How to Cite:
Juda, T. M., Mohammed, S. B., Al-Aaraji, A. J., & Al-Koofee, D. A. F. (2022). Genotyping of FOXO3a gene polymorphism rs13217795 (C > T) in asthmatic Iraqi patients. *International Journal of Health Sciences*, *6*(S6), 6074–6085.
https://doi.org/10.53730/ijhs.v6nS6.11675

Genotyping of FOXO3a gene polymorphism rs13217795 (C > T) in asthmatic Iraqi patients

Thana Mohammed Juda
University of Babylon, faculty of Medicine
Email: moh.thana@uobabylon.edu.iq

Seenaa Badr Mohammed
University of Babylon, faculty of Medicine
*Corresponding author email: bdr.seenae@uobabylon.edu.iq

Ameera Jasim Al-Aaraji
University of Babylon, faculty of nursing
Email: nur.ameera.jasim@uobabylon.edu.iq

Dhafer A. F. Al-Koofee
University of Kufa, faculty of Pharmacy
Email: dhafera.faisal@uokufa.edu.iq

Abstract---Background: Asthma is a chronic inflammatory process that affects the airways of the lung. It has a greater impact on public health. It is a polygenic disease, many factors play a role implicated in triggering its that starts like genetic, environmental, and geographic factors. Genetic studies have focused on the forkhead box O3 (*FOXO3a)* gene, mainly polymorphism at the specific sites which is thought may contribute to exacerbating the attack and complications of asthma. Methods: The study was designed as a case-control and involved 138 of asthmatic patients and 120 seemingly healthy people used as a control group. The diagnosis was carried out by consultants at a special respiratory center in Babylon governorate. Genotyping of all subjects was done for *FOXO3a* (rs13217795; C/T) analysis by PCR-RFLP technique. Multinominal regression was used to calculate Odds ratios (ORs) and 95% confidence intervals (CIs) by IBM. SPSS software version 20.0. Results: Analysis of the results that obtained form this work a considerable modification in forms of rs13217795; C/T polymorphism between asthmatic and healthy individuals. elevation in the risk of asmathic patient was seen in T allele of FOXO3a gene (odds ratio of 2.0313, 95% CI: 1.5493 - 2.6631, P<0.0001). Conclusions: We concluded that patients who carry the T/T allele are at high risk for developing illness and complications compared with

other forms which included C/T and C/C alleles, respectively (P < 0.0001), and patients with the C/T allele showed increased susceptibility with border-line significance. The results suggest that *FOXO3a* (rs13217795; C/T) polymorphism has a significant role in the development and progression of asthma.

Keywords---Asthma, FOXO3a, SNP, allele frequency, polymorphism, Iraq.

Introduction

Asthma is an allergic and non-allergic disease that causes obstruction of the airways, leading to attacks of shortness of breath that do not continue indefinitely and have some degree of reversibility (1). The condition has a genetic factor; therefore, children from an asthmatic family have a greater chance of developing asthma than those from families with no asthmatics (2)(3). Episodes of asthma usually feature wheezing, coughing, a tightening of the chest, and difficulty of breath (4)(5). Spirometry is an extremely useful investigation for respiratory diseases, but it is grossly under-utilized in many countries. For the clinical diagnosis of asthma, the demonstration of airway obstruction and its reversibility (a <12% and 200-ml increase in forced expiratory volume in 1 s [FEV1]) following the inhalation of a bronchodilator is recommended. A reduced ratio of FEV1 to forced vital capacity (FVC) indicates airflow obstruction (6)**.** Bronchial asthma is a complex disease and, because of there are multiple factor regard as both genetic and environmental factors may affect its clinical presentation . Genetic elements have an important role in asthma and indicate whether a many person may develop asthma and this accrue as the result of this genetic map (7).

