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Autophagy-related 5 gene mRNA expression and ATG5 rs510432 polymorphism in children with bronchial asthma

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Abstract

Purpose: Bronchial asthma is a common chronic respiratory disease in children with complex pathogenesis, characterized by airway hyper-responsiveness, obstruction, mucus hyperproduction, and airway remodeling. Autophagy is important for cellular physiology, and the ATG5 rs510432 has recently been implicated in several fundamental characteristics of childhood asthma pathogenesis and may play a role in the disease progression. This study aims to assess the expression of ATG5 messenger RNA (mRNA) according to rs510432 polymorphism in asthmatic children and to evaluate their possible relation with the development of the disease.

Methods: ATG5 mRNA expression and rs510432 polymorphism were measured using real-time polymerase chain reaction in 57 asthmatic children patients and 46 healthy controls.

Results: ATG5 level was significantly higher in asthmatic children than in controls and a significant increase in the frequency of TT and CC genotype of ATG5 rs510432 gene polymorphism was found in asthmatic patients when compared to control subjects ($p < 0.001$; and $p = 0.01$, respectively), and there was a statistically significant decrease in the frequency of CT genotype of ATG5 rs510432 gene polymorphism in asthmatic patients when compared to control subjects ($p < 0.001$).

Conclusion: ATG5 rs510432 gene polymorphism plays an important role in childhood asthma pathogenesis.

KEYWORDS

ATG5, autophagy, childhood bronchial asthma, rs510432 gene polymorphism

1 | INTRODUCTION

Bronchial asthma has become the world's most common chronic respiratory disease. Its incidence and mortality have risen in recent years.[1] To improve its burden, studies have been directed to understand its pathogenesis which is complex and depends upon the interaction of genetic, environmental, and hormonal factors.[2] We are in a new era in personalized asthma treatment according to each patient's phenotype and his/her underlying pathophysiology and genetics.[3]

The 2016 Nobel Prize in Physiology or Medicine was awarded to Professor Yoshinori Ohsumi for the discovery of the molecular

Abbreviations: ATG, autophagy-related gene; Atg, autophagy-related protein; AUC, area under the curve; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; GINA, global initiative for asthma; IgE, immunoglobulin E; SNP, Single-nucleotide polymorphism.

mechanisms of autophagy and considerable interest has emerged in autophagy modulation as a potential target in clinical medicine. Autophagy plays a critical role in cellular physiology including adaptation to metabolic stress, removal of dangerous cargo (e.g., protein aggregates, damaged organelles, intracellular pathogens), and prevention of genomic damage.[4]

Autophagy includes a large number of autophagy-related (Atg) proteins that are regulated by at least 604 ATG genes that have been identified up till now.[5] ATG5 is a key autophagy regulatory gene that is essential for autophagosome formation. The autophagosomes then fuse with the lysosomes to form autophagolysosomes. The cargo present within the autophagosome is then delivered into the lysosomes where they get degraded by the lysosomal enzymes.[6]

Several essential characteristics of asthma etiology, including eosinophilic airway inflammation, airway hyper-responsiveness, and airway remodeling, have recently been linked to the autophagic system. Environmental pollutants exposure has been proven to cause cell autophagy and airway inflammation via various immunological and molecular mechanisms. Furthermore, allergen exposure has been proven to activate autophagy.[7]

Previous studies yielded conflicting results regarding the link between ATG5 and the susceptibility and the severity of bronchial asthma in children. This study aimed to assess the expression of ATG5 mRNA and rs510432 gene polymorphism in Egyptian asthmatic children and to evaluate their possible relation with the development of the disease.

2 | SUBJECTS AND METHODS

2.1 | Subjects

A cross-sectional study was done on 57 children with bronchial asthma and 46 healthy age and sex-matched control subjects. Patients were between 3 and 17 years old. The asthmatic group was selected during their follow-up visits to the Pediatric Chest Clinic, Ain Shams University Hospital, while control subjects were selected from children free from any acute or chronic respiratory symptoms coming for well-child care visits. Patients who had underlying cardiorespiratory illness or malignancy were excluded from the study.

