CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR GENE MUTATIONS IN PATIENTS WITH MASSIVE NASAL POLYPOSIS

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ABSTRACT

Objective: *Isolated features of cystic fibrosis can be seen without classical cystic fibrosis clinical symptomps. In some instances, cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations can result in male infertility, pancreas involvement or lung problems with negative sweat test. Nasal polyposis is also one of the prominent components of cystic fibrosis clinic and theoretically may occur alone with CFTR gene mutations. In this study, we aim to detect the frequency of common cystic fibrosis transmembrane conductance regulator gene mutations in massive nasal polyposis patients.*

Material and Method: *The study group consisted of 30 severe nasal polyposis patients with high computed tomography scores (≥10 on each side, a total of ≥20) and the control group consisted of 30 healthy adults. None of the patients or controls had clinical characteristics of cystic fibrosis. Blood samples of all study and control group members were screened for most common 19 CFTR gene mutations.*

Results: *Only one CFTR gene mutation (G542X) was detected in the study group. In the control group no CFTR gene mutations were detected.*

Conclusions: *In massive nasal polyposis patients, CFTR gene mutations are not common.*

Key Words: *Nasal polyps, cystic fibrosis, cystic fibrosis transmembrane conductance regulator, mutation* ***Nobel Med 2013; 9(1): 17-20***

MASİF NAZAL POLİPOZİS HASTALARINDA KİSTİK FİBROZİS TRANSMEMBRAN İLETİM DÜZENLEYİCİSİ GENİ MUTASYONLARI

ÖZET

Amaç: Kistik fibrozisin izole özellikleri klasik kistik fibrosis kliniği olmaksızın da görülebilir. Bazen ter testi negatifliği ile birlikte kistik fibrosis transmembran iletim düzenleyicisi (KFTD) geni mutasyonları erkek infertilitesi, pankreas tutulumu veya akciğer problemlerine yol açabilmektedir. Nazal polipozis de kistik fibrosis hastalığının önemli bileşenlerinden biridir ve teorik olarak KFTD gen mutasyonları ile birlikte görülebilir. Bu çalışmada masif nazal polipozis hastalarında sık görülen KFTD gen mutasyonlarının sıklığının araştırılması amaçlanmıştır.

Materyal ve Metod: Çalışma grubu yüksek bilgisayarlı tomografi skoru (her bir tarafta ≥10, toplamda ≥20) bulunan 30 masif polipozis hastasından, kontrol grubu ise 30 sağlıklı yetişkinden oluşmuştur. Kistik fibrozisin klinik özellikleri hastaların ve kontrol grubu bireylerinin hiçbirisinde bulunmuyordu. Çalışma grubu ve kontrol grubunun tümünden kan örnekleri alındı ve en sık görülen 19 KFTR gen mutasyonu için tarama yapıldı.

Bulgular: Çalışma grubunda bir kişide KFTD geni mutasyonu (G542X) belirlendi. Kontrol grubunda KFTD gen mutasyonu belirlenmedi.

Sonuç: Masif nazal polipozis hastalarında KFTD geni mutasyonları sık değildir.

Anahtar Kelimeler: Nazal polipler, kistik fibrosis, kistik fibrozis transmembran iletim düzenleyicisi, mutasyon **Nobel Med 2013; 9(1): 17-20**

INTRODUCTION

As a common health problem, prevalence of nasal polyposis (NP) was reported to be as high as 2-4% in previous population based studies and questionnaires.[1-3] NP significantly reduces quality of life and despite medical treatment and aggressive surgical removal, nasal polyps are found to recur in most cases.[4] The cause(s) of this relatively common and resistant to treatment situation still remains mysterious in terms of etiology and etiopathogenesis. Several intrinsic and extrinsic etiologic factors were thought to have a role in NP formation but none of these were proved to have a leading role in NP etiology. Some of these are allergy, fungi, superantigens, viral infections and genetic factors.[5-9] Many factors probably contribute to formation of nasal polyposis, however, chronic persistent inflammation is at the heart of the disease process whatever the main etiology or triggering factor is. Sometimes, NP may occur with some other chronic diseases. As a genetic disorder, cystic fibrosis (CF) is one of the well known NP-related disorders. This disorder is known as the most lethal autosomal recessive disorder affecting Caucasians. The cause of CF is mutations of the cystic fibrosis transmembrane regulator (CFTR) gene which is located on the chromosome 7 (q31-q32).[10] Product of this gene forms a cyclic adenosine monophosphate-regulated chloride channel.

