensitization[13, 15], risk of asthma[13, 16,21], asthma severity[16], bronchodilator/steroid response in asthma[14, 16, 19,20], and the arginase-NOS pathway [17,18], although some of these results have not been replicated.

To date, associations between polymorphisms in *ARG* genes and PSW phenotypes are unknown. Therefore, this study aimed to investigate whether there is any relationship between polymorphisms in *ARG*genes and PSW phenotypes among Turkish children and compare these results with those of asthmatic children over five years old.

METHODS

Study population

This cross-sectional study was conducted at the Pediatric Allergy Department of Trakya University between May 2017 and May 2019 and included 102 patients and 86 healthy controls.

The patient group included three subgroups: EW (n = 42; median age 41 months), MW (n = 41; median age 39 months), and asthma (n = 19; median age 72 months). Asthma diagnoses were based on the Global Initiative for Asthma (GINA) guidelines[22]. Wheezing phenotypes in young children were discriminated, as proposed by the European Task Force[4]. The control group included 86 (median age 20 months) non-allergic, unrelated, healthy outpatients with no history of recurrent wheezing and no individual or family history of allergic illness (e.g., asthma, atopic dermatitis, allergic rhinitis, allergic rhinoconjunctivitis, and food allergy). The inclusion criteria were the presence of [?] 4 EW or MW episodes in a year for children up to five years of age or having asthma for children over five years of age. Patients with any other chronic disease or wheezing-associated diseases, such as cystic fibrosis, ciliary dyskinesia, anatomic abnormalities, and immunodeficiency disorder, and preterm-born children were excluded from the study.

Study forms were completed by pediatric allergists during patient visits at our pediatric allergy outpatient clinic. These forms included demographic data (i.e., age and gender) tobacco exposure, wheezing or asthma exacerbations per year, history of atopic dermatitis, presence of allergic rhinitis symptoms (i.e., nasal congestion, nasal itching, rhinorrhea, and sneezing), parental asthma, or other allergic diseases.

Laboratory tests

Serum total IgE levels in the patient group were measured by chemiluminescent immunometric assay (AU5800; Beckman Coulter Inc., CA, USA). Complete blood count analysis was performed by an automatic analyzer (UniCell DxH 800 Coulter Cellular Analysis System; Beckman Coulter Inc.) within one hour

of blood sampling. The original manufacturer's kits were utilized for the laboratory tests.

Skin tests

Aeroallergen and food allergen sensitivity were determined using a skin prick test. The following aeroallergens, common in Turkey, and food allergens were used: Dermatophagoides pteronyssinus and farinae, Felis domesticus, Canis familiaris, Blattella germanica, Dactylis glomerata, Festuca pratensis, Poa pratensis, Lolium perenne, Phleum pratense), Corylus avellana, Betula verrucosa, Alnus glutinosa, Artemisia vulgaris, Chenopodium album, Plantago lanceolata, Parietaria judaica, Alternaria alternata, Aspergillus fumigatus, Cladosporium herbarum, Penicillium, milk, egg white, egg yolk, wheat, hazelnuts, peanuts, walnuts, almond, and cacao (ALK-Abelló, Madrid, Spain). Histamine was used as a positive control and saline as a negative control. Skin reactions were evaluated 15 minutes after the skin test. A positive reaction was defined as wheal diameter [?] 3 mm.

Genetic analyses

DNA isolations and real-time PCR studies were carried out in the Genetic Diseases Diagnosis Center's Molecular Genetics Laboratory of Trakya University Medical Faculty.

SNPs selection

We searched PubMed and open-access databases[10-12] for the keywords "arginase, asthma genetics, atopy, asthma severity, SNP, bronchodilator response." SNPs in *ARG1* and *ARG2* were found associated with an increased risk of atopic sensitization, risk of asthma, asthma severity, and bronchodilator/steroid response in asthma in previous studies[13,16,20]. These were selected for investigation in our study.

Genomic DNA isolation

Peripheral blood samples from both the patient and control groups were collected using tubes containing EDTA. Genomic DNA isolation from peripheral blood samples was performed using a PureLink Genomic DNA Mini Kit (Invitrogen, Catalog number: K182001; ThermoFisher Scientific) according to the optimized protocol specified in the manufacturer's manual. Quality control and the purity of the isolated genomic DNA samples were determined using a DNA spectrophotometer (NanoDrop 2000C; ThermoFisher Scientific Inc., MA, USA). High-quality samples that had A260/280 values of 1.8–2.0 were included in the study, while low-quality samples were re-isolated from the blood samples.