Asthma exhibits the characteristics of inflammatory disease; this inflammatory reaction is mainly due to contributing effect of different cytokines that act as a mediator in an inflammatory and allergic reaction, which include a group of inflammatory markers such as interleukin-4 (IL-4), interleukin-5 (IL-5), and interleukin-13 (IL-13) which secreted mainly through specific T lymphocyte mainly T helper 2 (Th2) cells (1). Airway hyperresponsiveness and tissue transformation are due to the connection between asthma and Th2 cell inflamation. The activation of inflammatory cells comes from an unusual response to specific “aerial elements”(7).The approach to the pathogenesis of developing asthma indicates that the etiological factor participating are mainly through interaction of environmental with molecular and genetic effects (8).

The forkhead box O (*FOXO*) genes responsible for regulating important pathways, including the action of “insulin” signaling, the regulation process of programmed cell death or “apoptosis”, fixing and correcting the error in DNA that cause mutation through control repair mechanism, and control the effect of oxygen free radicals that cause an imbalance of prooxidant and antioxidant control mechanism, and longevity (9). *FOXO3* is located on the long arm of chromosome six (6q21) responsible for controlling the process of transcription of certain factors of the transcription process of RNA. These transcription factors play important

roles in controlling the action and mechanism of “immune homeostasis”, and different function losses may be linked to chronic inflammatory processes(10).

The primary mechanisms regulating the activity of the *FOXO3a* gene involve controlling theprocess of “translocation of FOXO3”a between the “nucleus and cytoplasm”, and this action are completed by tadding phosphate groups through action of of enzyme mainly of “kinases:. The phosphorylation of FOXO3a by P38 mitogen-activated protein kinases, macrophages stimulating 1 (MST1), and 5' AMP-activated protein kinase (AMPK) promote its nuclear entry and increase its transcriptional activity (11). Because balancing nuclear import and export is critical for maintaining FOXO3a functions, the loss of this balance leads to the increase and improvement of several types of diseases involving cancer. FOXO3a through its effect on transcription factor which is important for controlling many physiological actions inside the cell and disturbance of this process trigger for developing abnormal event in the cell as cancer and disease (12). Furthermore, FOXO3a is a core regulator of cellular homeostasis, stress response, and longevity, as it can modulate a variety of cellular processes, integrating inputs from energy metabolism, growth factors, and stress signaling cascades. The main processes regulated by FOXO3a include cell cycle progression, DNA repair, autophagy, reactive oxygen species detoxification, and apoptosis. In this research, we check out if the rs13217795 (C > T) polymorphism of the *FOXO3a* gene is embroiled and related to asthma in the Iraqi population (13). In this research, the aim was investigate *FOXO3a* (rs13217795) gene polymorphism to assess if genetic variants are associated with asthma in an Iraqi population.

Methods

Study Participants

Before beginning the study, we gained approval from the Ethics Board. This research included 258 participants: 138 patients with well-diagnosed asthma and 120 volunteers considered as a control healthy group.

Inclusion criteria

All patients with pure asthma were pre-diagnosed by consultants following Global Imitative for Asthma global initiative for asthma (GINA) guidelines (14).

Elimination conditions

Indiviuals suffering from diabetes mellitus or hypertension, those who smoked, were pregnant, or had any inflammatory diseases or autoimmune diseases, such as rheumatoid arthritis (RA), were excluded from this study.

Blood sample collection

Samples (5 ml) of peripheral blood from 120 healthy individuals and 138 patients suffering from asthma who were admitted to the special center for respiratory diseases were collected in EDTA tubes. DNA was separated from the lymphocyte of blood using ReliaPrep Blood gDNA Miniprep System (Cat. no. A508, Promega,

USA) using the manufacturer's instructions and guidelines. The extracted DNA was assessed for concentration and purity using a spectrophotometric assay (NANO drop-1000, Thermo Fisher Scientific, USA) and stored at −20°C until the date of analysis. At the time of the assay, the DNA was detected using agarose gel electrophoresis with the aid of ethidium bromide and purple gel-loading dye to visualize and track the DNA under a UV Gel Documentation System (Photo documentation E-Graph, Japan)

