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Strong Association of *STIP1* Gene rs2236647 Polymorphism and Serum Magnesium Level with Bronchial Asthma in a Population from Iraq

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Abstract:

Bronchial asthma causes the airways of the lungs to constrict and enlarge and create an overload of mucus, which makes it harder to breathe. The STIP1 gene polymorphisms have recently been linked to asthma by some researchers. The goal of this study was to whether there is an association between an SNP (rs2236647) in the *STIP1* gene and asthma, as well as to see if blood magnesium level is linked to bronchial asthma in the Iraqi population. A total of 80 subjects were enrolled in the study, including 40 bronchial asthma sufferers and 40 healthy controls. The mean age of patients diagnosed with bronchial asthma was 35.7±11.2 years, while the average age of the control group was 34.1±9.1 years. The mean magnesium level in people with asthma (1.75 mg/dl) was lower than that of the controls (2.10 mg/dl), however, the difference was not statistically significant (p value= 0.07). A significant genetic relationship was discovered between the rs2236647 CT and T allele of the *STIP1* gene and bronchial asthma in the studied population (p=0.001). Furthermore, allelic interaction model analysis revealed that the T allele is dominant on the C allele and the heterozygote genotype CT might be over-dominant on the homozygote genotypes TT and CC. Overall, we found that the CT genotype and T allele of the rs2236647 of the *STIP1* gene and lower levels of blood magnesium might be considered risk factors for bronchial asthma.

Keyword: Bronchial Asthma, rs2236647, SNP, *STIP1*- gene

Introduction

Asthma is a disease characterized by physiological, clinical, and pathological features. Shortness of breath, especially at night, is the most common symptom in the clinical history, which is frequently accompanied by a cough (1). Bronchial asthma is a prevalent chronic respiratory condition that affects people all over the world. Exacerbation of asthma has been linked to magnesium deficiency (2). Magnesium is the eleventh most common element in the human body, and its intracellular activity is the second most common (3). The total amount of magnesium in the body is about 25 grams, with 60% of it in the bones, 39% intracellular (about 20% in the muscle of skeletal), and only 1% in the extracellular fluid (4). Low dietary magnesium intake is linked to poor lung function, bronchial-hyper reactivity, and wheezing, according to epidemiological studies. In stable asthmatic individuals, it was discovered that a high magnesium intake is associated with improvement in symptom score, but not in objective measurements of airflow or airway reactivity (5). Magnesium affects how muscle cells handle calcium, which has a significant impact on muscular contraction and

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relaxation. Low magnesium intake and Hypomagnesemia may be linked to several illnesses, such as osteoporosis, cardiovascular disease, and diabetes (6,7). Studies show that magnesium is used to treat severe asthma (8,9). According to different studies on serum magnesium levels in asthma patients, hypomagnesemia is common among asthmatics (10-12). However, there is no research linking serum Mg levels and the management of asthma symptoms. The stress-induced phosphoprotein 1(STIP1) gene (11q13.1), encodes the STIP-1 protein that controls heat shock because it coordinates the activities of the heat shock proteins HSP90 and HSP70 (13). STIP-1 contains three tetratricopeptide repetition (TPR) domains that can bind with Hsp to create complexes involved in a variety of biological activities. STIP-1 involves glucocorticoid receptor (GR)-heterocomplex activation (14). The STIP1 gene has 14 exons and is a member of the steroid pathway (15). Recent studies link STIP1 SNPs to lung function in asthmatic patients receiving inhaled corticosteroids. This raises the possibility that this gene may serve as a predictor of glucocorticosteroid responses in those with compromised lung function (16). A candidate gene association study found that the SNPs rs6591838 (intron 1), rs4980524 (intron 1), rs2236648 (intron 5), and rs2236647 (intron 5) in the STIP1 gene were associated with an improvement in lung function in response to Inhaled corticosteroids (ICS) drug (15). There is a significant genetic association between the STIP1 rs2236647 SNP and the prevalence of asthma. The asthmatic group showed significantly greater frequency of the (C) allele and (CC) genotype of this SNP compared to controls (16). The objective of this research was to determine the relationship between magnesium levels and asthma in the Iraqi population and investigate about association of the rs2236647 polymorphisms in the STIP1 gene with asthma.