Genotyping with real-time PCR

The real-time polymerase chain reaction (RT-PCR) method was used for allelic discrimination for the genotyping. The discrimination of the genotypes in the SNPs was performed using a PCR machine (Applied Biosystems StepOnePlus^{TM} Real-Time PCR system; ThermoFisher Scientific) and TaqManAssays$^{(r)}$(ThermoFisher Scientific). The RT-PCR reaction was performed according to the manufacturer's recommended protocols for six SNPs in the*ARG1* gene and six *SNPs* in the *ARG2* gene, respectively (rs2781668, rs3756780, rs2781659, rs2781665, rs2781667, and rs2246012 and rs3759757, rs742869, rs3742879, rs17249437, rs3742880, and rs6573788) (see Figure 1).

Each PCR reaction was prepared in a 20 µl total volume of 10 µl 2xPrecision MasterMix, 1 µL TaqManAssay, 4 µL RNAse/DNAse free water, and 5 µL genomic DNA. The PCR conditions were 95 °C for eight minutes for enzyme activation, 95 °C for 10 s for denaturation, and 60 °C for 60 s for the first extension in 10 cycles followed by 95 °C for 10 s for a second denaturation and 68 °C for 60 s for the second extension in 35 cycles. The ROX^{TM} channel and VIC^{TM}channel of the RT-PCR machine were coded for the wild-type probe or mutant-type probe with sequences determined by the TaqManAssay® manufacturer. According

to the signals from the canals, the SNPs were considered either homozygous mutant, homozygous wild, or heterozygous. Fluorogenic data for each of the 12 polymorphisms were collected through the ROX and VIC channels at the end of each cycle of the second extension and determined individually.

Haplotype analysis

After determining the homozygote and heterozygote genotypes for the selected SNPs in *ARG1* and *ARG2* , 18 haplotypes for*ARG1* and 31 haplotypes for *ARG2* were constituted. The GVSc names and H19 localizations of the selected SNPs and haplotypes in the*ARG1* and *ARG2* genes are shown in Figure 2.

Ethical considerations

This study was approved by Trakya University's ethical committee (approval number 2016/264). Written informed consent was obtained from either parents or children more than seven years old.

Statistical analyses

Mean, standard deviation, median, minimum, maximum, and number (percentage) values were used for the descriptive statistics. Variable distribution was checked using the one-sample Kolmogorov–Smirnov test. Kruskal–Wallis and Mann–Whitney U tests with Bonferroni correction were used for a comparison of the quantitative data. Comparisons of genotype and haplotype frequencies and other categorical data were performed using Pearson's chi-squared test or Fisher's exact test when the sample size was small. The Hardy–Weinberg equilibrium test was used to calculate genotype frequencies. P -values < 0.05 were considered statistically significant. IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp) was used for the statistical analyses.

RESULTS

Demographic characteristics and laboratory results

The mean age of the total patient group was 46.3 ± 25.9 months (median 46), and the mean age of the control group was 23.9 ± 13.4 months (median 20) ($p < 0.001$). In the patient group, 37 (36.3%) of the cases were female, and 35 (40.7%) of the patients in the control group were female; ($p = 0.534$). A comparison of the demographic characteristics and laboratory results of the patient subgroups is shown in Table 1.

Genetic workup with real-time PCR

1. Genotype analysis of the studied SNPs

1a. Comparison of total patient group and controls

The genotypes were grouped as homozygote, heterozygote, or wild. After determining the genotypic distribution, we performed a genetic comparison according to the autosomal recessive inheritance pattern of the *ARG1* and *ARG* genes between the patients, controls, and patient subgroups. There was no difference between the total patient group and the healthy controls in terms of the homozygote genotype of the SNPs in the *ARG1* and *ARG2* genes (Table 2).

1b. Comparison of patient subgroups

The homozygote cytosine-cytosine (CC) genotype of the *ARG1*rs2781667T>C SNP in the EW group was significantly lower than for the controls ($p = 0.006$) [OR (95% CI): 0.26(0.10–0.66)], the MW group ($p = 0.002$) [OR (95% CI): 0.19(0.06–0.52)], and the asthmatics ($p = 0.025$) [OR (95% CI): 0.22(0.06–0.75)]. There

was no difference in the distribution of the other studied SNPs in the *ARG1* and *ARG2* genes between the patient subgroups and the controls (Table 2).

