

Association of the 5HTR2C gene Ser23 variation with childhood allergic asthma

Sehime Gulsun Temel^{1,2,3*}, Mahmut Cerkez Ergoren^{4,5}, Izel Yilmaz⁶, Ozel Yuruker⁷, Havva Cobanogullari⁸, Ozgur Tosun⁹, Haluk Barbaros Oral¹⁰, Nerin Nadir Bahceciler¹¹

Abstract

Objective: Allergic asthma is the most frequently observed subtype of individuals with asthma. The effects of serotonin plays in the pathophysiology of asthma has not been clearly determined. Thus, this study aimed to investigate the association between the 5HTR2C gene rs 6318 G>C polymorphism and allergic asthma in pediatric patients in Cyprus.

Methods: This study included total number of 177 individuals with 118 control and 59 pediatric patients (43 atopic and 16 non-atopic asthma patient). A skin prick test was performed for each patient to confirm asthma diagnosis and to evaluate atopic status. Genotyping for the 5HTR2C was completed by Real Time-PCR analysis.

Results: The genotype distribution frequencies were not in agreement with the Hardy-Weinberg Equilibrium in the patients' group ($p < 0.00001$). The frequency of the risk allele (allele C) was not significantly different between the patient and control groups ($p = 0.255$). The genotypic distribution between atopic asthma and non-atopic asthma within the patients groups was not in agreement with the Hardy-Weinberg Equilibrium ($p = 0.006$). However, risk allele presence showed a statistically significant association with atopy-related asthma ($p = 0.037$).

Discussion & Conclusion: Overall, despite the finding of no association between the 5HTR2C rs6318 C allele and childhood asthma, the current results indicated that there is a strong association between the 5HTR2C rs6318 C variant and childhood atopic asthma.

Keywords: Childhood Allergic Asthma, Atopy, 5HTR2C, rs6318

¹Department of Medical Genetics, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

²Department of Histology and Embryology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey.

³Department of Translational Medicine, Institute of Health Sciences, Bursa Uludag University, Bursa, Turkey.

⁴Department of Medical Genetics, Faculty of Medicine, Near East University, Nicosia, 99138, Cyprus

⁵Near East University, Faculty of Medicine, Research Center of Experimental Health Sciences (DESAM), Nicosia, 99138, Cyprus

⁶Department of Medical Immunology, Bursa Uludag University, Institute of Health Science, Bursa, Turkey

⁷Department of Immunology, Faculty of Medicine, University of Kyrenia, Kyrenia, Cyprus

⁸Department of Medical Biology, Faculty of Medicine, Near East University, Nicosia, Cyprus

⁹Department of Biostatistics, Near East University, Faculty of Medicine, Near East University, 99138, Nicosia, Cyprus

¹⁰Department of Immunology, Faculty of Medicine, Bursa Uludag University, Bursa, Turkey

¹¹Department of Pediatrics, Faculty of Medicine, Near East University, Nicosia, 99138, Cyprus

*Corresponding author:

*Corresponding author: Sehime Gulsun Temel

Address: Bursa Uludag University, Faculty of Medicine, Department of

Medical Genetics, Bursa, Turkey

e-mail: sehime@uludag.edu.tr

ORCID NO: 0000-0002-9802-0880

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Introduction

The serotonin (5-HT)_{2C} receptor (5HTR2C) is located at Xq24 and is a member of the seven-transmembrane spanning the G-protein-coupled receptor that signals through phospholipase C superfamily (1). The 5HTR2C gene is mainly expressed on principal neurons and GABAergic interneurons in the prefrontal cortex (2) and is implicated in the regulation of key brain functions such as food intake, stress response, sleep, anxiety, and psychiatric disorders (3). Therefore, importance of the 5HTR2C gene has been highlighted in many studies due to its role in different critical neural circuits (1-3). 5HTR2C receptor plays a critical role in the mediation of stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation and stress-induced release of serotonin in the central nervous system (CNS) (4).