Materials and Methods

Study population

The study included two groups, 40 asthmatic patients and 40 healthy people as a control group. A doctor's clinical examination in the Baghdad Center for Allergy was used to determine each patient's diagnosis, and they were all chosen according to the GINA guideline. And, healthy individual criteria include they do not suffer from chronic diseases such as diabetes and high blood pressure, having no family history of allergy, being a non-smoker, and with a serum total IgE value less than 100 IU/ml. Blood was obtained from subjects with an asthmatic visit to the allergy center in Baghdad Alrusafa City during the period from November 2020 to January 2021. Informed consent was obtained from the patients and controls before the commencement of the study. Blood samples are divided into 2 portions: 1 ml of whole blood is collected into tubes containing ethylene demine tetra acetic acid (EDTA), kept at–20ºC, for genomic DNA extraction, and 2 ml into gel tubes to obtain serum are separated immediately for magnesium test.

Extraction of Genomic DNA

Asthma patients and healthy controls had two ml of peripheral venous blood drawn out of them using disposable EDTA syringes. Using the gSYNCTM DNA Extraction Kit (blood) from Geneaid, Inc. (Taiwan), genomic DNA was purified from the Blood samples. The Qubit 4 Fluorometer was then used to gauge the purity and concentration of the DNA.

Genotyping of the DNA samples

A DNA seqment containing the *STIP1* SNP (rs2236647, C–T transition), was amplified using primers; Forward; 5 - TGCTTCAAGTCGAAGGGATT-3 and reverse; 5 - ATCGTTCCCCAGCTCTTTTT-3 (Tsai et al., 2018). The reaction conditions for the amplification of target DNA are listed in Table (1). The amplicons were assessed at Gel Doc. (Biorad) after electrophoresis on 2% agarose gel stained with ethidium bromide. PCR products were sent to Macrogen Corporation (South Korea) for sequencing. The sequencing was done using the ABI Big Dye v.3.2 terminator sequencing kit based on the kit instructions. Geneious software was used to compare the sequence data to the NCBI RefSeq reference sequence (http://www.ncbi.nlm.nih.gov) (ID for *STIP1 gene*: 10963).

Table (1): The PCR program used for amplification of the *STIP1* gene segment

Step	Temperature	Time	Cycle
Initial-denaturation	94ºC	5 minute	1
Denaturation Annealing Extension	94ºC 54.2ºC 72ºC	30 seconds 1minute 30 seconds	35
Final extension	72ºC	7 minutes	1

Measurement of Serum Magnesium Level:

All participants had a two-milliliter venous blood sample obtained without any anticoagulant. The coagulated samples were centrifuged for 10 minutes at 3000 rpm and the serum samples were analyzed by an ELISA to assess Mg level (Microwell ELISA, Diagnostic Automation Inc., USA). Reference magnesium concentrations in adult male blood serum should be between 1.8 and 2.6 mg/dl, while total magnesium concentrations in adult female blood serum should be between 1.9 and 2.5 mg/dl. Hypomagnesaemia was defined as a serum Mg level of less than 1.6 mg/dl (17).

Statistics Analysis

Statistical Software for Social Sciences, version 13 (IBM-Corporation, USA) was used to analyze the demographic and genetic data. The chi-square test was used to estimate deviations from the Hardy-Weinberg equilibrium. WinPepi software estimated the odds ratio and 95% confidence interval for the genotypes and allele frequencies. The results were expressed as (Mean ± S.D.) and the p-value ≤0.05 was considered significant.

Results

Demographic and Clinical Data:

This study included 40 asthmatic patients and 40 persons who appeared to be in good health as a group. The demographic information of the participants is summarized in (Table 2). As stated in the table, there were no significant differences between the case and control groups regarding age and gender. However, family history was significantly different between the two groups.