1c. Association between the SNP analysis and allergic rhinitis, aeroallergen sensitivity, number of aeroallergens detected as positive in the skin prick test, and tobacco exposure

The homozygote CC genotype of the *ARG1* rs2781667T>C SNP was more frequent in patients with AR than in those without AR (49.1% and 21.3%, respectively, $p = 0.007$) [OR (95% CI): 3.56(1.48–8.56)].

There was no difference in the presence of aeroallergen sensitivity, the number of aeroallergens detected as positive in the skin prick test, tobacco exposure, and homozygote genotype distribution of any SNPs among all the patient groups.

2. Haplotype analysis

2a. Comparison of total patient group and controls

There was a statistically significant difference in terms of *ARG* 1 haplotype 5 (CT/T/G/T/T/CT) and *ARG* 1 haplotype 9 (C/T/G/T/T/T) between the healthy controls and the patient group. The frequency of the*ARG1* haplotype 5 in the patient group was 9.8% and 1.2% in the control group ($p = 0.028$) [OR (95% CI): 9.23(1.15–73.70)]. The frequency of *ARG1* haplotype 9 in the patient group was 2% and 11.6% in the control group ($p = 0.016$) [OR (95% CI): 0.15(0.03–0.71)]. There was no difference between the patient groups and the controls in terms of other haplotypes in the *ARG1* and*ARG2* genes (see Tables 3A and 3B).

2b. Comparison of patient subgroups

The frequency of the *ARG1* haplotype 2 (CT/T/AG/AT/CT/CT) was significantly higher in the EW group than in the healthy control and MW groups ($p = 0.041$) [OR (95% CI): 2.76(1.13–6.76)] and ($p = 0.014$) [OR (95% CI): 5.88(1.49–25.0)], respectively. The frequency of haplotype 5 was significantly higher in the EW group than in the controls ($p = 0.005$) [OR (95% CI): 14.16(1.64–121.93)]. The *ARG1* haplotype 4 (C/T/A/A/C/T) was more frequent in the MW, asthmatics, and control groups than in the EW group ($p < 0.0001$) [OR (95% CI): 7.77(2.54–23.7)], ($p = 0.036$) [OR (95% CI): 4.31(1.15–16.15)], and ($p = 0.030$) [OR (95% CI): 3.44(1.20–10.0)], respectively (see Tables 3A, 3B).

2c. Association between SNP analysis and allergic rhinitis, aeroallergen sensitivity, number of aeroallergens detected as positive in the skin prick test, and tobacco exposure

ARG1 haplotype 4 was more frequent in patients with allergic rhinitis than those without allergic rhinitis, 41.8% and 21.3%, respectively ($p = 0.046$) [OR (95% CI): 2.65(1.10–6.41)].

There was no difference in terms of haplotype distribution and presence of aeroallergen sensitivity, the number of aeroallergens detected as positive in the skin prick test, and tobacco exposure among all the patient groups.

DISCUSSION

To our knowledge, this is the first study to look at PSW phenotypes and*ARG* gene polymorphisms. We hypothesized that there would be relationships between a PSW phenotype and polymorphisms of the*ARG1* and *ARG2* genes. Our study revealed that the frequency of the homozygote C-allele rs2781667 in *ARG1* was significantly higher in the MW and asthma groups than in the EW group. Li et al.[13] reported that four SNPs in *ARG1*(rs2781659, rs2781665, rs2781667, rs2246012) and rs 3742880 in*ARG2* were associated with the number of positive skin tests, and carrying two copies of the minor allele for rs3759757 and rs6573788 SNPs in *ARG2* were found to be associated with the presence and severity of asthma. Litonjua et al.[14] reported that rs2781659 in *ARG1* was significantly associated with BDR in asthmatic children. In another study[15], each variant allele of rs3742879 in *ARG2* was found to be associated with increased asthma risk.

Vonk et al.[16] reported that two polymorphisms in *ARG2* (rs17249437 and rs3742879) were associated with both asthma and more severe airway obstruction, and increased airway hyperreactivity (AHR) and lower beta-2-agonist reversibility were reported as associated with both *ARG1* and *ARG2*. Hing Yee et al.[20] reported that rs7216389 in *ORMDL3* and rs3756780 in *ARG1* interactions might be associated with a risk of asthma, and rs2749935 in *ARG1* and rs2190242 in *CRHR2*interactions might be associated with BDR. A recent study from India showed that high arginase activity and IL-13 concentration in serum and the *ARG1* rs2781666 G/T genotype might increase the risk of asthma[21]. Vonk et al.[16] reported that the protective effect of inhaled corticosteroids (ICS) against annual FEV1 decline was significantly lower in homozygote carriers of the C-allele of rs2781667 in *ARG1* in a longitudinal cohort of 200 adult asthma patients. Based on this, our finding of a higher frequency of homozygote C-allele rs2781667 in *ARG1* in the MW and asthma groups may be important for the management of patients with PSW. We think it might be a useful marker for the differentiation of the PSW phenotype. In addition, the association between this SNP and ICS or BDR could be addressed in future studies.