Abnormality of chloride channel results in electrolyte transport dysfunction across the apical membrane of epithelial cells of ducts in many organs such as lung, pancreas, sweat gland, liver, salivary glands. Mucus becomes thicker and leads to reproductive, respiratory and digestive system obstructions. When upper respiratory system is affected, recurrent otitis media, rhinosinusitis and nasal polyposis are most common manifestations. In CF patients, NP frequency is reported approximately 40%.[11,12] In young patients with massive nasal polyposis, investigations for CF is strongly recommended. Clinical spectrum of the disease can be wide and severity of the disease may show great variability. Isolated mild features of cystic fibrosis such as mild lung disease, pancreatic insufficiency or male infertility can be seen without abnormal sweat duct function which are postulated as a monosymptomatic forms of CF.[13-15] Although nasal polyposis is a common manifestation of CF, it is still unknown whether NP may be a monosymptomatic form of the disease or not. In our study we aimed to detect common CFTR gene mutations in massive NP patients and control group.

MATERIAL and METHOD

Study and control group

Patients presented to our clinic with massive nasal polyposis were included in the study group. Inclusion criteria for the study group were primary or recurrent massive nasal polyposis with computed tomography score higher than or equal to 20 (≥10 on each side) according to Lund & Mackay staging system and absence of previous CF diagnosis or any CF symptoms.[16] Control group consisted of healthy individuals without any nasal or systemic pathology. 3 ml sample of peripheral venous blood was collected from each study and control group subject and kept in tubes containing calcium-ethylenediaminetetraacetate (EDTA) at -20°C. Informed consent was obtained from each study and control group subjects.

DNA extraction

Constitutional DNA was extracted from peripheral whole blood of each subject using an EZ-10 Spin Column Genomic DNA Minipreps Kit (for blood) (Bio Basic Inc.).

PCR Strip Assay

Inno-Lipa assays (Innogenetics- CFTR19 and CFTR12) were used for the detection of 20 known mutations in the CFTR gene. This assay is a reverse blot hybridization and post-PCR analysis based on biotin–streptavidin-peroxidase sandwich hybridization and colorimetric detection with a chromogen. The PCR conditions were as follows: Denaturation at 95°C for 15 min and then 30 cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min and extension at 68°C for 1 min and final extension at 68°C for 10 min. The results were evaluated after electrophoresed in a 2% agarose gel. Inno-Lipa CFTR19 assay included ΔF508, G542X, N1303K, W1282X, G551D, 1717-1G→A, R553X, CFTRdele2, 3 (21 kb), ΔI507, 711+1G→T, 3272-26A→G, 3905insT, R560T, 1898+1G→A, S1251N, I148T, 3199del6, 3120+G→A, Q552X.

Statistical Analysis

Data were analyzed using the software SPSS 10.0 and chi-square test was applied for comparison. A *p* value less than 0.05 was considered as significant.

RESULTS

The study group consisted of 30 patients (14 male, 16 female; mean age 46; range 16-70) affected by massive NP. Only one patient had family history of NP. Twelve of the patients had previous sinus surgery history for NP. The mean CT scores of nasal polyposis were 10.91 and 10.67 for the right and left sides respectively. Genetic evaluation of the peripheral venous blood samples revealed only one positive result (3.3%). This was G542X mutation. The patient with G542X mutation was a 63 year-old female without any family history→

or previous operation and had no comorbid diseases. The control group consisted of 30 healthy subjects (18 male, 12 female; mean age 34; range 18-65). No CFTR mutations were found in any of the control group subjects. Mutation rates between the study and the control groups were not statistically significant (p>0.05).

DISCUSSION

Polyps are characterized by stromal edema and variable cellular infiltration to intercellular space as a result of a chronic inflammatory process. This chronic inflammatory disease almost always presents in conjunction with chronic rhinosinusitis. The etiology of NP or triggering factor of the inflammatory process is still unknown and may differ individually. Exact role of genetic background in NP formation is also a mystery. There are some families whose several members suffering from nasal polyposis suggests a genetic predisposition however finding strong evidences could not be possible to date. Genetic susceptibility for NP formation has studied by some researchers and an increase in formation of NP with some HLA alleles were reported.[17-19] Liu et al. have studied gene expression profiles of NP patients by DNA microarray technology and reported overexpressed and underexpressed genes in polyp tissue.[20] The development of nasal polyps may be more common with positive family history.[21] NP can also be related with some inherited diseases like Kartagener's syndrome, CF, and primary immune deficiencies. Among them, CF is known as the most lethal autosomal recessive disorder affecting Caucasians.