Magnesium level in the serum of the subjects

A comparison of the Mg levels between the case and control groups revealed asthmatic patients had lower mean magnesium levels than the controls (1.75 vs. 2.10 mg/dl). And, significant differences in serum magnesium levels were observed according to the gender of asthmatic patients compared with control (P- value=0.04), as summarized in Table 3.

Diagnostic value of the serum Mg level

To understand whether the Mg level in serum could differentiate asthmatic patients from healthy controls, a Receiver Operating Characteristic (ROC) curve analysis was conducted. This graph was used to discriminate whether Mg level is a marker of asthma or not. The results are summarized in Table (4) and Figure (1). The Area under the ROC curve (AUC) =was 0.705 with a Cut-off = ≤ 1.75(P<0.014), implying that the Mg level of serum might be considered a good marker for diagnosis of asthmatics.

Table (2): Participants' demographics and clinical features.

Demographic	Patient N (%) (N=40)	Controls N (%) (N=40)	P-value
Age (Mean ± S.D.)	35.7±11.2	34.1±9.1	0.1 NS
Gender: Male	22 (55%)	16 (40%)	0.2 NS
Female	18 (45%)	24 (60%)	
Family history: Yes	30 (75%)	0 (0%)	0.03*
No	10 (25%)	40 (100%)	

S.D.= Standard Deviation, N= Frequency, % = percentage , NS= Not Signficent differences, *= Significant differences.

Table 3: Mean Serum Magnesium Levels of Asthmatic Patients and Controls.

Groups	Asthmatic (Mean ± S.D.) (mg/dl)	Control (Mean ± S.D.) (mg/dl)	P-value
Total	1.75± 0.39	2.10 ± 0.45	0.07 NS
Subgroup: Male	1.78±0.26	2.11±0.26	
Female	1.70±0.19	2.00±0.19	0.04*

mg/dl= milligram/deciliter, S.D.= Standard Deviation, N= Frequency, NS= Not Signficent differences *= Signficent differences.

Table 4: ROC curve analysis of Mg (mg/dl) serum level in asthma.

ROC TEST	
AUC	0.705
Standard Error	0.0838
95% CI	0.573 to 0.816
Z value	2.448
P-value	0.0144
Accuracy	0.7000
Cut-off	≤1.79
Sensitivity	100.00
Specificity	70.00