We also found that the homozygote CC genotype of the *ARG1*rs2781667T>C SNP was more frequent in patients with allergic rhinitis than in those without allergic rhinitis ($p = 0.004$). While it is known that the arginase-NOS pathway is associated with allergic rhinitis [23,24], we were unable to find any study related to the association of allergic rhinitis and *ARG*genes until now. Furthermore, we have no data for whether *ARG1*rs2781667 is a functional polymorphism and associated with changes in arginase expression in the nasal mucosa.

Our haplotype analyses revealed that the frequencies of the *ARG1*haplotype 2 and haplotype 5 were significantly higher in the EW group than in the control group. Two recent studies suggest an interaction between arginase expression and viral airway infections[25,26]. Santiago-Olivares et al.[25] investigated the impact of Respiratory Syncytial Virus (RSV) infection on nitric oxide production in an experimental model and found that *ARG1* overexpression contributes to the maintenance of the RSV genome in the host in persistent infection. Wieczfinska et al.[26] found that the expression of TGF-β1 and arginase was increased due to rhinovirus infection. To date, we have no data for viral-induced wheezing and genetic variations in*ARG* genes, but new studies could advance this. The *ARG1*haplotype 4 was more frequent in the MW group than in the EW group. Interestingly, *ARG1* haplotype 4 carried the variant C-allele for rs2781667 in *ARG1* , so it may be useful for differentiating between EW and MW. Salam et al.[15] reported that the association between arginase variants and childhood asthma may be related with haplotypes rather than SNPs. Duan et al.[19] studied haplotypes were identified according to the SNPs (rs2781659, rs2781663, rs2781665, and rs60389358) in the*ARG1* and they have reported that two haplotypes may alter BDR and differentially regulate gene expression. The haplotypes that we estimated according to the studied SNPs were different from those used in these studies, so it is impossible to make a comparison between haplotype-based results.

The diagnoses of asthma cases and the classification of patients according to a PSW phenotype were performed by pediatric allergists at a tertiary pediatric allergy specialty clinic, these were strengths of the our study. However, there were some limitations. First, the sample size was smaller than previous studies have referred to; additionally, it does not represent all Turkish children. Second, this was a case-control study; thus, we have no longitudinal data about the progress and bronchodilator or ICS responses of patients with a homozygote CC genotype of the *ARG1* rs2781667T>C and/or *ARG1*haplotype 4. Third, we included no information about the functionality of the homozygote CC genotype of the *ARG1*rs2781667T>C and/or *ARG* 1 haplotype 4 in lung and nasal mucosa.

In conclusion, our study revealed that *ARG1*rs2781667T>C and/or *ARG* 1 haplotype 4 were more frequent in patients with MW and asthma than in the EW group. The frequencies of the *ARG1* haplotype 2 and haplotype 5 were significantly higher in the EW group, while haplotype 9 was more frequent in the healthy controls. Taken together, we conclude that variations in *ARG1* gene may be potentially important for differentiating PSW phenotypes. Our findings need to be confirmed in larger, different PSW populations, preferably with different ethnic backgrounds.

Acknowledgement

The study was funded by the Research Council of Trakya University (STUDY NUMBER 2017/86).

REFERENCES

Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. N Engl J Med 1995;332:133–138.

2-Stevens CA, Turner D, Kuehni CE, Couriel JM, Silverman M. The economic impact of preschool asthma and wheeze. Eur Respir J 2003;21:1000–1006.

3- Savenije OE, Kerkhof M, Koppelman GH, Postma DS. Predicting who will have asthma at school age among preschool children. J Allergy Clin Immunol 2012;130(2):325–331.

4-Brand PL, Baraldi E, Bisgaard H, et al. Definition, assessment and treatment of wheezing disorders in preschool children: An evidence-based approach. Eur Respir J 2008;32(4):1096–1110.

5-Brand PL, Caudri D, Eber E, et al. Classification and pharmacological treatment of preschool wheezing: Changes since 2008. Eur Respir J 2014;43(4):1172–1177.

6-Esposito S, Ierardi V, Daleno C, et al. Genetic polymorphisms and risk of recurrent wheezing in pediatric age. BMC Pulm Med 2014;14:162.

