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Original Article

A Negative Correlation Between *MEFV* Mutations and Allergic Diseases

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ABSTRACT

Aim: Atopy is associated with a genetic predisposition to develop allergic diseases such as allergic rhinitis, asthma, and atopic dermatitis. In this study, we aimed to compare the prevalence of Familial Mediterranean Fever (FMF) mutations in asthma and allergic rhinitis patients with controls in the pediatric population and to analyse the positive or negative effect of *MEFV* mutations in the development of atopy.

Materials and Methods: For the detection of FMF mutations, 88 pediatric patients (51 allergic asthma, 17 allergic rhinitis and 20 both asthma and allergic rhinitis cases) and 92 controls were included in our study. Total genomic DNA was extracted from peripheral blood samples using DNA isolation kit. Then, the patient and control groups were screened for *MEFV* gene mutations by Reverse Hybridization procedure (Strip Assay).

Results: There were 9 carriers (heterozygous mutation) in the patient group. The control group had 21 carriers and 1 individual with a compound heterozygous mutation. It was not detected any homozygous mutation in both two groups. The number of individuals with mutation was statistically higher in the control group than in the patients of asthma and allergic rhinitis (p=0.015) and the mutation number (allelic frequency) in the control group was also higher than in the patients (p=0.014).

Conclusion: We suggest that FMF mutations are less frequent in allergic rhinitis and asthma cases than in the normal population. Asthma and allergic rhinitis may be more common in individuals without FMF mutation. It can be thought that *MEFV* gene mutations are effective to prevent allergic reactions on the basis of T helper 2 (Th2) suppression.

Keywords: Allergic rhinitis, asthma, FMF, *MEFV* gene, mutation

Introduction

Allergy is among the most common chronic disorders of childhood and it is manifested primarily by symptoms associated with the nose, lungs, sinuses and skin. Allergic diseases such as asthma, eczema and allergic rhinitis affect approximately 20% of the population all over the world (1). These are complex conditions that are affected by different genetic and environmental factors (2). Asthma is a chronic, inflammatory disorder of the lower airways characterized by airflow obstruction, airway hyperresponsiveness and remodeling (3). Allergic rhinitis is a disease of the upper airways resulting from IgE-mediated inflammation caused by intolerance to allergens in the external environment (4). Allergic rhinitis and asthma often coexist in the same patients and constitute a significant global health problem (5). Immunotherapy is an important form of treatment in such allergic diseases (6). The main drugs used for the treatment of allergic rhinitis are oral antihistamines

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and nasal corticosteroids (7). These allergic disorders are associated with the activity of T helper 2 (Th2) cells involved in the production of interleukin 4 (8). Asthma is a Th2-cell driven inflammatory disease accompanied by eosinophilic inflammation, cytokine production and airway hypersensitivity (9).

Familial Mediterranean Fever (FMF) is an autosomal recessive genetic disorder that has the highest prevalence in people of Mediterranean origin (10). It is characterised by recurrent episodes of pain and fever, serosal inflammation including peritonitis, pleuritis, synovitis and erysipelas like erythema (11). Amyloidosis is an important problem in FMF and it can cause renal failure. Mutations in the *MEFV* gene cause FMF. This gene encodes a protein called pyrin (12). FMF mutations cause T helper 1 (Th1) polarization. The defective pyrin cannot suppress Th1 mediated inflammation. Th1 cells produce interferon-γ (IFN-γ) and they are associated with immune responses against intracellular viral and bacterial infections (13). IFN-γ inhibits IgE dependent reactions.

Atopy may be rare in people with MEFV gene mutations due to Th1 dominance. In a study by Yildiz et al. (14), the frequency of asthma, atopic dermatitis and allergic rhinitis in patients with FMF were found to be lower than their general prevalence in Turkey. However, Aydoğmuş et al. (15) found no difference in terms of the prevalence of asthma and allergic rhinitis between the FMF patients and control group. Therefore, they claimed that there was no proven antagonistic relationship between atopic diseases and FMF. Yazici et al. (16) stated that atopy was at a low frequency in FMF patients.