The research was accepted by the Faculty of Medicine's Ethics Committee, Ain Shams University. Informed consent for enrollment was obtained from all participants, family members, or legal guardians, according to the organization's ethical guidelines.

All subjects in the study were subjected to full history taking (age, family history, and disease duration), clinical evaluation, and other data were collected from patients' records. Asthma severity and control were assessed according to Global Initiative for Asthma Guidelines (GINA).[8] Spirometry was done for those above 5 years of age using VIASYS Healthcare GmbH. Leibnizstrasse 7 to measure FEV_1, FVC, FEV_1/FVC ratio. Laboratory investigations included serum IgE and the molecular assessment of ATG5 rs510432 gene polymorphism (measurement of the ATG5 mRNA expression level was done by real-time polymerase chain reaction [PCR] method, and analysis of ATG5 rs510432 gene polymorphism by TaqMan single nucleotide polymorphism [SNP] Genotyping Assay).

2.2 | Sample collection

2.2.1 | Blood sample

Two milliliters of peripheral venous blood samples were withdrawn from patients and control subjects under complete aseptic condition, collected in ethylenediamine tetraacetic acid vacutainer tubes then divided into two parts: 1 ml of blood was used for RNA extraction for mRNA expression. The second portion of blood (1 ml) was used for DNA extraction for genotyping of the studied SNP.

3 | METHODS

Molecular determination of ATG5 expression by real-time PCR method

(1) Purification of total RNA, including ATG5 (mRNA): Total RNA from blood samples was extracted using QIAamp RNA blood Mini Kit (Catalog number: 52304) (Qiagen) according to the manufacturer's instructions. Detection of RNA purity and concentration: by Nanodrop 2000 spectrophotometer (Thermo-Fisher Scientific).
(2) Relative quantitation of ATG5 (mRNA) by two-step real-time PCR using SYBR Green: The two steps of real-time PCR included reverse transcription (complementary DNA [cDNA] cDNA synthesis) and quantitative PCR (RT-qPCR) (amplification).

The first step of real-time PCR was reverse transcription which was performed using a High Capacity cDNA Reverse Transcription Kit (Invitrogen, Thermo Fisher Scientific), so the synthesis of DNA from the RNA template, via reverse transcription results in cDNA. Ten microliters of template RNA sample were added to 2 µl of 10X RT Buffer, 0.8 µl of 25X dNTP Mix (100 mM), 2 µl of 10X RT Random Primers, 1 µl of MultiScribe Reverse Transcriptase, 1 µl of RNase Inhibitor, and 3.2 µl nuclease-free H_2O into each well of a 96-well reaction plate and mixed gently. Reverse transcription was performed in a thermal cycler. cDNA was stored at (−80°C) to be continued with real-time PCR. The second step of PCR was a quantitative PCR (amplification) which was performed using (LightCycler 480 system; Roche) using SYBR® Green PCR Master Mix and SYBR® Green RT-PCR Reagents Kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's instructions. For accurate results in mRNA quantitation by real-time PCR, it was necessary to normalize the amount of target mRNA by using a suitable endogenous reference RNA. In our study, the values were normalized based on the expression level of the endogenous housekeeping gene

glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primer sets were used for ATG5 PCR

ATG5 Gene:

(1) Forward primer (5′-AAAGATGTGCTTCGAGATGTGT-3′).
(2) Reverse primer (5′-CACTTTGTCAGTTACCAACGTCA-3′).
(3) Amplicon size (152 bp).

GAPDH Gene:

(1) Forward primer (5′-GAGTCAACGGATTTGGTCGT-3′).
(2) Reverse primer (5′-GACAAGCTTCCCGTTCTCAG-3′).
(3) Amplicon size (185 bp).