5HTR2C rs6318 variant detected as X-linked nonsynonymous coding single nucleotide polymorphism (SNP) comprised of G to C switch at base pair 68, which leads to the substitution of serine for cysteine at codon 23 (Cys23Ser) (5). In addition, this variation was also observed with important effects on stress response which may lead to development of different diseases. For instance, 5HTR2C Ser23 C allele detected as an impact on visual hallucinations and psychotic symptoms in late onset Alzheimer's disease (AD) (6,7). Furthermore, in laboratory tests, 5HTR2C Ser23 C allele hemizygotes males showed two-fold increase in plasma cortisol when recalling conditions that made them angry or sad compared to those carrying the Cys23 G allele. Thus, their finding suggests that this variation may have an impact on HPA axis activation and dysregulation of stress response which may increase the risk of depression (8). Furthermore, individuals who carry the 5HTR2C Ser23 C allele are more likely to have higher cortisol levels, central

obesity, glucose, insulin resistance, blood pressure and lipids so these carriers are under risk of type-2 diabetes and cardiovascular disease (CVD) development (9).

The association between chronic psychosocial stress and asthma was clarified with changes in the methylation and expression of genes that regulate behavioral, autonomic, neuro-endocrine, and immunologic responses to stress (10). In addition, an allergy can be defined as the result of an IgE-mediated immune response to specific antigens known as allergens. Allergic diseases such as asthma, allergic rhinitis, and food allergy are among the most commonly observed diseases worldwide, especially in Westernized countries (11,12). Both genetic and environmental factors play a crucial roles in the etiology of allergic diseases, while various microbial and dietary exposures, pollution, and smoking are suggested to be possible protecting and driving factors (11). Additionally, exacerbation of allergic conditions have been suggested to be associated with emotional or psychosocial stress (10,13,14). For instance, behavioral problems and family conflicts preceded the development of asthma in multiple pediatric cases (13). Likewise, Barnthouse and Jones (2019) indicated that chronic psychosocial stress is additional environmental exposure for allergic asthma (14). Moreover, chronic stress was shown to affect some biological processes such as changes in cortisol levels, alterations in the HPA, cytokine regulation, and the expression of immunologic genes (9,14).

According to all the mentioned points, the *5HTR2C* rs6318 G>C variation was shown to be associated with stress responses, as well as the development of psychosocial and physiological diseases. More importantly, as stress was also identified as one of the environmental risk factors for the development of allergic diseases, few studies have been undertaken to show its effect on allergic asthma (14-18). In addition to these, in literature there is no other study for now found for investigation the effect of *5HTR2C* rs6318 G>C variation on allergic disorders. Therefore, our study aimed to investigate the association of *5HTR2C* rs6318 G>C polymorphism in stress induced childhood allergic asthma to clarify possible underlying mechanism which may also provide information for potential prognosis and therapies.

Materials and Methods

Sample selection

The study protocol was approved by the Near East University Ethics Review Board (YDU/2019/71-866) and informed consent was obtained from all parents of patients and controls. Patients with asthma were selected from children and adolescents (< 18 years old) who were under follow-up at the Department of Pediatric Allergy & Immunology of Near East University Hospital, whereas controls were selected from age-matched healthy children (< 18 years old) with no chronic disease attending the Department of Pediatrics for routine control. Demographic, clinical data, and allergic sensitizations of all patients were gathered from the hospital database system retrospectively. Subsequently, all patients were re-evaluated to

confirm asthma diagnosis and atopic status using skin prick tests, and finally, a blood sample was obtained.

Skin-Prick test

A total of 177 individuals were included, of whom, 118 were allocated to the control group and 59 of them with confirmed atopic or non-atopic asthma were assigned to the patient groups. Patients were categorized as atopic and non-atopic asthmatic groups based on allergic sensitization established by skin prick tests which were performed annually with locally common aeroallergens including *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria Alternata*, *Aspergillus mix*, *Cladosporium mix*, *Penicillium mix*, a mixture of four kinds of cereal, a mixture of 12 types of grass, *Phleum pratense*, *Compositae*, *Urtica dioica*, *Parietaria officinalis*, Tree mix, Cypress, *Acacia dealbata*, *Olea Europea*, *Pinus sylvestris*, cat hair and dog hair (Allergopharma, Reinbek, Germany). Histamine and dihydrochloridesaline were used as positive and negative controls respectively. A drop of each allergen extract was introduced via special lancets into the skin on the volar side of the left forearm. After 15 min, the wheal reaction was measured as the mean of the longest diameter and the length of the perpendicular line through its middle. A wheal size of at least 3 mm was considered positive. Those with no allergic sensitizations according to the skin prick test were grouped as non-atopic asthmatics, while those with allergic sensitizations detected by the skin prick test were categorized as atopic asthmatics.