Primer design and genotyping of rs13217795 in *FOXO3a*

The polymorphism rs13217795 in *FOXO3a* was detected by amplification of the segment containing" the polymorphic region using polymerase chain reaction (PCR)". The work depends on the principle of the method apply for detection the mutation by "a PCR restriction fragment length polymorphism (PCR-RFLP)" (15), by the action of the specific enzyme "restrictive endonuclease".action first by amplifying the polymorphism region using PCR, the following specific primers were designed for this research as follows (16) were used: "forward primer" 5'-CTCCTTGGTCAGTTTGGTG-3' and "reverse primer" 5'-ATGAGTGAAGATGGAAGTAAGC-3'. The amplification was completed using a 25 µl total volume PCR mix containing 100 ng of genomic DNA, 5 µl of genomic extracted DNA, with a volume of 2.5 µl from primer sspecific for this study all these elements were added to a volume of 12.5 µl of "hot start green master mix: Promega, and 2.5 µl of ddH_2O. In a thermal cycler, the reaction was run with the following optimized protocol:" initial denaturation (95°C, 5 minutes, 1 cycle)," 30 cycles of denaturation (95°C, 30 seconds), annealing (58°C, 30 seconds), extension (72°C, 30 seconds), final extension: (72°C, 5 minutes)", and a final hold (12°C, 15 minutes)". The fragment of 667 bp which considered as "amplicon" gestated at 37°C per nuclease (New England Biolabs, UK) endonuclease for 90 minutes in a final volume of 20 µl containing a volume of 4 µl of ddH_2O, 5 µl of specific "NEB Buffer", a volume of 10 µl of the amplicon, and 1 µl "*Bsp*H1". The digested amplicon was subjected to 2% agarose gel electrophoresis with a safe stain and visualized by UV light using gel documentation (Photo documentation E-Graph, Japan)

.

Statistical analysis

Analyzed statistically using IBM SPSS statistics version 2.0 for Microsoft Windows (SPSS, Chicago, Illinois, USA) to verify the association between polymorphism and disease condition using Fisher's exact test, and https://www.easycalculation.com/statistics/odds-ratio.php was used for odds ratio (OR) calculations. Genotype frequencies consistent with the Hardy–Weinberg equilibrium (HWE) were assessed by using HWE SNPs Analysis Hardy from the university Magna Graecia (UMG) depending on Pearson's x2 test. At statistically significant *P*-value <0.05 (17)

Results

Research focused on the genotype frequencies of 138 asthmatic patients (78 males, 60 females) compared with 120 healthy participants (67 males, 53 females) using a PCR-RFLP assay to investigate the presence or absence of the designed

mutation single nucleotide polymorphism (SNP)of the *FOXO3a* gene. The rs13217795 SNP of the *FOXO3a* gene was recognized via the C/T allele. The the genotype frequencies, as shown in Figure 1. Our results indicate that the homozygous recessive allele (T/T) has a frequency of 43.5%, while the homozygous reference allele (C/C) and heterozygous allele (C/T) appeared less often at 30.4% and 26.1%, respectively. Furthermore, the frequency of the mutant T allele was increased in asthmatic patients (57%) compared with the controls, while the wild type C allele had a frequency of 43%. These frequencies were much higher compared with the control group (38% and 62%, respectively), as shown in Figure 2.

The associations of each genotype with asthma and under different models of inheritance were further tested (Table 1). The data from the HWE exact test revealed that only the control group genotype frequency follow the HWE (P < 0.18), while a significant deviation was recorded in the distribution of the asthmatic patients' genotypes (P < 0.0001), which failed to coincide with HWE, as shown in Table 2. The analysis of the rs13217795 SNP revealed a significant association with the occurrence of asthma. Specifically, the T/T homozygous recessive genotype increased the risk of asthma fourfold with respect to those with the wildtype C/C allele (OR = 4.28, CI 95%, 2.08-8.82, P = 0.000) and the heterozygous C/T allele (OR = 0.57, CI 95%; 0.31-1.03, P = 0.05). Further analysis regarding the recessive and over-dominant models indicated a significant association (P < 0.0001) between the recessive mutant model and the risk of asthma, with a risk factor of about 5.82 fold. The frequency of the minor allele (T) was higher in the asthma patients than those in the control group, however.