Two microliters of template cDNA was dispensed into the individual plate wells, then the reaction mix containing 12.5 µl of 2x SYBR® Green PCR Master Mix, 2.5 µl of the Forward Primer, 2.5 µl of Reverse Primer, and 5.5 µl of RNase-free water added to each plate well-containing template cDNA. The thermal cycler was programmed, with initial activation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Data analysis was determined using the light cycler 480 real-time PCR system. The relative quantification of gene expression was performed using fold expression changes were calculated using the equation $2^{-\Delta\Delta c^t}$. Where the amount of the target (ATG5) mRNA is normalized to an endogenous reference gene (GAPDH) and relative to a control. Each run was completed using melting curve analysis to confirm the specificity of the amplification and absence of primer dimers.[9]

Analysis of ATG5 rs510432 gene polymorphism by TaqMan SNP Genotyping Assay

(1) Purification of total DNA: DNA was extracted from the peripheral blood using a DNA extraction kit supplied by QIAamp DNA Blood Mini Kit (Catalog number: 51104) (Qiagen, Hilden) according to the manufacturer's instructions. The extracted DNA concentration in the eluate was measured by absorbance at 260 nm using Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific).

(2) SNP Genotyping of ATG5 rs510432 gene polymorphism: was performed by the Roche real-time PCR system (light cycler 480) using TaqMan® Genotyping Master Mix kit, A predesigned probe set for the genotyping was used (Applied Biosystems, Thermo Fisher Scientific) according to manufacturer's instructions. The reaction mix containing,12 µl of 2x TaqMan® Genotyping Master Mix, 1.5 µl of 20x SNP Genotyping Assay mix (ATG5 rs510432 gene), and 6.5 µl of Nuclease-free water then 5 µl of genomic DNA was dispensed into each plate well containing the reaction mix. The real-time cycler was programmed, polymerase activation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing/extension at 60°C for 1 min. Data analysis was determined using the light cycler 480 real-time PCR system. It plotted the results of the allelic discrimination data that represented each sample well as an individual point on the plot, as the sample which was homozygous for allele (C), the fluorescence readout showed mostly VIC fluorescence, while the sample which was homozygous for allele (T), the plot showed signal from the fluorescein amidites dye. And the heterozygous sample, there was an equal signal for each dye.

TABLE 1 General characteristics of the studied group

Factors	Patients	($n = 57$)	Control	($n = 46$)	p
Age	Mean	SD	Mean	SD	0.3[a]
	8.82	3.6	9.64	4.19	
Sex	*N*	%	*N*	%	
Female	20	35.09	9	19.57	0.08[b]
male	37	64.91	37	80.43	

Note: $p < 0.05$ considered significant.

[a]Independent *t*-test.

[b]χ^2 test.

3.1 | Statistical analysis

Results were statistically analyzed using Minitab 17.1.0.0 for windows (Minitab Inc.), Quantitative variables were summarized as mean and standard deviation or as median (interquartile range [IQR]), while categorical variables were summarized as numbers and percentages. Comparisons between groups were analyzed using the Independent *t*-test, Mann–Whitney, Kruskal–Wallis test, and χ^2 test. Spearman's correlation was done between two quantitative variables where r's was the correlation coefficient. A $p < 0.05$ was considered significant.

4 | RESULTS

The study was conducted from March 2020 to July 2021 on 57 asthmatic children and 46 control subjects. It included 37 (64.91%) males and 20 (35.09%) females diagnosed as asthmatic with a mean age of 8.82 ± 3.6 years, while controls included 37 (80.43%) males and 9 (19.57%) females with a mean age of 9.64 ± 4.19 years. In terms of age and gender, there was no statistically significant difference between patients and controls ($p = 0.3$, and 0.08, respectively) (Table 1).

The majority of cases were uncontrolled according to GINA Guidelines control classification (47.37%) and had a positive family history (57.89%). Moderate and severe asthma were the most frequent presentation (43.84% and 36.84%), respectively (Table 2).

