ORIGINAL STUDY

Association of TNF-alpha gene polymorphism with nasal polyposis in Romanian asthmatic patients

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ABSTRACT

BACKGROUND. Nasal polyposis is one of the most common chronic inflammatory diseases of the nasal and paranasal sinus mucosa, frequently associated with asthma and aspirin intolerance. Increased concentration of tumour necrosis factor-α (TNF-α), demonstrated in nasal polyps and airways of asthmatic patient have suggested possible pathogenic role of cytokines in both diseases. TNF-α gene polymorphisms have been investigated worldwide in association with nasal polyposis or asthma, with variable results in different populations. Our study aimed to investigate the association between TNF-α single nucleotide polymorphisms T with nasal polyposis in Romanian patients with asthma.

MATERIAL AND METHODS. TNF-α -308 G/A, -238G/A, -857C/T single nucleotide polymorphisms was investigated in 106 unrelated subjects with asthma with (45 patients) and without nasal polyposis (61 patients).

RESULTS. Important allelic differences were obtained for TNF-α -857C/T gene polymorphism; the minor allele T was more frequent in asthmatic patients with nasal polyposis than in those without polyposis, with borderline statistical significance (0.288 vs. 0.188, p=0.08). The difference was more significant when analyzing the subgroups of asthma tolerant (0.237 vs. 0.125, p=0.01), respective intolerant to aspirin (0.248 vs. 0.198, p=0.05). We identified a tendency of association of haplotype TGG -857/-308/-238 with nasal polyposis (p = 0.08). The same tendency was observed for allelic pairs TNF-α TG -857/-308 (p=0.08) and TNF-α -857/-238 TG (p = 0.1). There were no significant differences in the distribution of genotypes and alleles frequencies for the SNPs TNF-α -238G/A, -308G/A in our patients, related to the presence of nasal polyposis.

CONCLUSION. This study suggests a possible linkage of a SNP in the TNF-α promoter region with nasal polyposis. The results need to be confirmed with multicentre studies for more precise interpretation and corroborative studies for investigating the influence of polymorphism on nasal polyposis pathogenesis.

KEYWORDS: TNF-α, gene polymorphisms, nasal polyposis, asthma

INTRODUCTION

Nasal polyposis (NP) is a chronic inflammatory disease, one of the most common lesions of the nasal and paranasal sinus mucosa, affecting up to 4% of the population[1]. The etiology is still unclear, but association with asthma and aspirin intolerance are frequently described, 26% of patients with NP having asthma and 6% of asthmatic patients having NP, with an earlier onset in aspirin-intolerant asthma (AIA)

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than in aspirin-tolerant asthma (ATA) patients[2]. Association of nasal polyps and asthma constitutes one of the most severe form of unified respiratory tract disease, characterized by older age of the patients, greater duration of nasal symptoms, extent of sinus radiological changes, more severe bronchial obstruction and some authors reported also an increased incidence of perennial allergic rhinitis[3]. Older studies have failed to show an increased prevalence of NP in patients with atopy or allergic disorders, compared with the general population[4]. There are authors underlying a genetic predisposition due to positive family history of NP, which could be responsible for 14% and 52% of cases[5]. Genetic and molecular alterations required for its development and progression are still unclear.

The pathogenesis of NP is not totally understood, partially due to a lack of a widely accepted classification, which includes both clinical history and histology to differentiate between the various forms of NP. Different inflammatory mediators and cellular characteristics were demonstrated in chronic rhinosinusitis and NP mucosal tissue. Most of the studies confirm that eosinophils and related inflammatory products are the hallmark of NP-associated inflammation. For example, interleukin-5 (IL-5), a cytokine involved in eosinophil survival and differentiation, and eosinophil cationic protein (ECP), eotaxin, the indicators for eosinophil chemotaxis and activation, immunoglobulin E, have been found to be significantly increased in NP vs. chronic rhinosinusitis without NP and controls. Expression of cytokines, proinflammatory enzymes, may play different roles in the pathogenesis of NP[3].

Tumour necrosis factor-α (TNF-α) is a potent proinflammatory cytokine involved in many inflammatory events characteristic to allergic diseases. Increased concentration of TNF-α was demonstrated in asthmatic airways[6] and in lavage fluid from asthmatic lungs[7]. It was shown that the contents of TNF-α and TNF-α (+) cells is markedly increased in NP nasal mucosa, suggesting that TNF-α may contribute to the pathogenesis of NP[8].