Table 3 shows the genotype and allele frequency of the *FOXO3a* gene polymorphism in males and females. The frequency of the C/T genotype in males was significantly higher in control individuals than asthmatic patients (53.7% *versus* 23.1%), and the T/T genotype frequency was significantly higher in the patient than the control group (46.2% *versus* 10.5%). The OR for those with the T/T allele indicated they had a 5.1 times higher risk of developing asthma compared with people with the C/C wildtype allele. The C allele was more prevalent in the healthy control group (63%) than the asthma group (42%), and the mutant T allele frequency was the opposite, being detected in 58% of asthma patients and 37% of healthy participants.

The frequency of the heterozygous allele (C/T) in females did not exhibit any significant difference between the studied groups (P = 0.32), but there was a significant difference in the frequency of the homogenous mutant (T/T) genotype between patients and healthy volunteers (40% *versus* 13%). The OR (3.42) for the mutant homozygous T/T allele indicates that it confers a 3.42 times higher risk of developing asthma compared with the wildtype C/C allele. The C allele was more frequent in the control group (60%) than the patient group (45%), while the T allele frequency showed the opposite trend, manifesting in 55% of patients and 40% healthy people.

Discussion

In the present research, we assessed the association between *FOXO3a* gene polymorphism (rs13217795) and the risk of asthma in a population in Iraq. A significant association ($p< 0.05$) among intended SNP from the *FOXO3a* gene. Many genes are known and implicated in the airway inflammation in asthma involving *FOXO3a.* Our results indicated that highst frequency in control group for hetroygous CT rather than asthma patient. Genotype frequencies of mutant type TT for cases were 43.5% while healthy subjects were 11.7% respectively. On the other hand, CC genotypes were 30.4% for asthmatic patients and 35% for the healthy group respectively. Minor allele frequency (T allele) were 57% and 38% for patients and the control group, while major allele frequency were 43% and 62% for patients and the control group respectively. Nihal et. al, (18) study on the Egyptian children reached the highest frequency was seen for the heterozygous CT in both cases and controls groups, whereas the genotype frequencies of mutant type TT for cases and controls were 12 % and 16% respectively also distribution of CC genotype was present in 37.3% of asthmatic patients and 22.6% in the control group and this is opposite to our results.

Asthma is a long-term inflammatory disorder of the bronchial tree in which several cells, especially eosinophils, mast cells, and T lymphocytes, play a key role. Asthma is also recognized as having a solid genetic component (19). *FOXO3a* plays a central role in maintaining immunoregulation and is associated with several inflammatory diseases, including arthritis and chronic obstructive pulmonary disease (20). In this study, we explored the association between an intronic single-nucleotide substitution in *FOXO3a* (rs13217795) and asthma, and we found a significant association between the SNP and the disease ($P < 0.05$).

FOXO3a is known as a tumor suppressor gene, as it is a trigger for apoptosis (21). It encodes a protein, a transcription factor with forkhead DNA-binding domains. *FOXO3a* also has roles in the expression regulation of several genes involved in diverse biological processes, such as the duplication of nuclear DNA content before cell division, senescence, oxidative stress resistance, and vascular remodeling (22)(23). However, FOXO3 is involved in innate immune homeostasis and inflammation, controlling the T cell response and regulating neutrophil functions during inflammation (24). The link between *FOXO3a* gene polymorphism and RA disease was investigated by Ludikhuize et al., who found that mutation of the *FOXO3a* gene increased the survival of neutrophils in RA samples compared with healthy samples. As neutrophils are one of the main components involved in eliciting an immune response, their survival and maintenance are fundamental to asthmatic inflammation (25). A deficiency in FOXO3a has been associated with spontaneous lymphoid proliferation, inflammation in various organs, and increased hyperactivated T helper cells (26).