Molecular genotyping

QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA) was used to isolate the genomic DNA from peripheral blood samples by following the instructions provided by the manufacturer. 5-hydroxytryptamine receptor 2C (rs6318) polymorphism was investigated by the TaqMan System (Applied Biosystems, Life Technologies) Primer Prob Mix and qPCR Probes Master (Jena Bioscience, Germany, PCR-360). The interested sequence of the rs6318 variant is CTAATTGGCCTATTGGTTTGGCAAT[G/C]TGATATTTCTGTGAGCCCAGTAGCA. First reporter dye VIC® was used to label one probe for the interested SNP major allele G and second reporter dye FAM was used to label the minor allele C. Real time PCR (RT-PCR) reaction was performed in a 20 µl volume containing 10 µl qPCR Probes Master, 1 µl Primer Prob Mix, 7 µl PCR Grade water, 2 µl DNA mix for each well. The reaction was carried out on a Biorad CFX machine. Cycling conditions for rs6318 polymorphism were set as: at 95°C, 2 minutes, 1 cycle followed by 95°C, 15 seconds, 40 cycles, 60°C, 1 minute, 1 cycle. Lastly, the study was completed by analyzing fluorescence measurements of the reporter dyes at the end of the RT-PCR reactions.

Statistical analysis

Statistical analysis was performed by using a commercial software program (Graphpad Software, Inc. California, USA). The

standard deviation (SD) and mean values of the descriptive

Results

Demographic characteristics of the studied subjects

The age and gender characteristics of the individuals are shown in Table 1. The subjects who participated in this study comprised a total of 177 Turkish Cypriot children, including 59 patients with atopic and non-atopic asthma, and 118 Turkish Cypriots as the control group. The asthma group showed no statistically significant difference from the control group concerning age and sex ($p=0.269$ and $p=0.535$, respectively). Table 2 demonstrates the atopy distribution among the asthmatic group. Among 59 asthma patients, atopy was detected in 43, and 16 patients were non-atopic.

Table 1. General Characteristics of studied groups.

Variables		Control	Astma + allergy	p-value
		n=118	n=59	
Age (years)	Mean $\pm$ SD	8.04 $\pm$ 3.99	7.59 $\pm$ 4.50	0.269
	Median (Min - Max)	8.00 (2.00 - 18.00)	6.00 (1.00 - 18.00)	
Sex	Female	47 (39.8%)	21 (35.6%)	0.585
	Male	71 (60.2%)	38 (64.4%)	

Table 2. Atopy distribution within patients group.

Atopy	Asthma n (%)
Negative	16 (27.1%)
Positive	43 (72.9%)

Atopy distribution within patients group

The allele and genotype frequency distributions of the 5HTR2C rs6318 G>C polymorphism among asthma and control groups are demonstrated in Table 3. Regarding the genotype distributions of the 5HTR2C rs6318 G>C, the results within each group showed statistical significance in both groups (HWE $p<0.001$).

However, the comparison of both allele and genotype distributions between the groups showed no statistical significance ($p=0.255$ and $p=0.850$, respectively).

Genotype distribution and allele frequencies of both control and patients groups

The comparison of atopic asthma and non-atopic asthma pediatric patients in terms of their allele frequency and genotype distribution were detected as significantly different as respectively; $p=0.004$ and $p=0.038$. Furthermore, the genotype distribution of the 5HTR2C rs6318 within groups showed discordance to HWE ($p=0.006$ and $p=0.017$).

Genotype distribution and allele frequencies among asthma atopic and non-atopic pediatric cases.

Different genotype combination risk score analysis showed that there was a strong association between the 5HTR2C gene rs6318 C allele and atopy-related asthma risk ($p=0.037$), whereas no significance was observed between the rs6318 C allele and asthma ($p=0.191$) (Table 5).

Discussion

Serotonin is a key CNS neurotransmitter that plays a very crucial role such as controlling appetite, mood, cognitive behaviors, and sleep (20). Additionally, serotonin transporter gene 5-HT is implicated in the pathogenesis of many diseases such as autoimmunity, depression, inflammatory bowel disease, and asthma (21-23).