TNF-α gene polymorphisms have been investigated worldwide in association with NP or asthma; the results are variable in different populations and sometimes discordant. Tumor necrosis factor (TNF) gene is located on chromosome 6p21 within the major histocompatibility complex class III region[9], which has been linked to asthma by several genome-wide linkage studies. Altered levels of TNF-α may occur due to polymorphisms in the TNF-α promoter region and may also be associated with NP susceptibility.

Starting from this general information, we investigated the possible association of the TNF-α -308G/A, -238G/A, -857C/T single nucleotide polymorphisms (SNPs) with NP in Romanian patients with asthma.

MATERAL AND METHODS

Subjects

A total number of 106 unrelated subjects with asthma with and without nasal polyposis were recruited for the study in the period 2005 - 2010. Ethical approval was obtained from the institutional review board of the hospital. A full verbal explanation was provided to all patients who gave their informed consent to participate in this study.

Personal and familial histories of asthma, allergic rhinitis, aspirin sensitivity and NP were recorded. The diagnosis of asthma was based on a previous medical history, positive clinical symptoms, spirometry tests. Patients were classified in controlled, partially controlled or uncontrolled in the light of actual guidelines recommendations[10]. Starting from the documented history of bronchospasm induced by aspirin or other nonsteroidal anti-inflammatory drug, patients were further grouped in those with aspirin-intolerant (AIA) or aspirin-tolerant asthma (ATA). Due to ethical and safety reasons, we did not perform the challenge test in order to demonstrate the intolerance to aspirin or other nonsteroidal anti-inflammatory drugs (NSAID); we have included in the group of aspirin/NSAID intolerant asthma only the patients with at least two episodes of bronchospasm NSAID-induced or an episode described by a physician. NP diagnosis was confirmed by ENT examination. Polyps were diagnosed following the criteria of the European Position Paper on Rhinosinusitis and Nasal Polyps[11]. Skin prick tests were performed on all patients. Each patient was evaluated for sensitivity to 14 common allergen extracts (*Dermatophagoides pteronyssinus, Dermatophagoides farinae, Alternaria alternata, Aspergillus fumigatus, Penicillium notatum, Cladosporium cladosporioides, cat, dog, Blattella germanica, Betula verucosa, Salix caprea, grasses pollen, Ambrosia artemisifolia, Artemisia vulgaris*) (Alyostal, Stallergenes) and to positive and negative control substances. A test result was considered positive for sensitivity when at least 1 of the induration diameters was 3 mm higher than that in the negative control. Table 1 illustrates the clinical characteristics of patients included in the study.

Nasal polyposis was present in 45 patients (42.45%) (82.61% of them being diagnosed with aspirin-intolerant asthma) and absent in 61 subjects (57.55%).

Genomic DNA analysis and genotyping of the TNF-α -308G/A, -238G/A, -857C/T polymorphisms

A peripheral blood sample was taken from all patients; blood samples were collected in microcentrifuge tubes containing EDTA (Ethylenediaminetetraacetic acid). DNA extraction was performed from 200-400µl blood with commercial kits (mi-Blood Genomic DNA Isolation Kit - Metabion, Germany and QIAamp DNA Blood Mini Kit Qiagen, Germany) ac-

Table 1
Clinical characteristics of study groups

	Patients with aspirin-intolerant asthma (AIA)	Patients with aspirin-tolerant asthma (ATA)	p
Number	46	60	NS
Median age	44.17 (±15.05)	42.75 (±15.08)	NS
SEX M/F	15 M / 31 F (32.6% / 67.4%)	23 M / 37 F (38.33% / 61.67%)	NS
Nasal polyps present (NP+)	38 (82.61%)	7 (11.7%)	0.0001
Nasal polyps absent (NP-)	8 (17.39%)	53 (88.3%)	
Positive skin prick test to one or more allergens	16 (34.78%)	39 (65%)	0.02

cording to manufacturer protocol. Genotyping of TNF gene polymorphisms was done by Real-Time PCR method, using system 7300 Real Time PCR (Applied Biosystems, USA) with minor modifications from the manufacturer's instructions[12].

Statistical analysis

Statistical analysis was performed with Kruskal–Wallis H to investigate differences in the levels of numerical variables examined in relation to the studied polymorphisms. Allele and genotype frequencies were compared between patients and control groups and between patients' subgroups and controls in some cases. Chi-square analysis was used in order to test any deviation of genotype distribution from Hardy-Weinberg equilibrium (HWE) for every single nucleotide polymorphism studied, for both control and patients groups. HWE reflects a situation in which a defined population displays constant genotype frequencies from generation to generation, and those genotype frequencies can be calculated from the allele frequencies based on the HWE formula. Association tests for each polymorphism and haplotype frequency estimations were performed with the software package PLINK v1.07 and p-values ≤0.05 were considered statistically significant. The magnitude of the association was assessed using the odds ratio and confidence intervals of 95% (95% CI) were calculated.