RA studies revealed that the phosphorylation of FOXO3a occurs in lymphocytes. FOXO3a deficiency in mice resulted in spontaneous lymphoproliferation along with inflammation of various organs, such as salivary glands and lungs, and increased helper T cell activity, suggesting FOXO3a has an important role in preventing T cell hyperactivity—one of the primary causes of asthmatic inflammation (26). The hyperactivity of mast cells is associated with *FOXO3a*

polymorphism, as mast cells are key mediators of smooth muscle inflammation in asthma (27), and FOXO3a keeps mast cells alive (25). Therefore, increased mast cell proliferation may result from the phosphorylation or mutation(s) of FOXO3a (28). The function of FOXO3 is affected by post-translational modifications, such as acetylation, phosphorylation, and ubiquitination, which may affect post-transcription protein levels (29)(30).

Additionally, the *FOXO3a* gene regulates the production of IL-10, NF-κB, IFN-α, and IL-2. The genes for IFN-γ and IL-2 are pro-inflammatory targets of the FOXO3a. SNP of *FOXO3a* may result in the upregulation of these genes, thereby facilitating continuous inflammation (31)(32). An Egyptian cross-sectional case-control study done by Nihal et al. on 75 asthmatic children following up in the pulmonology outpatient clinic, and 75 age and sex-matched healthy control reported no association between *FOXO3a* gene polymorphism (rs13217795) and asthma. Their results were the C allele frequency was 53.3% in the healthy group and 62.8% in the patients group, whereas T allele frequencies were 37.2% in asthmatic cases and 46.7% in the healthy subjects while the CC genotype has appeared in 37.3% of asthmatic patients and 22.6% in the controls, (p value>0.05) (33), which disagrees with our findings. In contrast, Shravani et. al.'s documented the results of a case-control study designed with the lowest CC genotype (9.65%) in asthmatic patients and 50.7% in the control group while the TT genotype was high (51.75%) in asthmatic patients compared to the control group (12.67%), also T allele frequencies were distributed with71.05% in asthmatic patient and 30.99% respectively, which consistent with the present study, as they found a significant relationship between asthma and the C/T and T/T genotypes ($P < 0.0001$) (16).

Conclusions

Our results demonstrated that the rs13217795 "polymorphism" of the *FOXO3a* gene can be considered a potential threat factor for increasing the susceptibility and exacerbation of the attack of asthma. The rs13217795 C < T SNP in *FOXO3a* was associated with the development of asthma, with the T allele being more frequent in asthma patients than the C allele. We also found asthma risk to be affected by gender.

Acknowledgements

The authors would like to thank the staff and nurses in the respiratory center in Babylon governorate Hospitals for their cooperation and help in implementing this work as well as the special thanks for patients..

Abbreviations

FOXO3a: Forkhead box O3
FVC: Forced vital capacity
FEV1: Forced expiratory volume in 1 s
MST1: Macrophages stimulating 1
AMPK: 5' AMP-activated protein kinase
ROS: Reactive oxygen species

GINA: Global initiative for asthma
RA: Rheumatoid arthritis
PCR: Polymerase chain reaction
RFLP: Restriction fragment polymorphism
HWE: Hardy-Weinberg equilibrium
IFN-*α*: Interferons type1
IL-2: Interleukin-2
IFN-γ: Interferons- γ

Conflicts of interest

The authors declare there is no conflict for this work.

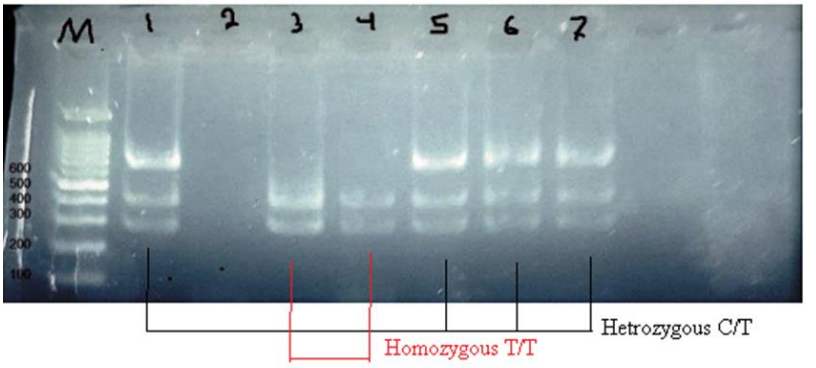

Figure 1: PCR product digestion with BspHI endonuclease demonstrating the different genotypes of *FOXO3a* on 2% agarose, 100 V; lanes 1,5, 6, 7 represent heterozygous C/T allele (3 bands 667+391+275 bp); lanes 3, 4: represent homozygous recessive T/T allele (2 bands of 391+275 bp). Homozygous wildtype; C/C allele (1 band 667 bp) does not appear here. Lane M contains the 100 bp DNA marker.