In recent years, the incidence of allergic diseases has increased rapidly (24). Allergic (atopic) asthma is the most commonly observed asthma subtype. Allergic asthma is characterized by the presence of allergic sensitization and interaction between allergen exposure and clinical symptoms, while non-atopic asthma is a phenotype with no allergic sensitization (25). Until now, several risk factors including family history, exposure to microbial agents at early ages, and genetic/epigenetic, and environmental factors have been investigated to learn more about the etiology of asthma (26,27). For instance, knowing the family history will reveal more information about allergic asthma etiology when compared to non-allergic asthma (25). Only a few genes; beta2 adrenergic receptor, cytokine secretion and their signaling pathways related and transcription factors that involve Th1 and Th2 differentiation were found as-

Table 3. Genotype distribution and allele frequencies of both control and patients groups.

		Control n (%)	Asthma n (%)	Chi Square	p-value
Allel	G	139 (79.35)	70 (83.78)	1.297	0.255
	C	41 (20.65)	14 (16.22)		
		Observed (Expected)	Observed (Expected)		
Genotype	GG or G/-	90 (80.56)	50 (45.83)	1.140	0.850
	GC	15 (33.88)	4 (12.34)		
	CC or C/-	13 (3.56)	5 (0.83)		
	HWE p-value	<0.001	<0.001		

Table 4. Genotype distributions and allele frequencies of among asthma atopic and non-atopic children.

		Asthma + Atopic n (%)	Asthma + Non-Atopic n (%)	Chi Square	p-value
Allel	G	56 (79.35)	14 (83.78)	8.326	0.004
	C	6 (20.65)	8 (16.22)		
Genotype		Observed (Expected)	Observed (Expected)		
	GG or G/-	39 (37.21)	11 (9.0)	4.320	0.038
	GC	2 (5.58)	2 (6.0)		
	CC or C/-	2 (0.21)	3 (1.0)		
	HWE p-value	0.006	0.017		

Table 5. The genotype comparison of the 5HTR2C gene rs6318 variant with asthma and atopy related risk.

Asthma Related Risk Measures of Different Genotype Combinations for rs6813			
Genotypes	Odd Ratios	Chi-Square	p-value
GG and G/- vs GC, CC and C/-	1.728 (0.781-4.121)	1.708	0.191
CC and C/- vs GC, GG and G/-	1.137 (0.461-3.538)	0.278	0.793
GC vs GG, G/-, CC and C/-	2.002 (0.660-5.751)	1.445	0.306
Atopy Related Risk Measures of Different Genotype Combinations for rs6813 in Asthma Patients			
Genotypes	Odd Ratios	Chi-Square	p-value
GG and G/- vs GC, CC and C/-	4.432 (1.009-15.970)	4.345	0.037
CC and C/- vs GC, GG and G/-	4.731 (0.861-27.970)	2.988	0.118
GC vs GG, G/-, CC and C/-	2.929 (0.419-19.620)	1.137	0.295

sociated with development of atopic asthma (27).

The serotonin (5-HT)_{2c} receptor (5HTR2C) is known to be expressed on GABAergic interneurons in the prefrontal cortex (2). Its role in different critical neural circuits especially in stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation (4) attracts the attention in terms of development of diseases. During researches conducted for understanding of 5HTR2C gene effect on disease, 5HTR2C rs6318 G>C SNP which leads to the substitution of serine for cysteine at codon 23 (Cys23Ser) detected (5) and this variation has been associated with Alzheimer's disease (6,7), increase in cortisol level

(9), depressive behavior (8), risk of type-2 diabetes and cardiovascular disease (CVD) development (9). Moreover, there are few studies which considers the functional difference of this Cys23Ser SNP. For instance, Ser23 expression detected with low affinity binding to 5-HT ligand although intracellular Ca²⁺ peak did not differ between the alleles. In the same research they also observed that Ser23 allele compared to Cys23 constitutively more active (28). On the other hand, most recently under in vitro conditions with 5-HT promotion, in Ser23 variant cell lines decreased potency and provide peak response in intracellular calcium release compared to Cys23 cell lines. In the same study they also detected decreased plasma membrane localization and O-linked glycosylation of 5-HT_{2C}R Ser23 variant so there were distinct functional pharmacological aspects between 5-HT_{2C}R variants (29).

In addition to all the mentioned relevant diseases and 5HTR2C rs6318 variant, there is no information in the literature about this variant and its association between asthma (30). Nevertheless, the 5HTR2C receptor has been recognized in the mediation of HPA stimulation by stress (4) and it plays an immunomodulatory role by releasing different inflammatory cytokines from bronchial epithelial cells (22). Moreover, as stress has been identified as a critical environmental factor for allergic diseases such as asthma, many researchers have attempted to show the relationship between stress and asthma (15-17).