RESULTS

All SNPs in TNF were successfully genotyped. The test for Hardy-Weinberg equilibrium suggested that the genotypes for all the SNPs were in Hardy-Weinberg proportions and there was no deviation from Hardy-Weinberg equilibrium for the subgroups of patients.

Tables 2 and 3 mention the results for genotypes distribution and minor allele frequencies for the SNP TNF-α -857C/T for the total group of asthma patients and for the subgroups AIA, ATA. Statistical analysis revealed a tendency of association of the minor allele T of TNF-α -857C/T with nasal polyposis; minor allele frequency was higher in patients with NP compared to those without NP, with borderline statistical significance (0.288 vs. 0.188, p=0.08) in the total group of asthma patients. In the subgroups analysis, there was an association of polymorphism of TNF-α-857C/T with nasal polyposis (see Table 3) with a statistical significance in both subgroups (p=0.01 for the ATA patients, respectively p=0.05 for the AIA patients), the minor allele being most commonly present in patients with nasal polyposis compared to those without nasal polyposis (for the patients with aspirin-intolerant asthma, T allele frequency was 0.237 in those with NP vs. 0.125 in those without NP; 0.248 vs. 0.198 in the ATA patients).

There were no significant differences in the distribution of genotypes and alleles frequencies for the SNPs TNF-α -238G/A, -308G/A in our patients related to the presence of nasal polyposis (Table 4).

Analysing the relationship between haplotypes of the studied polymorphisms and the presence of nasal polyposis, we have found an association of haplotype TGG-857/308/238 of TNF-α (which contains minor allele T of TNF-α-857 polymorphism) with nasal polyposis, with borderline statistical significance (p=0.08), while the haplotype CGG occurs more frequently in patients without polyposis (p=0.08) (Table 5).

Table 2
Genotypes and allele frequencies of the TNF-α -857C/T in asthma patients with and without nasal polyps

GENOTYPE TNF-857* (rs1799724)	CC	CT	TT	Minor allele T frequency	p
Asthma (n=106)	64 (60.4%)	36 (34%)	6 (5.6%)	0.226	0.32
SEX					
F (n=68)	40 (58.9%)	24 (35.3%)	4(5.8%)	0.235	0.87
M (n=38)	23 (60.5%)	13 (34.2%)	2 (5.3%)	0.223	
NP+ (N=45)	22(48.8%)	19(42.2%)	4(9%)	0.288	0.08
NP- (N=61)	41 (51.7%)	17 (35%)	3 (13.3%)	0.188	

Table 3
Genotypes distribution of TNF-α -857C/T in ATA, AIA patients with and without nasal polyps

GENOTYPE	CC	CT	TT	p
ATA (n=60)	35 (58.3%)	21 (35%)	4 (6.7%)	0.24
NP- (53)	34(64.1%)	17(32.1%)	2(3.8%)	
NP+ (7)	1(14.29%)	4(57.14%)	2(28.57%)	0.01
AIA (n=46)	28(60.8%)	16(34.8%)	2(4.4%)	0.56
NP+(38)	21(55.2%)	16(42.1%)	1(2.7%)	0.05
NP- (8)	7(87.5%)	0	1(12.5%)	

Table 4
Genotypes and allele frequencies of the TNF-α -238G/A, -308G/A in asthma patients with and without nasal polyps

GENOTYPE TNF-308* (rs1800629)	GG	GA	AA	Minor allele A frequency	p
NP+ (N=45)	33(73.3%)	9(20%)	3(6.7%)	0.166	0.95
NP- (N=61)	44(72.1%)	14(22.9%)	3(5%)	0.163	
GENOTIP TNF-238 * (rs361525)	**GG**	**GA**	**AA**		
NP+ (N=45)	42(93.3%)	3(6.7%)	0	0.033	0.42
NP- (N=61)	59(96.7%)	2(3.3%)	0	0.16	

Table 5
Haplotypes of TNF-α -238G/A, -308G/A, -857 C/T with significant frequency in patients with nasal polyposis

Haplotype	Haplotype frequency		p
857/308/238	NP+	NP-	
CAG	0.16	0.16	0.95
TGG	0.28	0.18	0.08
CGG	0.51	0.63	0.08
857/308			
CA	0.16	0.16	0.95
TG	0.28	0.18	0.08
CG	0.54	0.64	0.12
308/238			
AG	0.16	0.16	0.96
GG	0.80	0.81	0.72
857/238			
TG	0.28	0.18	0.1
CG	0.69	0.79	0.08

Analysing the allelic pairs possible to be constructed, there is the same tendency of association as in the case of haplotypes described. Thus, TNF-α TG -857/-308 occurs more frequently in patients with nasal polyps (p=0.08), while TNF-α - 857/-308 CG occurs more frequently in patients without polyps (p=0.1). There is a tendency of association of TNF-α -857/-238TG with nasal polyposis (p=0.1).

