

Original article

The *IL1A* genotype is associated with nasal polyposis in asthmatic adults

Background: Nasal polyposis (NP) is a chronic inflammatory disease often found coexisting with asthma. As this disorder tends to cluster in families, a genetic predisposition has been suggested. Interleukin-1 (IL-1) has been proposed to play a role in the pathogenesis of NP.

Methods: We analysed the single G-to-T base exchange polymorphism in exon 5 at +4845 of the gene encoding IL-1 α (*IL1A*) and the C-to-T base exchange polymorphism at -511 of the gene encoding IL-1 β (*IL1B*) in a population-based sample of adult asthma patients ($n = 245$). The data were assessed for correlation with data on history of NP and other phenotype-related characteristics.

Results: The prevalence of NP in our study group was 14.3%. The distribution of the *IL1A* genotype differed significantly between asthmatics with and without NP ($P = 0.005$). The risk of NP was markedly increased in allele G homozygous subjects (OR = 2.73; 95%CI = 1.40–5.32). In the case of *IL1B* we found no significant associations. Asthmatics with NP had more symptoms than others, but lung function and blood eosinophil counts were similar.

Conclusions: Our study demonstrates an association of *IL1A* with NP in asthmatic patients and addresses the role of IL-1 α as an inflammatory modulator in the pathogenesis of this disease.

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Nasal polyposis (NP) is a chronic inflammatory disease of the nasal mucosa. It is clinically characterized by oedematous masses in the nasal and paranasal cavities, causing symptoms such as nasal obstruction, anosmia, sneezing, rhinorrhoea and itching. It often coexists with asthma (1, 2). Nasal polyposis impairs the quality of life more seriously than perennial allergic rhinitis and the impairment is even greater in asthmatics (3). As this disorder is seen to cluster in families, a genetic predisposition has been suggested (4). However, the current understanding of genetic factors underlying NP is minimal.

Concentrations of many cytokines and other inflammation-related mediators are found to be increased in nasal polyps when compared with nasal tissue in subjects without NP, indicating the inflammatory nature of this disease (2, 5). Inflammatory cells found in nasal polyps are mainly composed of eosinophils capable of releasing an abundance of inflammatory proteins. However, the presence of eosinophilia does not appear to depend on the presence of allergy (6). Rather it would seem to be related to the appropriate cytokines and chemokines, which

attract eosinophilic precursors and contribute to cell apoptosis, leading to increased survival of eosinophils (2).

Interleukin-1 (IL-1) is a highly inflammatory cytokine found in two forms, IL-1 α and IL-1 β , sharing a similar profile of functions by binding to the same receptors with different affinity (7). Interleukin-1 activates T cells and monocytes, upregulates expression of adhesion molecules, and induces expression of a number of other cytokines and inflammation-associated proteins, which form an amplifying cascade of inflammatory response. Interleukin-1 is regularly expressed in the polyps (2, 8, 9) and activity of IL-1 β has been proposed to play a role in the development of NP (10). Moreover, *in vitro* findings have implied that a genetic influence on the strength of inflammation is exerted by polymorphisms of the cytokine genes, and several of these polymorphisms have been shown to be associated either with the severity of or susceptibility to many inflammatory diseases (11).

Here we studied the polymorphism of the *IL1A* and *IL1B* genes in asthmatics with or without NP in order to establish whether IL-1, one of the major regulators of inflammation, has an effect on susceptibility to NP.

Material and methods

Study subjects were participants in a Finnish population-based case-control study aimed at identifying risk factors and predictors of outcome in adult asthma. In all there were 245 asthma patients. Inclusion criteria for asthmatics have been described in detail elsewhere (12). The participants were classified as having NP according to their answer to the question: ‘Have polyps been found in your nose?’ This question has previously been used in an epidemiological study (13). Subjects giving an affirmative answer were classified as NP and their medical history was further examined to find out if they had undergone a surgical removal of nasal polyps. The ethnic origin of the cohort was uniform (Finnish Caucasian). Demographic and clinical characteristics of subjects with and without NP at study entry are summarized in Table 1. Approval for this study was obtained from the ethical committee at Tampere University Hospital. All subjects gave informed consent to participate.

Allergy testing was carried out by the skin prick method and measurement of total serum immunoglobulin E (IgE) concentration. Skin prick tests were performed by specially trained nurses with a panel of 22 common allergen extracts (ALK A/S, Copenhagen, Denmark). The patient was considered prick test-positive if at least one allergen elicited a wheal with a diameter at least 3 mm larger than that of the negative control. Total serum IgE levels were analyzed by the immunoluminometry method in an accredited laboratory according to the manufacturer’s instructions (Ciba Corning Diagnostics, Halstead, UK).