One of the clinical characteristics of CF is NP. Morphological characteristics of NP in CF patients are not different from the others and pathogenetic mechanisms are thought to be similar in both.[22] However in CF patients, the polyps arise in young ages and tend to run an aggressive course. Although other isolated forms of CF were reported, isolated NP as a clinical variant of CF is not a well-known entity. Only a few reports and studies investigating this issue were published to date. Varon et al. reported a new mutation of CFTR in male twins of Turkish origin presenting with recurrent nasal polyposis without any other characteristics of CF.[23] It has been suggested by Bürger et al. that isolated NP without other clinical features of CF can be seen with CFTR mutation G551D in adults.[24] In another clinical study, Irving et al. screened 55 adults with severe nasal polyposis but without other characteristics of CF and found three CFTR mutations.[25] These were one R006H and two DF497 mutations. The authors concluded that NP and CF are unrelated conditions and did not recommend routine screening of adults with severe nasal polyposis unless they exhibit additional features of cystic fibrosis. Kostuch *et al.* reported the frequency of CFTR mutations in adult recurrent NP patients as 11.4%.[26] They have reported the same mutation, heterozygous ΔF508 mutation, in five of the 44 subjects and concluded as this mutation may participate in the formation of recurrent NP.

Table 1: Frequency of most common CFTR gene mutations in Turkish and some Mediterranean populations

Mutation	Frequency (%)				
	Onay et al.[31] (Turkey)	Kılınç et al.[30] (Turkey)	Kanavakis et al.[32] (Greece)	Tanackovic et al.[33] (Croatia)	Guilloud-Bataille et al.[34] (France)
ΔF508	25	23.5	53.4	65	67.9
1677delTA	5.3	7.2	0.9	-	-
G542X	4.1	3.6	3.9	5	2.5
2183AA→G	3.5	4.2	1.4	-	-
N1303K	1.8	2.4	2.6	3.3	2.0
2043delG	1.8	-	-	-	-
F1052V	1.2	3.0	0.2	-	-
W1282X	0.6	3.0	0.7	-	0.6
E822X	-	-	1.4	-	-
1717-1G→A	-	0.6	-	-	1.2
R553X	-	-	-	-	0.8
G551D	-	-	0.3	-	0.7
R1162X	-	-	0.1	1.7	0.4

So far, over 1800 mutations have been identified and the most common mutation types are missence, frameshift and splicing.[27] Most common CFTR gene mutation is ΔF508, in which 3 base pair deletion occurs that removes phenylalanine at codon 508. The most common genotype is also homozygosity for this mutation. The other common CFTR gene mutations are G542X, G551D, R553X, W1282X and N1303K mutations.[28] Frequency of CFTR gene mutations have diversity in different regions of the world. In a study carried out in Europe, heterogenity in CFTR gene mutation is found to be highest in Mediterranean region.[29] In a study by Kılınç et al., CFTR gene mutations in Turkish population were found to be similar with other Mediterraneaen nations.[30] Main differences were higher frequency of 1677delTA mutation in Turkish population and absence of G551D mutation which is among the most frequent CFTR gene mutations in European population (Table 1). The results of another study in Turkish population by Onay et al. also confirmed the results of this study and revealed high diversity of CFTR gene mutations.[31]

In this study, we have studied CFTR gene mutations in massive NP patients and in control group subjects. We could detect CFTR gene mutation in only one patient in the study group. The detected mutation was G542X mutation which is different from previous→

studies done by Bürger et al, Irving et al and Kostuch et al. G542X mutation constitutes 3.6-4.1% of the CFTR mutations found in Turkish population.[30,31] The most common CFTR mutation in Turkish population is ΔF508 (23.5-25%) and G542X mutation is one of the most common CFTR gene mutations in Turkish population.[30,31] When study and control groups compared, mutation rates were not statistically significant.

We conclude that CFTR gene mutations are not common in massive NP patients. Main limitation of our study is small size of our study group. With this small sample we cannot completely exclude the possible role of CFTR gene mutations in NP formation.

Another limitation is number of studied CFTR gene mutations. When presence of more than 1800 CFTR mutations are considered, 19 mutations studied may be considered insufficient to make a conclusion. However 19 CFTR gene mutations studied are among the most common ones in Turkish population and cover a high percentage of CFTR gene mutations. Besides these limitations our results support that NP is not an isolated manifestation or monosymptomatic form of CF.

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