There was an 84% significant increase in the expression of ATG5 mRNA in the patient group ($p = 0.021$) (Table 3). While the correlation between ATG5 mRNA expression and rs510432 gene polymorphism in the control group was negative in direction but with an insignificant effect ($p = 0.819$).

There was a statistically significant increase in the frequency of TT and CC genotype of ATG5 rs510432 gene polymorphism in asthmatic patients when compared to control subjects ($p < 0.001$ and $p = 0.01$), however, there was a statistically significant decrease in the frequency of CT genotype of ATG5 rs510432 gene polymorphism in asthmatic patients when compared to control subjects ($p < 0.001$). Allele C was 43.8% in patients and

51% in control ($p = 0.57$). Allele T was 56.2% in patients and 49% in control ($p = 0.49$) (Table 4).

The TT polymorphism of ATG5 rs510432 resulted in significantly higher mRNA expression in asthmatic patients than the CC and CT polymorphisms ($p = 0.019$).

The correlations between ATG5 rs510432 genotype and asthma severity ($p = 0.291$), asthma control ($p = 0.294$), FEV1/FVC ($p = 0.574$), and serum IgE ($p = 0.264$) were statistically insignificant. Also, the correlation between ATG5 mRNA expression and asthma severity ($p = 0.248$), asthma control ($p = 0.406$), FEV1/FVC ($p = 0.459$), and serum IgE ($p = 0.572$) were statistically insignificant (Table 5).

The performance of ATG5 mRNA expression in predicting asthmatic patients was shown to be good, the AUC was 63.3%, $p = 0.021$. The sensitivity, specificity, PPV, and NPV at cutoff point = 0.85 were 63.2%, 58.7%, 65.5%, and 56.3%, respectively.

The regression analysis of ATG5 rs510432 gene polymorphism for asthma was highly significant ($p = 0.001$), OR = 10.432. Also, the regression analysis of ATG5 mRNA expression for asthma was significant ($p = 0.025$), OR = 1.318. So, the rs510432 gene polymorphism and ATG5 mRNA expression are good predictors for asthma in children (Table 6).

TABLE 2 Clinical characters of the studied asthmatic children

		Total ($n = 57$)
Asthma Control (*N*, %)		
Well controlled	11	19.3
Partly controlled	19	33.33
Uncontrolled	27	47.37
Asthma severity (*N*, %)		
Mild	11	19.3
Moderate	25	43.86
Severe	21	36.84
Family history of asthma (*N*, %)		
No	24	42.11
Yes	33	57.89
Spirometry (mean, SD)		
FEV1 (percent of predicted)	96.53	16.91
FVC (percent of predicted)	98.55	16.24
FEV1/FVC	98.34	9.15
Serum IgE level (median, IQR)	103.5	(57.075–185)

Abbreviations: FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E; IQR, interquartile range; *N*, number; SD, standard deviation.

TABLE 4 Genotype frequency of ATG5 rs510432 gene polymorphism in both studied groups

Variable rs510432 gene	Patients *N*	($n = 57$) %	Control *N*	($n = 46$) %	*p*
TT	21	36.84	2	4.35	<0.001[a]
CC	14	24.56	3	6.52	0.01[a]
CT	22	38.6	41	89.13	<0.001[a]
Allele C	50	43.8	47	51	0.57
Allele T	64	56.2	45	49	0.49

Note: $p < 0.05$ considered significant.

Abbreviations: ATG, autophagy-related gene; N, Number.

[a]χ^2 test.

TABLE 5 Correlation between ATG5 mRNA expression, rs510432 gene polymorphism, and asthma severity, asthma control classification, FEV1/FVC, and Serum IgE

	rs510432 gene polymorphism		ATG5 mRNA expression	
	r_s[a]	*p*	r_s[a]	*p*
Asthma severity	0.142	0.291	−0.156	0.248
Asthma control	−0.141	0.294	0.112	0.406
FEV1/FVC	0.076	0.574	−0.100	0.459
Serum IgE	−0.150	0.264	0.076	0.572

Abbreviations: IgE, immunoglobulin E; FEV1, forced expiratory volume in one second; FVC, forced vital capacity.