In this study, we aimed to evaluate the frequency of FMF mutations in allergic rhinitis and asthma cases in comparison to the normal population in Central Anatolia, Turkey where FMF is very common. Unlike the literature evaluating the atopy status in FMF patients, we screened *MEFV* mutations in allergic patients on the basis of Th1 and Th2 connections.

Materials and Methods

Study Design

This study was carried out between 2014 and 2019 in Cumhuriyet University Research Hospital, Department of Medical Genetics in Sivas, Turkey. Eighty-eight children diagnosed with allergic asthma and/or allergic rhinitis and ninety two healthy controls were included in the study in consultation with the biostatistics department. Respectively, 51 patients (58%) had allergic asthma, 17 patients (19.3%) had allergic rhinitis and 20 of them (22.7%) were suffering from both asthma and allergic rhinitis. This study was approved by the Cumhuriyet University Ethics Committee (approval number: 2014-06/09, date of the approval: 18.06.2014). Informed consent was obtained from the parents of the patients and control group.

Atopy Examination

The levels of total serum IgE were measured for the patients and a skin prick test panel including *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria*, cat dander, grass mix, tree mix, *Blattella germanica*, (histamine phosphate as positive control and 0.9% serum physiologic as negative control) was used to confirm the presence of atopy.

Mutation Screening

In our molecular laboratory, total genomic DNA was extracted from peripheral blood samples with a DNA isolation kit (Invitek Invisorb Spin Blood Kit, Germany). Multiplex polymerase chain reaction (PCR) amplification was performed with biotinylated primers. Those patients with asthma and allergic rhinitis and the controls were screened for *MEFV* gene mutations (E148Q, P369S, F479L, M680I(G/A), M680I(G/C), I692del, M694V, M694I, K695R, V726A, A744S, R761H) using a Reverse Hybridization procedure (Vienna Lab, FMF StripAssay, GMBH, Austria). PCR products were incubated on nitrocellulose strips and the process was completed with colour development and the detection of signals in Auto-LIPA (Auto-LIPA Innogenetics).

Statistical Analysis

Statistical analysis was performed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) to evaluate the differences between the patients and controls. Mean age was analysed via t-test and chi-squared test was used to compare the frequencies of *MEFV* mutations in the two groups. The odds ratios were calculated at a 95% confidence interval.

Results

The patient group consisted of 56 males (63.6%) and 32 females (36.4%). The mean age of the patients was 8.46±2.4 years (range: 5-14 years). The control group consisted of 56 males (60.9%) and 36 females (39.1%). The mean age of the controls was 9.02±1.6 years (range: 6-15 years). There was no difference between the patients and controls in terms of age and gender (Table I).

The distribution of FMF mutations in the patient and control groups is shown in Table II. There were 9 individuals with a single mutation (10.2%) in the patient group. There was no homozygous or compound heterozygous mutation in this group. The number of controls with mutation was 22 (23.9%). While 21 of them had a heterozygous mutation, only one had a compound heterozygous mutation. The controls also had no homozygous mutation. The most frequent mutation in both the patient and control groups was E148Q (3.4% in patients, 6.5% in controls). The compound heterozygous mutation in the control group was M694V+M680I (G/C).

There was no significant difference between asthma, allergic rhinitis and asthma+allergic rhinitis cases in terms of mutation frequency (Table III). The difference between the patients and the controls was significant in terms of the number of individuals with mutation (p=0.015). The mutation ratio of the controls was also significantly higher in comparison to the patients (p=0.014). Those individuals with mutation and the number of total mutations were presented in Table IV. The proportion of individuals with FMF mutations was 10.2% in the patient group and 23.9% in the control group. Allelic frequency was 5.1% in the patients and 12.5% in the controls. The mutation ratio of the control group was more than two-fold of each patient group (Figure 1).