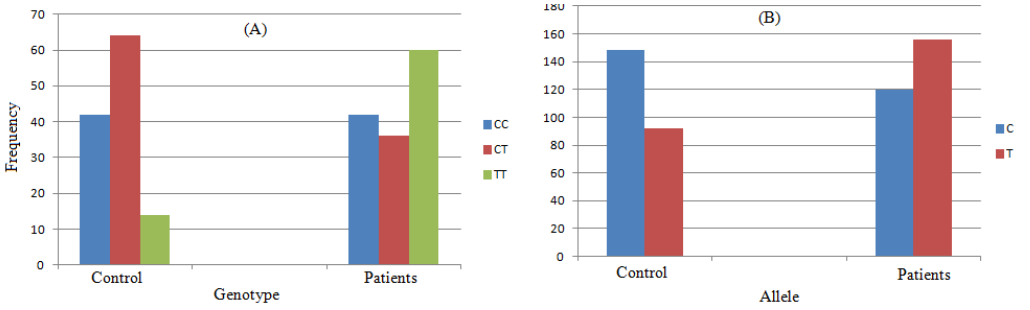

Fig. 2. Frequency distribution of (rs13217795) SNP of *FOXO3a* genotypes (A)/alleles (B) in asthmatic patients and controls

Table 1
Genotype and allele frequency of *FOXO3a* gene polymorphism in the studied groups

SNP (C/T) rs13217795	*Control (%) #120*	*Patient (%) #138*	*OR (95% CI)*	*P value*
Co-dominant				
CC	42(35%)	42(30.4%)	1.00	
CT	64(53.3%)	36(26.1%)	0.5714 0.3161 - 1.0331	0.05
TT	14(11.7%)	60(43.5%)	4.2857 2.0818 - 8.8228	<0.0001
Dominant				
CT+TT	78(65%)	96(69.6%)	1.2308 0.7305 - 2.0738	0.4354
Recessive				
CC+CT	106(88.3%)	78(56.5%)	1.00	
TT	14(11.7%)	60(43.5%)	5.8242 3.0372 - 11.1686	< 0.0001
Overdominant				
CC+TT	56(46.7%)	102(7.9%)	1.00	
CT	64(53.%)	36(26.1%)	0.3088 0.1831 - 0.5207	< 0.0001
Additive				
C	148(62%)	120(43%)		
T	92(38%)	156(57%)		
Frequency of T allele	92 (38%)	156 (57%)	2.0313 (1.5493 - 2.6631)	< 0.0001

Table 2: Hardy-Weinberg equilibrium results of *FOXO3a* gene polymorphisms in healthy subjects and patients with asthma group

	CC	*CT*	*TT*	*C*	*T*	*P value*
All Subjects	84	100	74	268	248	<0.0003
Control	42	64	14	148	92	0.18
Patients	42	36	60	120	156	<0.0001

Table 3
Genotype and allele frequency of *FOXO3a* gene polymorphism in the studied groups according to gender interaction

Gender	*Allele*	*Control (%)*	*Patients (%)*	*OR (95 % CI)*	*P Value*
Male	CC	24 (35.8)	24 (30.7)	-	
	CT	36 (53.7)	18 (23.1)	0.5 (0.2246 - 1.113)	0.089
	TT	7 (10.5)	36 (46.2)	5.1 (1.9153 - 13.8095)	0.0012
	C	84 (63)	66 (42)		
	T	50 (37)	90 (58)		
	MAF%	37	57		
Female	CC	18 (34)	18 (30)	-	
	CT	28 (53)	18 (30)	0.64 (0.2662 - 1.5526)	0.32
	TT	7 (13)	24 (40)	3.4286 (1.1811 - 9.953)	0.02
	C	64 (60)	54 (45)		
	T	42 (40)	66 (55)		
	MAF%	39.5	55		