[a]r_s: Spearman correlation.

TABLE 3 ATG5 mRNA expression in both studied groups

Variable	Patients	($n = 57$)	Control	($n = 46$)	*p*
ATG5 mRNA expression	Median	IQR	Median	IQR	
	1.84	(0.63–4.98)	0.83	(0.38–1.93)	0.021[a]
	Min.–Max.		Min.–Max.		
	0.0034–56.12		0.0281–3.928		

Note: $p < 0.05$ considered significant.

Abbreviations: ATG, autophagy-related gene; IQR, interquartile range.

[a]Mann–Whitney test.

TABLE 6 Regression analysis of ATG5 rs510432 gene polymorphism & ATG5 mRNA expression for asthmatic children

	Wald regression	Significant	OR	95% CI for OR	
				Lower	Upper
Asthma					
ATG5 rs510432 genotypes	10.656	0.001*	10.432	2.552	42.637
ATG5 mRNA expression	5.006	0.025*	1.318	1.035	1.678

Abbreviation: ATG, autophagy-related gene.

*$p < 0.05$ considered significant.

5 | DISCUSSION

Asthma is a multifactorial disease with different phenotypic types due to various genetic and epigenetic mechanisms. The different phenotypes of asthma are due to different molecular and cellular biomarkers.[10] It is characterized by airway inflammation, remodeling, and increased susceptibility to viral infection.[11,12]

Autophagy, which means in Greek "self-eating," is the delivery of cytoplasmic cargo to the lysosome for degradation.[4] Autophagy is involved in both airway inflammation and remodeling.[13] It is also important for immune response, such as antigen presentation, immune cell differentiation, and T cell responses, as well as the establishment of pathogen-specific protective immunity.[14]

Autophagy is regulated by Atg. Among these proteins, Atg-5 is involved in a complex called (the Atg12-Atg5-Atg16 complex) which is essential for the formation of the membrane of the autophagosome.[13,15] The autophagy pathway responds to cigarette smoke exposure and viral infection, both of which are important asthma cofactors, indicating that autophagy plays a role in respiratory diseases. It was also discovered that ATG5 is a key component of the autophagy pathway and plays a role in viral clearance.[16]

In this study, there is a significant increase in ATG5 mRNA expression in asthmatic patients when compared to the control group ($p = 0.021$). These results matched with Martin et al.[16] who reported that ATG5 mRNA expression is upregulated during an acute asthma attack and significantly increased in the nasal epithelial cells obtained from children with acute asthma compared to controls ($p = 0.0057$), and this is in concordance with McAlinden et al.,[17] Lv et al.,[18] and Jyothula and Eissa.[12] However, Poon et al.[19] reported that no significant difference was detected in the ATG5 expression between nonasthmatic control and asthmatic subjects ($p = 0.1$) and that there was no association discovered between the level of ATG5 protein and the occurrence of asthma, the severity of asthma, or pulmonary function testing. This study focused on ATG5 proteins in the submucosal part of the airways, suggesting that gene expression correlations may not be transferred to protein expression in different cell types.

Considering the genotype frequency of ATG5 rs510432 gene polymorphism, this study revealed that the frequency of TT and CC genotype was significantly increased in asthmatic patients when compared to the control group, ($p < 0.001$ and $p = 0.01$) respectively, however, the frequency of CT genotype was significantly decreased in asthmatic patients when compared to control group, ($p < 0.001$). This is in agreement with Zeki et al.,[20] Jyothula and Eissa,[12] Poon et al.,[19] and Martin et al.[16] who reported an association of SNPs in ATG5 rs510432 with childhood asthma. However, Pham et al.[21] reported no association of ATG5 polymorphism in adults with neutrophilic asthma with the disease susceptibility or severity, and the percentage of CC genotype was 43.6% ($p = 0.442$), the percentage of CT genotype was 44.8% ($p = 0.097$), the percentage of TT genotype was 11.6% ($p = 0.998$) among cases, which means they did not reach statistical significance.