Psychosocial stress stimulates the HPA and consequently increases (4) the cortisol levels (8) to stimulate the immune system, which might have an impact on inducing allergic inflammation and asthma pathogenesis (10). Subsequently, more studies are required to explore the interaction between the atopic immune reaction, dysregulated neuronal physiology, and their association with asthma (32). Therefore, we aimed to investigate the possible association between the 5HTR2C rs6318 G>C variant and childhood atopic and non-atopic asthma. In this study, the patient groups comprised 59 individuals with atopic and non-atopic asthma, while the control group constituted 118 healthy individuals. The individuals who were involved in this study were Turkish Cypriots who were younger than 18 years old. These groups were evaluated based on the atopy development status. Importantly, out of the 59 asthma patients, atopy was observed in 45 patients, meaning that 16

patients were classified as having non-atopic asthma.

The 5HTR2C rs6318 G>C variation was analyzed in both patient and control groups. Allele frequency in the patient groups was not in agreement with the Hardy-Weinberg Equilibrium ($p < 0.001$) which shows that there is pressure of certain genotype due to the effect of mutations, possibility of isolated population or different genetic background in the population (33). In addition, when the frequency of the risk allele (C allele) was compared between the patient and the control groups, no significant difference was observed between the two groups ($p=0.255$). The genotypic distribution of 5HTR2C rs6318 G>C polymorphism between atopic asthma and non-atopic asthma patients showed no concordance with HWE, as expected ($p=0.038$, respectively). The allele frequency of the disrupting allele 5HTR2C rs6318 C showed a statistical significance when we compared both atopic and non-atopic asthma patient groups ($p=0.004$). To the best of our knowledge, this is the first study which investigate the association between the 5HTR2C rs6318 G>C and childhood and adolescence asthma in the Turkish Cypriot population and results showed an association between the 5HTR2C rs6318 C variant and childhood atopic asthma.

A limited number of studies have attempted to investigate the role of serotonin in asthma; for instance, Farjadian et al., (2013) aimed to investigate the association between the 5-HTT gene polymorphisms (rs35521, 5-HTTLPR, and STin2.VNTR) and asthma but found no association (23).

Conversely, since the serotonin transporter gene-linked promoter region polymorphisms (5-HTTLPR) plays a modifier role in depression in asthma, and diabetes, Omari-Asor et al., (2015) focused on analyzing the association between the 5-HTTLPR polymorphism and these diseases in French individuals aged between 65-100 yrs. The study showed an association between the 5-HTTLPR S allele and asthma and depression (32). A comprehensive study was designed to investigate the association between *hydroxy tryptamine receptor 4 (HTR4)* gene variations (32 SNPs) and asthma in the Korean population, which showed that +142828 G>A and +122769 G>A SNPs were significantly associated with asthma (21).

Finally, from this current study, some limitations should be noted. Especially the number of individuals included in this study seems relatively small due to total size of Turkish Cypriot population. However, as it is very important to identify population-specific SNPs while defining unique phenotypes and disease susceptibility/protective variants, these findings are specific to the Turkish Cypriot population and may not be observable in other populations or ethnic groups (34). More importantly, the Turkish Cypriot population is small, the genetic makeup of the Turkish Cypriot population has not been broadly studied before and polymorphism data is absent for Turkish Cypriots. Therefore, the results obtained from this study are still very valuable. Moreover, our study is significant since non-of the studies which were found in literature were examined the association between 5HTR2C rs6318 C variant and childhood asthma.

Conclusion

To conclude, allergic diseases such as asthma are highly heterogeneous and asthma has a very complex etiology that involves gene-gene interactions, gene environmental interactions, and epigenetic regulation. To date, several studies have been undertaken to show the effect of serotonin molecule plays in many diseases. However, the role of serotonin in asthma has not been comprehensively studied. In this study, a strong association was found between the 5HTR2C rs6318 G>C polymorphism and childhood asthma. Overall, this study can provide valuable insights for scientists to better understand the mechanisms involved in allergic diseases, so medical treatment alternatives can be tailored according to each individual's unique genetic profile, emphasizing better prognosis options developed for childhood and adulthood allergic diseases in the future.

Ethical approval

All procedures performed in this study were by the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (Approval number: ydu/2019/71-866Tit).

Informed consent

Informed consent was obtained from all individual participants included in the study

Conflict of Interest

The authors do not have any conflict of interest to declare.

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