References

1. Al-Aaraji AJ, Aesa Al-Qaysi S, Salihbaay A. Haplotype in ABCC4 gene by PCR-SSCP technique in Iraqi Asthmatic patients. J Phys Conf Ser. 2019;1294(6):4–9.
2. Ali Abdulla A, Abdulaali Abed T, Razzaq Abdul-Ameer W. Impact of IL-21 Gene Polymorphisms (rs2055979) and the Levels of Serum IL-21 on the Risk of Multiple Sclerosis. Arch Razi Inst. 2022;77(1):71–6.
3. Asouri M, Rokni HA, Sahraian MA, Fattahi S, Motamed N, Doosti R, et al. Analysis of Single Nucleotide Polymorphisms in HLA-DRA, IL2RA, and HMGB1 Genes in Multiple Sclerosis. Reports Biochem Mol Biol. 2020;9(2):199–208.
4. Becker T, Loch G, Beyer M, Zinke I, Aschenbrenner AC, Carrera P, et al. FOXO-dependent regulation of innate immune homeostasis. Nature [Internet]. 2010;463(7279):369–73. Available from: http://dx.doi.org/10.1038/nature08698
5. Chen YF, Pandey S, Day CH, Chen YF, Jiang AZ, Ho TJ, et al. Synergistic effect of HIF-1α and FoxO3a trigger cardiomyocyte apoptosis under hyperglycemic ischemia condition. J Cell Physiol. 2018;233(4):3660–71.
6. Dejean AS, Beisner DR, Ch'en IL, Kerdiles YM, Babour A, Arden KC, et al. Transcription factor Foxo3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. Nat Immunol. 2009;10(5):504–13.
7. Elrifai N, Al-Wakeel H, Osman H, El Taweel R. FOXO3a gene polymorphism and bronchial asthma in Egyptian children. Egypt J Pediatr Allergy Immunol. 2019;17(1):31–6.

8. Fasano C, Disciglio V, Bertora S, Lepore Signorile M, Simone C. FOXO3a from the Nucleus to the Mitochondria: A Round Trip in Cellular Stress Response. Cells. 2019;8(9):1110.
9. Halapi E and USB. Overview on the current status of asthma genetics. Clin Respir J. 2009;
10. Ho KK, McGuire VA, Koo CY, Muir KW, De Olano N, Maifoshie E, et al. Phosphorylation of FOXO3a on Ser-7 by p38 promotes its nuclear localization in response to doxorubicin. J Biol Chem. 2012;287(2):1545–55.
11. Huang H, Tindall DJ. Dynamic FoxO transcription factors. J Cell Sci. 2007;120(15):2479–87.
12. Jonathan Maslan, MD, James W. Mims M. What is A s t h ma ? P a t h o p h y s i o l o g y , D e m o g r a p h i c s , an d H e alt h C a re C o s ts. Otolaryngol Clin NA. 2014;47(1):13–22.
13. Kalemci S, Edgunlu TG, Turkcu UÖ, Çetin ES, Zeybek A, Candan H. FOXO3a gene polymorphism and serum FOXO3a levels in patients with chronic obstructive pulmonary disease and healthy controls: Effects of genetic polymorphism in chronic obstructive pulmonary disease. 2013;1–5.
14. Kim TH, Oh DK, Oh Y, Lee SW, Lee S Do, Lee JS. Fibrinogen as a potential biomarker for clinical phenotype in patients with chronic obstructive pulmonary disease. J Thorac Dis. 2018;10(3):5260–8.
15. Lin L, Hron JD, Peng SL. Regulation of NF-κB, Th activation, and autoinflammation by the forkhead transcription factor Foxo3a. Immunity. 2004;21(2):203–13.
16. Ludikhuize J, De Launay D, Groot D, Smeets TJM, Vinkenoog M, Sanders ME, et al. Inhibition of forkhead box class O family member transcription factors in rheumatoid synovial tissue. Arthritis Rheum. 2007;56(7):2180–91.
17. Martin KR, Ohayon D, Witko-Sarsat V. Promoting apoptosis of neutrophils and phagocytosis by macrophages: Novel strategies in the resolution of inflammation. Swiss Med Wkly. 2015;145(February):1–10.
18. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: Executive summary of the GINA Dissemination Committee Report. Allergy Eur J Allergy Clin Immunol. 2004;59(5):469–78.
19. Moheimani F, Hsu AC, Reid AT, Williams T, Kicic A, Stick SM, et al. The genetic and epigenetic landscapes of the epithelium in asthma. Respir Res [Internet]. 2016;17(119):1–15. Available from: http://dx.doi.org/10.1186/s12931-016-0434-4
20. Nakajima H, Hirose K. Role of IL-23 and Th17 Cells in Airway Inflammation in Asthma. immune Netw. 2010;10(1):0–3.
21. Osman NMERHA-WHM, Taweel RH El. FOXO3a gene polymorphism and bronchial asthma in Egyptian children INTRODUCTION Bronchial asthma is the most prevalent chronic immunological disorder in childhood period. It is characterized by airways inflammation and bronchial hyper-responsiveness where [Internet]. Molecular Biology International. 2019 [cited 2020 Oct 19]. p. 31–6. Available from: https://www.ajol.info/index.php/ejpai/article/view/185776
22. Parmin, P., Suarayasa, K., & Wandira, B. A. (2020). Relationship between quality of service with patient loyality at general polyclinic of kamonji public health center. International Journal of Health & Medical Sciences, 3(1), 86-91. https://doi.org/10.31295/ijhms.v3n1.157