All data suggests the possible role of the minor allele T of TNF-α -857C/T polymorphism in the pathogenesis of nasal polyposis, role modulated by the simultaneous presence of certain alleles of other TNF-α polymorphisms. All haplotypes containing the minor T allele of the TNF-α -857 demonstrated a tendency of association with nasal polyposis in the Romanian asthmatic patients. To our knowledge, these is the first time when the gene polymorphisms of TNF-α and their association with nasal polyposis are studied in Romanian asthma patients (the results were extensively presented in doctor's Camelia Berghea PhD thesis).

DISCUSSIONS

Nasal polyposis (NP) is an inflammatory chronic disease affecting the paranasal sinuses and nasal mucosa, particularly associated with the aspirin-intolerant asthma. Tumor necrosis factor (TNF-α) is a member of the proinflammatory cytokine gene family; they are produced by various cells, including epithelial cells and macrophages. Proinflammatory cytokines act synergistically in the process of chronic inflammation. TNF-α regulate the extravasation of eosinophils into the lamina propria by up-regulating adhesion molecule expression in nasal polyps[14]. TNF gene polymorphisms may influence the risk of developing NP or its severity.

In a study of 82 patients with NP and 106 subjects without sinus pathology, Erbek SS and collaborators have identified a significant association between the homozygous genotype AA of TNF-α-238G/A (p=0.05), respectively the GA genotype TNF-α-308G/A (p=0.001) and nasal polyposis, without association with asthma, atopy or intolerance to aspirin in their lot of study[14]. Another group of investigation

reported that TNF-α-308G/A SNP is an independent risk factor for the development of nasal polyposis[15]. Bernstein and his collaborators have reported also an association of minor allele of TNF-α-308G/A with nasal polyposis[16].

Two similar, independent studies, failed to demonstrate any association of TNF-α-308G/A, TNF-α-238G/A polymorphisms with nasal polyposis[17,18]. A recent study that evaluated the role of TNF-α -308G/A as a predisposing factor for nasal polyposis in patients of Hungarian ethnicity did not reveal differences in allelic frequency between patients and control group. However, they have noted a greater frequency of genotypes containing the minor allele that associates aspirin intolerance in patients[19].

We have not identified data published by other authors regarding the role of TNF-α-857C/T as a risk factor for nasal polyposis. We report that these new SNPs were not previously associated with NP. The results obtained by us showed a uniform distribution of genotypes of SNPs TNF-α -308G/A and TNF-α -238G/A in asthma patients with or without nasal polyposis, but with no association between gene polymorphisms TNF-α -238G/A, -308G/A and nasal polyposis in Romanian asthmatic patients.

For the polymorphism TNF-α-857C/T, we identified a higher frequency of minor allele (T) in patients with nasal polyposis (0.288) compared to those without nasal polyposis (0.188) in the group of asthma patients. Also, there was found a statistically significant association between the polymorphism TNF-α -857C/T and nasal polyposis when analysing the subgroups of patients with aspirin-intolerant asthma (p=0.05) or aspirin-tolerant asthma (p=0.01). Haplotype analysis in our study group revealed an association with borderline statistical significance (p=0.08) for haplotype TNF alpha TGG -857/-308/-238. There was also a tendency of association with nasal polyposis for allelic pairs containing minor allele (T): TNF-α -857/-308 TG, TNF-α -857-238 TG. We suggest that minor allele T of TNF-α -857C/T single nucleotide polymorphism could be a risk factor for NP in asthma patients.

CONCLUSIONS

In conclusion, all data suggest the possible role of the minor allele (T) of TNF-α -857C/T polymorphism in the pathogenesis of nasal polyposis. The influence of this polymorphism could be modulated by the simultaneous presence of certain alleles of the TNF-α polymorphisms, all haplotypes containing minor allele (T) of the TNF-α -857 demonstrating a tendency of association with nasal polyposis in Romanian asthmatic patients. This study suggests a possible linkage of a SNP in the TNF-α promoter region with nasal polyposis. These results need to be confirmed with multicentre studies for a more precise interpretation and corroborative studies for investigating the influence of polymorphism on nasal polyposis pathogenesis.

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