Considering the relation between ATG5 mRNA expression with ATG5 rs510432 gene polymorphism, the present study revealed that ATG5 mRNA expression in asthmatic patients showed statistically significant changes in the three different polymorphism of ATG5 rs510432 gene, $p = 0.019$ (TT polymorphism was significantly higher than CC and CT polymorphism). However, Martin et al.[16] found that the increased activity of the allele G is associated with an increased risk of asthma and consistent with increased gene expression of ATG5 in asthmatics ($p < 0.007$).

The mean serum IgE level in the current study was 169.5 ± 20.13 IU/ml, which is comparable to the studies of Ishak et al. (188.95 ± 167.75 IU/ml)[22] and Ahmed et al. (99.83 ± 233.81 = IU/ml)[23] on Egyptian asthmatic children. It is also comparable to that in asthmatic children in Iraq (189.59 ± 216.16 IU/ml)[24] but substantially lower than the mean serum total IgE levels in other countries, such as those of asthmatic children in India (700.97–571.01 IU/ml).[25] In concordance with the findings of Pham et al.,[21] neither serum IgE nor other parameters of the asthma severity were found to be correlated with ATG5 mRNA gene expression or rs510432 gene polymorphism in our study.

6 | CONCLUSION

ATG5 rs510432 gene polymorphism plays an important role in childhood asthma pathogenesis.

AUTHOR CONTRIBUTIONS

Conception and design of the work: Eman G. Behiry. *Data collection*: Sally Raafat Ishak. *Laboratory investigation*: Enas Sebaey Ahmad, Safia Mohamed Diab, and Abeer Ramadan. *Data analysis and interpretation*:

Shereen El-Bassyoni. *Writing the manuscript*: Shereen El-Bassyoni and Sally Ishak. All authors revised the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data are available upon request.