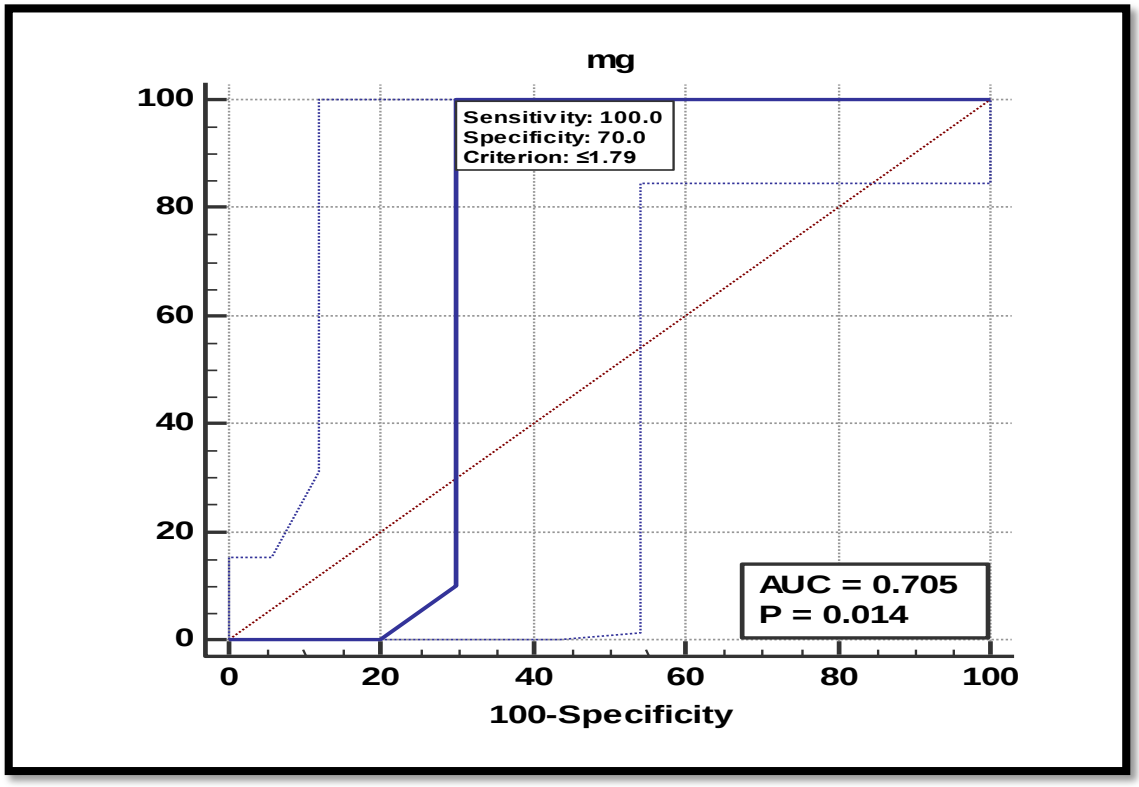

Figure (1): Receiver operating characteristic analysis of Mg (mg/dl) serum level in asthma.

Genotype and allele frequencies of the SNP in the case and control groups

The genotype of each participant for the STIP1 gene rs2236647 (C>T) was determined using direct sequencing of the PCR products. The location of STIP1 gene rs2236647 concerning chromosome 11 and Intron 5 is shown in Figure 2. The genotype and allele frequencies of the case and control groups are summarized in Table 5. To understand whether the case and control groups are in Hardy-Weinberg equilibrium (HWE), a Chi-squared test was conducted. As indicated in Table 6, the patient group revealed a departure in the observed genotypes from the anticipated genotypes (X2=10.15, P=0.001) and the patient group was not in the Hardy-Weinberg equilibrium. However, that control group was in the HWE (X2=0.78, P=0.37). The genotype and allele frequencies were compared between the case and control groups (Table 5). The results indicated that the CC genotype is significantly more prevalent in controls than in patients (OR=0.11, P=0.001). However, the CT genotype was significantly more prevalent in the patients than in the controls (OR=7.0, P=0.001). Although the TT genotype frequency was higher in the patient's group than in the controls, the difference was not statistically significant as shown in Figure 3. Comparing the allele frequencies between the case and control groups showed that the frequency of the T allele was higher in the case group than the controls and the difference was statistically significant (OR=3.12, P=0.002). These results indicated that the T allele might be related to asthma.

The CT genotype appears to be over-dominate

We further analyzed the genetic mode of action of the genotypes in the STIP1 gene rs22366474 (Table 7). The results showed that the T allele is dominant to the C allele and the genotypes (CT+ and TT) might be considered as a risk factor for asthma development. Besides, analyzing the(CC+TT) model revealed that the CT genotype probably acts in an over-dominant genetic mode where it might be considered a risk factor for asthma as indicated in Table (7**).**

Table (5): Genotype and Allele frequencies of the rs2236647 (C>T) of STIP1 gene in the asthmatic patients and healthy controls.

Genotype and Allele Frequencies	Patients (N=40)	Controls (N= 40)	OR	95%CI	P-value
	N (%)	N (%)			
CC	6 (15.0%)	25 (62.5%)	0.11	0.04-0.31	0.001
CT	30 (75.0%)	12 (30.0%)	7.00	2.62-18.51	0.001
TT	4 (10.0%)	3 (7.5%)	1.37	0.29-6.43	1.00
C	42 (52.5%)	62 (77.5%)	0.32	0.16-0.63	0.002
T	38 (47.5%)	18 (22.5%)	3.12	1.58-6.15	0.002

OR= odd ratio, C.I. = Confidence Interval

Table 6: Hardy-Weinberg equilibrium testing of the case and control groups regarding SNP (rs2236647) in the STIP1 gene