23. Peng SL. Forkhead transcription factors in chronic inflammation. Int J Biochem Cell Biol. 2011;42(4):482–5.
24. Ronnebaum SM, Patterson C. The FoxO family in cardiac function and dysfunction. Annu Rev Physiol. 2009;72:81–94.
25. Sela U, Dayan M, Hershkoviz R, Cahalon L, Lider O, Mozes E. The negative regulators Foxj1 and Foxo3a are up-regulated by a peptide that inhibits systemic lupus erythematosus-associated T cell responses. Eur J Immunol. 2006;36(11):2971–80.
26. Shah T JK. Analysis of FOXO3a Gene Polymorphism Associated with Asthma. Methods Mol Biol. 2019;1890:259–66.
27. Shravani Barkund, Tejas Shah, Nikhil Ambatkar, Maithili Gadgil KJ. FOXO3a Gene Polymorphism Associated with Asthma in Indian Population. Mol Biol Int. 2015;2015:6.
28. Stahl M, Dijkers PF, Kops GJPL, Lens SMA, Coffer PJ, Burgering BMT, et al. The Forkhead Transcription Factor FoxO Regulates Transcription of p27 Kip1 and Bim in Response to IL-2 . J Immunol. 2002;168(10):5024–31.
29. Stephen J. Galli, Mindy Tsai and AMP. The development of allergic inflammation. Nature. 2008;454(7203):445–54.
30. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. International Journal of Health Sciences, 5(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937
31. Thomsen SF. Genetics of asthma: an introduction for the clinician. Eur Clin Respir J. 2015;1:1–5.
32. Wang X, Hu S, Liu L. Phosphorylation and acetylation modifications of FOXO3a: Independently or synergistically? Oncol Lett. 2017;13(5):2867–72.
33. Wang Y, Zhou Y, Graves DT. FOXO transcription factors: Their clinical significance and regulation. Biomed Res Int. 2014;2014(Figure 1).
34. Zhang X, Tang N, Hadden TJ, Rishi AK. Akt, FoxO and regulation of apoptosis. Biochim Biophys Acta - Mol Cell Res [Internet]. 2011;1813(11):1978–86. Available from: http://dx.doi.org/10.1016/j.bbamcr.2011.03.010
35. Zhu Y, Yan X, Zhai C, Yang L, Li M. Association between risk of asthma and gene polymorphisms in CHI3L1 and CHIA : a systematic meta-analysis. BMC Pulm Med. 2017;17(193):1–10.