7-Genuneit J, Cantelmo JL, Weinmayr G, et al. A multi-centre study of candidate genes for wheeze and allergy: The International Study of Asthma and Allergies in Childhood Phase 2. Clin Exp Allergy 2009;39(12):1875–1888.

8-van den Berg MP, Meurs H, Gosens R. Targeting arginase and nitric oxide metabolism in chronic airway diseases and their co-morbidities. Curr Opin Pharmacol 2018;40:126–133.

9-Que LG, Kantrow SP, Jenkinson CP, Piantadosi CA, Huang YC. Induction of arginase isoforms in the lung during hyperoxia. Am J Physiol 1998;275:L96–L102.

10-Genetics Home Reference: https://ghr.nlm.nih.gov/gene/ARG1

11-OMIM: ARGINASE 1 http://omim.org/entry/608313

12-ARG2 arginase 2 https://www.ncbi.nlm.nih.gov/gene/384

13-Li H, Romieu I, Sienra-Monge JJ et al. Genetic polymorphisms in arginase I and II and childhood asthma and atopy. J Allergy Clin Immunol 2006 Jan;117(1):119–26.

14-Litonjua AA, Lasky-Su J, Schneiter K, et al. ARG1 is a novel bronchodilator response gene: Screening and replication in four asthma cohorts. Am J Respir Crit Care Med 2008;178(7):688–694.

15-Salam MT, Islam T, Gauderman WJ, Gilliland FD. Roles of arginase variants, atopy, and ozone in childhood asthma. J Allergy Clin Immunol. 2009 Mar;123(3):596–602.

16-Vonk JM, Postma DS, Maarsingh H, Bruinenberg M, Koppelman GH, Meurs H. Arginase 1 and arginase 2 variations associate with asthma, asthma severity and beta2 agonist and steroid response. Pharmacogenetics and Genomics 2010 Mar;20(3):179–86.

17-Breton CV, Byun HM, Wang X, Salam MT, Siegmund K, Gilliland FD. DNA methylation in the arginase-nitric oxide synthase pathway is associated with exhaled nitric oxide in children with asthma. Am J Respir Crit Care Med 2011;184(2):191–197.

18-Salam MT, Bastain TM, Rappaport EB, et al. Genetic variations in nitric oxide synthase and arginase influence exhaled nitric oxide levels in children. Allergy 2011;66(3):412–419.

19-Duan QL, Gaume BR, Hawkins GA, et al. Regulatory haplotypes in ARG1 are associated with altered bronchodilator response. Am J Respir Crit Care Med 2011;183(4):449–454.

20-Sy HY, Ko FW, Chu HY, et al. Asthma and bronchodilator responsiveness are associated with polymorphic markers of ARG1, CRHR2 and chromosome 17q21. Pharmacogenetics and Genomics 2012;22(7):517–524.

21-Donthi S, Neela VSK, Gaddam S, et al. Association of increased risk of asthma with elevated arginase & interleukin-13 levels in serum & rs2781666 G/T genotype of arginase I. Indian J Med Res 2018;148(2):159–168.

22-Global Initiative for Asthma, Global Strategy for Asthma Management and Prevention, 2016.

23-Cho WS, Kim TH, Kim KH, et al. Increased expression of arginase I and II in allergic nasal mucosa. Laryngoscope 2011;121:236–240.

24-Yasar H, Kiran B, Cagatay T, Ozkul H, Icten S. The effect of montelukast sodium on serum arginase levels in patients with seasonal allergic rhinitis. Am J Rhinol Allergy 2011;25(4):e153–e155.

25- Santiago-Olivares C, Rivera-Toledo E, Gómez B. Nitric oxide production is downregulated during respiratory syncytial virus persistence by constitutive expression of arginase 1. Arch Virol 2019;164(9):2231–2241.

26-Wieczfinska J, Pawliczak R. Thymic stromal lymphopoietin and apocynin alter the expression of airway remodeling factors in human rhinovirus-infected cells. Immunobiology 2017;222(8–9):892–899.

Hosted file

`Tables.docx` available at https://authorea.com/users/350887/articles/475602-the-relationship-between-arginase-genes-polymorphisms-and-preschool-wheezing-phenotypes

Hosted file

`Figure 1.docx` available at https://authorea.com/users/350887/articles/475602-the-relationship-between-arginase-genes-polymorphisms-and-preschool-wheezing-phenotypes

Hosted file

`Figure 2.docx` available at https://authorea.com/users/350887/articles/475602-the-relationship-between-arginase-genes-polymorphisms-and-preschool-wheezing-phenotypes