Genotypes	Observed	Expected	Observed	Expected
	Patients (N=40)		Controls (N=40)	
CC	6	11.0	25	24.0
CT	30	20.0	12	14.0
TT	4	9.0	3	2.0
X^2 value	10.15		0.78	
P value	0.001		0.37	

HWE- p value= Hardy-Weinberg Equilibrium, X^2= chi square

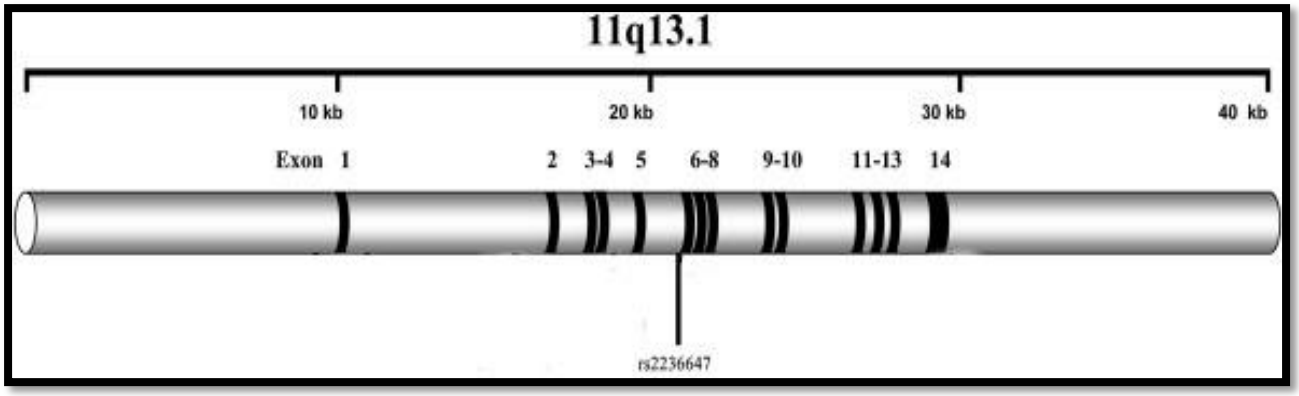

Figure (2): Diagram of STIP1 gene and Location of SNPs rs2236647 (C>T) ON chromosome11 /Intron 5