Discussion

Allergy is one of the most common chronic problems in the world with the symptoms of nose, lungs, sinuses and skin. It is a medical condition caused by the hypersensitivity of the immune system and is associated with genes and the environment. Allergic asthma is a chronic inflammatory disease with airway hyper-responsiveness and air-flow obstruction. The inflammatory process that enhances eosinophil accumulation and IgE production is driven by Th2 cells (17). In this context, asthma is associated with an excessive Th2 response to allergic stimuli that cause airway inflammation. Allergic rhinitis is also an inflammatory disease characterized by an IgE-mediated hypersensitivity reaction and mucosal infiltration with inflammatory cells (18). Antihistamines are frequently used in the symptomatic treatment of allergic rhinitis.

Th1 cells are associated with the host immunity and directed by IL-12 and IL-2. One of the main cytokines in this process is IFN-γ. Th2 cells are usually triggered by IL-4 and their potent cytokines are IL-4, IL-5, IL-9, IL-10 and IL-13. It was demonstrated that some myeloid dendritic cells are effective in the differentiation of Th1 and Th2 cells during the immune response (19). While IL-12 directs Th1 differentiation, IL-4 induces the development of Th2 (20). Th1-type cytokines can contribute the pro-inflammatory responses to kill intracellular microorganisms. Th2 cells have a responsibility in the development of allergic diseases (21). Th2-type cytokines are associated with eosinophilic activity in atopy. Th2 cells play an important role in the activation of IgE antibody producing B cells, mast cells and eosinophils (22). Th2 cytokines, especially IL-13 have a critical role in the pathogenesis of asthma (23).

FMF is an auto-inflammatory genetic disease caused by *MEFV* gene mutations leading to interleukin-1β activation

Table I. The characteristics of the patients and controls

Characteristics	Patients	Controls	p-value
Age	8.46±2.4	9.02±1.6	NS*
Gender			
Male	56 (63.6%)	56 (60.9%)	NS*
Female	32 (36.4%)	36 (39.1%)	
*Non-significant			

Table II. *MEFV* mutations in the patients and controls

Mutation	Patients (%)	Controls (%)
M694V	2 (2.3)	5 (5.4)
E148Q	3 (3.4)	6 (6.5)
P369S	1 (1.1)	2 (2.2)
M680I (G/C)	2 (2.3)	4 (4.3)
V726A	1 (1.1)	2 (2.2)
A744S	-	2 (2.2)
M694V+M680I (G/C)	-	1 (1.1)
Individuals with mutation	9 (10.2)	22 (23.9)
Individuals with no mutation	79 (89.8)	70 (76.1)
Total mutation number	9	23

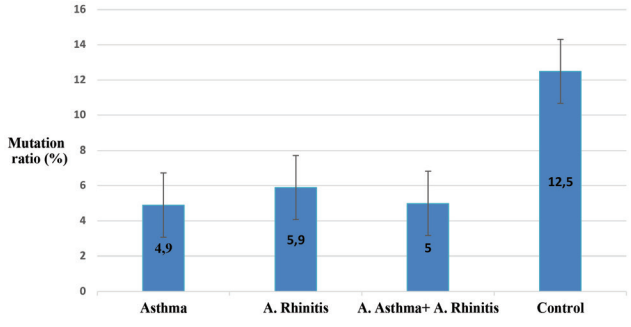

Figure 1. *MEFV* mutation ratio of the patients and control group

(24). This gene encodes pyrin, a protein that is effective in the regulation of apoptosis and inflammation (25). Pyrin is produced in some blood cells such as neutrophils, eosinophils, and monocytes. It may direct white blood cells to the site of the inflammation and reduce the inflammatory response if it is no longer needed.

FMF is an important cause of morbidity especially in the Jewish, Arab, Armenian, and Turkish populations (26). The etiology of FMF is not fully understood. Mutations in a single gene play a role in this disease, but some other factors may also be effective in its pathogenesis. Although FMF is regarded as an autosomal recessive disorder, some patients carry only one *MEFV* mutation and therefore, it can be thought that dominant inheritance is possible. On the other hand, some mutations may have not been identified yet and some rare mutations may be overlooked in the laboratory.