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REFERENCES

1. Hou C, Zhu X, Chang X. Correlation of vitamin D receptor with bronchial asthma in children. *Exp Ther Med*. 2018;15(3):2773-2776.
2. Shan L, Kang X, Liu F, Cai X, Han X, Shang Y. Expression of vitamin D receptor in bronchial asthma and its bioinformatics prediction. *Mol Med Rep*. 2018;18(2):2052-2060.
3. Guilleminault L, Ouksel H, Belleguic C, et al. Personalised medicine in asthma: from curative to preventive medicine. *Eur Respir Rev*. 2017;26(143):160010. doi:10.1183/16000617.0010-2016
4. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. *Cell*. 2019;176(1-2):11-42.
5. Bordi M, Berg MJ, Mohan PS, et al. Autophagy flux in CA1 neurons of Alzheimer hippocampus: increased induction overburdens failing lysosomes to propel neuritic dystrophy. *Autophagy*. 2016;12(12):2467-2483. Epub November 4, 2016. doi:10.1080/15548627.2016.1239003
6. Ravanan P, Srikumar IF, Talwar P. Autophagy: the spotlight for cellular stress responses. *Life Sci*. 2017;188:53-67.
7. Sachdeva K, Do DC, Zhang Y, Hu X, Chen J, Gao P. Environmental exposures and asthma development: autophagy, mitophagy, and cellular senescence. *Front Immunol*. 2019;10:2787.
8. Global Initiative for Asthma. *Global strategy for asthma management and prevention*. *Global strategy for asthma management and prevention*. 2020. Accessed March 29, 2022. www.ginasthma.org
9. Palizgir MT, Akhtari M, Shahram F, et al. Downregulation of autophagy-related genes in macrophages from patients with Behcet's disease. *Crescent J Med Biol Sci*. 2018;5(1):14-20.
10. Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. *Proc Am Thorac Soc*. 2009;6(3):256-259. doi:10.1513/pats.200808-087RM
11. Kabesch M, Tost J. Recent findings in the genetics and epigenetics of asthma and allergy. *Semin Immunopathol*. 2020;42(1):43-60. Epub February 14, 2020. doi:10.1007/s00281-019-00777-w
12. Jyothula SS, Eissa NT. Autophagy and role in asthma. *Curr Opin Pulm Med*. 2013;19(1):30-35. doi:10.1097/MCP.0b013e32835b1150
13. Li BB, Chen YL, Pang F. MicroRNA-30a targets ATG5 and attenuates airway fibrosis in asthma by suppressing autophagy. *Inflammation*. 2020;43(1):44-53. doi:10.1007/s10753-019-01076-0
14. Theofani E, Xanthou G. Autophagy: afriend or foe in allergic asthma? *Int J Mol Sci*. 2021;22(12):6314. doi:10.3390/ijms22126314
15. Arakawan S, Honda S, Yamaguchi H, Shimizu S. Molecular mechanisms and physiological roles of Atg5/Atg7-independent alternative autophagy. *Proc Jpn Acad*. 2017;93(6):378-385.
16. Martin LJ, Gupta J, Jyothula SS, et al. Functional variant in the autophagy-related 5 gene promotor is associated with childhood asthma. *PLoS One*. 2012;7(4):e33454. Epub April 20, 2012. doi:10.1371/journal.pone.0033454
17. McAlinden KD, Deshpande DA, Ghavami S, et al. Autophagy activation in asthma airways remodeling. *Am J Respir Cell Mol Biol*. 2019;60(5):541-553. doi:10.1165/rcmb.2018-0169OC
18. Lv X, Li K, Hu Z. Asthma and autophagy. *Autophagy*: *Biology and Diseases*. Advances in Experimental Medicine and Biology. 2020;5(1):581-584, Springer.
19. Poon AH, Choy DF, Chouiali F, et al. Increased autophagy-related 5 gene expression is associated with collagen expression in the airways of refractory asthmatics. *Front Immunol*. 2017;8:355. doi:10.3389/fimmu.2017.00355
20. Zeki AA, Yeganeh B, Kenyon NJ, Post M, Ghavami S. Autophagy in airway diseases: a new frontier in human asthma? *Allergy*. 2016;71(1):5-14. Epub October 20, 2015. doi:10.1111/all.12761
21. Pham DL, Kim SH, Losol P, et al. Association of autophagy related gene polymorphisms with neutrophilic airway inflammation in adult asthma. *Korean J Intern Med*. 2016;31(2):375-385. doi:10.3904/kjim.2014.390
22. Ishak SR, Abd El Sayed STK, Wahba NS. Prevalence of common sensitizing aeroallergens in Egyptian asthmatic patients. *World Allergy Organ J*. 2020;13(4):100115. doi:10.1016/j.waojou.2020.100115
23. Ahmed AE, Hassan MH, Toghan R, Rashwan NI. Analysis of 25-hydroxy cholecalciferol, immunoglobulin E, and vitamin D receptor single nucleotide polymorphisms (Apa1, Taq1, and Bsm1), among sample of Egyptian children with bronchial asthma: a case-control study. *Pediatr Pulmonol*. 2020;55(6):1349-1358. Epub April 20, 2020. doi:10.1002/ppul.24785
24. Hameed RM, Ahmed MM, Abood HA, Hussein AM. To evaluate total serum immunoglobulin E level and factors that effect on this level in Iraqi asthmatic children. *Biomed Biotechnol Res J*. 2019;3:240-244.
25. Chaudhary GS, Kumar A, Shashtri M, Chaudhary V. Comparison of total serum immunoglobulin E and absolute eosinophil count levels among asthmatic and non-asthmatic children. *Indian J Child Health*. 2017;4(3):345-347. doi:10.32677/IJCH.2017.v04.i03.016

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