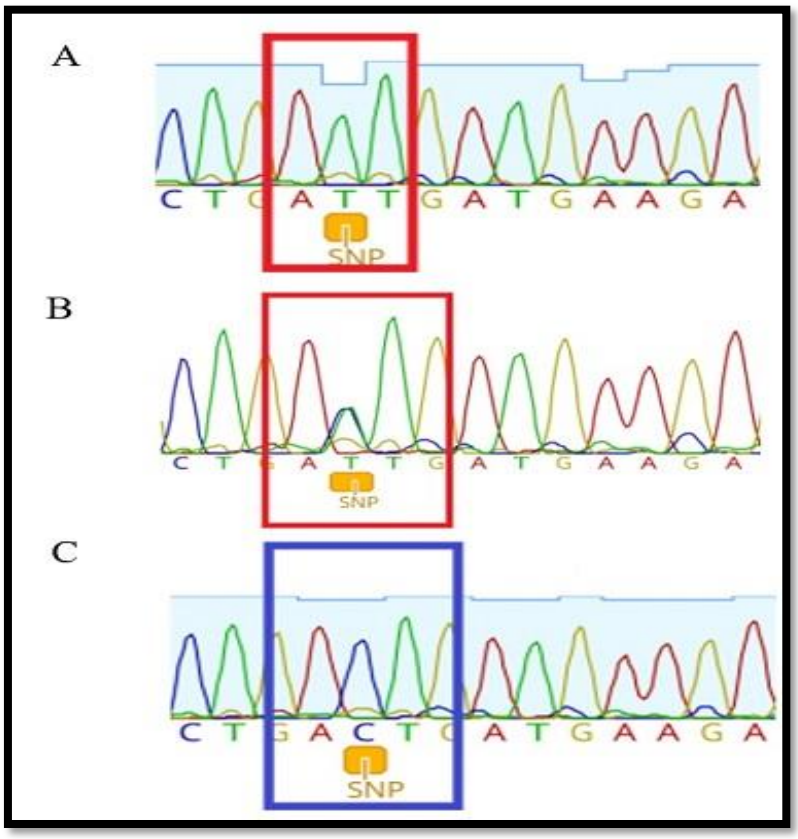

Figure3: Examples of sequencing results representing genotypes of the STIP1 Polymorphism (rs2236647 C>T). A) Represents a homozygote AA genotype, B) represents a heterozygote CT genotype, and C) represents a homozygote CC genotype.

Table (7): Genetic Model action of rs2236647 polymorphisms

Genetic Model	Genotypes	Patients(N=40)	Controls(N=40)	OR	95% C.I.	P-value
Dominant	**CT+TT**	30/4	12/3	9.44	3.26-27.3	0.001
	CC(Ref.)	6	25	1		
Recessive	**CT+CC(Ref.)**	30/6	12/25	1		
	TT	4	3	1.37	0.28-6.55	0.5
Over-dominant	**CC+TT(Ref.)**	6/4	25/3	1		
	CT	30	12	17.0	2.61-18.7	0.001

OR= odd ratio, C.I. = Confidence Interval

Discussion

Raised smooth muscle tone in the airways is the primary factor causing the obstruction that characterizes asthma. Mg is a crucial macro element that participates in the contraction of skeletal and smooth muscles as well as neuronal synapses (18). Patients with persistent asthma were shown to have a high prevalence of hypomagnesemia. Although the cause of hypomagnesemia in chronic asthma patients is unknown (19), it might be connected to asthmatics' low magnesium intake or elevated magnesium loss in the urine as a side effect of b2-agonist, corticosteroid, and theophylline medication (20). Blood Mg levels were found to be lower in allergic asthmatics than in controls in the present study. This result is in agreement with the studies done by Alamoudi in 2001 (19); Oladipo et al. in 2003 (21); Agin and Darjan, in 2005 (22); Shaikh et al., in 2016 (12); Yuvarajan et al., 2017 (23). Additionally, a number of genetic differences and a number of environmental factors interact to generate widespread asthma, asthma, and allergy illnesses. Candidate gene analyses have looked into a long variety of genes involved with asthma and allergy, and they have found over 100 loci to be functional. Genetic variations in STIP1 may affect how the body reacts to corticosteroids in asthmatics with weakened lungs (24-26). Between asthma patients and control subjects, there was a significant difference in the genotypic distribution of the rs2236647 polymorphism (p=0.001). The frequencies of CC (15.0% vs. 62.5%), and CT (75.0 vs. 30.0%) genotypes, and allele frequencies of allele C (52.5 vs. 77.5%) and allele T (47.5% vs. 22.5) were significantly different between the asthmatics and healthy controls. These findings revealed a strong correlation between the development of asthma and the allele T and the genotype CT of STIP1 rs2236647 SNP. Additionally, the genotype CC of the rs2236647 SNP showed a significant protective effect against developing asthma. However, the limited sample size of the study made it impossible to confirm a connection between the TT genotype and asthma risk. Only a few research have looked into the connection between STIP1 polymorphisms and the likelihood of developing asthma in populations with asthma from Iraq and the Arab world (including Tunisians and Jordanians ; Salhi et al. in 2021(27) as well as Chinese people ; Huang et al. in 2020) (28); Ratib and Saud, 2023)(29).

Conclusion:

Our results indicate that serum magnesium levels are lower in asthmatics, and depending on the patients' magnesium condition, these variations were substantial. In addition, the T allele and the CT genotype of STIP1 rs2236647 may be regarded as risk factors for the onset of asthma in the Iraqi population. To validate these results, larger sample sizes and additional research are required.

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Conflict of interest

There are no competing financial interests declared by the authors.

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