Pyrin coordinates caspase-1 activation and thus IL-1b production (27). Pyrin normally diminishes neutrophil-mediated inflammation by the downregulation of interleukin-1 (IL-1), but it is defective in FMF (28). The defective pyrin has been shown to enhance inflammation through IL-1β production but the precise physiopathology of FMF appears to be more complex (29). FMF inflammation is accompanied by Th1 polarization and the IFN-γ levels are usually higher in FMF patients. IFN-γ inhibits Th2 cells. On the basis of these facts, suppression of Th2 is expected in FMF.

The negative association between asthma and FMF mutations may originate from the suppression of Th2 activity with defective pyrin (30). Lidar et al. (31) suggest that normal pyrin is essential for eosinophil function and the mutated pyrin in patients with FMF attenuates eosinophil-mediated bronchial inflammation resulting in a reduction in the frequency of asthma. Similarly, on the basis of Th2 suppression, the prevalence of atopy in FMF patients was found to be significantly lower than in the normal population (8). However, Amet et al. (32) suggested that atopy frequencies were similar in children with or without FMF. On the other hand, Celiksoy et al. (33) asserted that FMF is a multi-systemic problem and atopic disorders are characteristic features of this disease. This claim does not appear to be compatible with the literature. According to Aypar et al. (34), Th1 polarization in patients with FMF and carriers may be protecting them from diseases of pronounced Th2 response but the decreased allergic responses in those patients with FMF are a result and not the cause of the underlying pathophysiology. The frequency of MEFV mutations in our study was significantly lower in the patient group compared to the control group. The relationship of *MEFV* mutations with atopy is a topic of great interest. The subject of allergy has been studied in FMF patients in the past. However, the screening of *MEFV* mutations in allergic patients is likely to be the first with this study.

Conclusion

Our study demonstrated that the allelic frequency of *MEFV* mutations in asthma and allergic rhinitis patients is statistically lower in comparison to the healthy population in Sivas, Central Anatolia. The mutation ratio in the healthy

Table III. The frequency of *MEFV* mutations in the patients (A. Asthma, A. Rhinitis and A. Asthma + A. Rhinitis)

Disease	A. asthma (n=51)	%	A. rhinitis (n=17)	%	A. asthma+A. rhinitis (n=20)	%	p-value
Mutation	5	9.8	2	11.8	2	10	>0.05*
Wild	46	90.2	15	88.2	18	90	
*Binary comparisons							

Table IV. The comparison of individuals with mutations and the allelic frequency in the patients and controls

Individuals	Patients	%	Controls	%	p-value
With mutation	9	10.2	22	23.9	0.015 p<0.05
Without mutation	79	89.8	70	76.1	
Odds ratio: 0.362 (0.157-0.839)					
Allelic frequency	**Patients**	**%**	**Controls**	**%**	**p-value**
Mutation	9	5.1	23	12.5	0.014 p<0.05
Wild	167	94.9	161	87.5	
Odds ratio: 0.377 (0.169-0.840)					

group was more than twice that of the patient groups. These data suggest that individuals with FMF mutations may be more resistant to allergic diseases. Although the current results were obtained with a different study plan (*MEFV* mutation screening in allergic diseases), they are compatible with most of the previous studies in this area. *MEFV* mutations may be an advantage for the protection against allergic diseases in the context of their relationship with Th2 activity.

Ethics

Ethics Committee Approval: This study was approved by the Sivas Cumhuriyet University Ethics Committee (approval number: 2014-06/09, date of the approval: 18.06.2014).

Informed Consent: Informed consent was obtained from the parents of all participants included in the study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Project: M.E.Y., Molecular Study: M.E.Y., H.K.K., Analysis: M.E.Y., H.K.K., H.K., Writing the Manuscript: M.E.Y., Literature Search: H.K.K, F.D., Statistics: H.K., Patient Supply and Diagnosis: F.D.

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