Lung function was measured with the Vitalograph® (Vitalograph Ltd., Buckingham, UK) spirometer, and the results were corrected for BTPS (body temperature and pressure, saturated with water vapour). Results of two technically faultless measurements were recorded, and the highest readings for forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were taken as the final results, whether obtained at the same measurement or not. The only reason for rejecting a measurement was a technically incomplete or otherwise unsuccessful blow. Calibration was performed according to manufacturer’s instructions. Finnish reference values (14) were used when converting results to per cent predicted form.

Analysis of IL1A and IL1B polymorphism

The DNA was isolated from blood samples by the salting out method (15). *IL1A* (+4845G>T). G-to-T base exchange at position +4845 of the *IL-1α* gene was analysed by the modified method of van den Velden (16). Polymerase chain reaction (PCR) primers 5'-ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA- 3' and 5'-AAT GAA AGG AGG GGA GGA TGA CAG AAA TGT- 3' amplified exon 5 but introduced a mutation (T→G) in the flanking primer which facilitates analysis of this polymorphism by *Sat I* (MBI Fermentas, Vilna, Lithuania) digestion. *IL1B* (-511C>T). The region containing the *AvaI* polymorphic site at position -511 (C-to-T base exchange) of the *IL-1β* gene was amplified by PCR (17). The oligonucleotides 5'-TGGCATTG-ATCTGGTTCATC-3' and 5'-GTTTAGGAATCTTCCCACTT-3' were used as primers. Fragments were analysed by electrophoresis on 9% polyacrylamide gel electrophoresis (PAGE) and stained with ethidium bromide. Genotyping was successful in all subjects with NP – 243 of 245 subjects overall. The allele distribution of the cytokine genes studied followed Hardy–Weinberg equilibrium.

Participants with and without NP were compared. The statistical method selected is indicated in ‘Results’ as used. Findings were considered statistically significant at *P* < 0.05. Statistical calculations were carried out with SPSS (ver. 10.1, SPSS Inc., Chicago, IL). Odds ratios (OR) with 95% confidence interval (CI) were calculated

by CIA software (ver. 1.1, copyrighted by MJ Gardner and the British Medical Journal, 1989).

Results

The prevalence of NP in our study group was 14.3%. From the 35 subjects with NP, 27 (77%) had undergone surgical removal of nasal polyps. Asthmatics with NP had a history of allergic rhinitis more often than those without, but otherwise the basic characteristics of the groups were similar (Table 1). As expected, NP was associated with symptom severity in asthma. Patients with NP had more nightly symptoms and used inhaled steroids more often than those without NP. In addition, there was greater need for short peroral steroid courses within the last 12 months in the NP group. However, whether this was the result of nasal or asthmatic symptoms was not recorded. No clear association was found with lung function, atopy or blood eosinophil count (Table 2).

Table 1. Basic characteristics of the study group

	Nasal polyposis		<i>P</i> -value
	Yes (<i>n</i> = 35)	No (<i>n</i> = 210)	
Gender, male (%)	40.0	37.6	0.788*
Age, years (mean)	57.7	59.2	0.317‡
Duration of asthma, years (media)	12.9	7.0	0.087‡
Smoking (non/ex/current) (%)	48.6/28.6/22.8	50.5/33.3/16.2	0.604†
Allergic symptoms			
Rhinitis (%)	71.4	51.9	0.032*
Eye symptoms (%)	48.6	41.4	0.429*
Skin symptoms (%)	34.3	34.3	1.000*

P-values calculated by chi-square test in * 2 × 2 table, † 3 × 2 table or ‡ Mann–Whitney *U*-test.

Table 2. Characteristics of asthmatics with and without nasal polyposis

	Nasal polyposis		<i>P</i> -value
	Yes (<i>n</i> = 35)	No (<i>n</i> = 210)	
FEV ₁ (% pred)	88.2	86.1	0.861*
IgE (IU/L) (median)	71.0	42.0	0.872*
Eosinophil count (10 ⁹ /L) (median)	0.20	0.16	0.079*
Atopy (%)	47.1	57.9	0.237
History of ASA-related asthma symptoms (%)	20.0	9.0	0.051
History of night symptoms at least once a week (%)	34.3	18.6	0.034
Inhaled steroid treatment (%)	82.9	65.6	0.042
Continuous peroral (p.os) steroid treatment (%)	–	3.8	0.240
More than two rescue p.os steroid courses in last 12 months (%)	14.3	5.2	0.045
Self-assessed symptom severity moderate or severe (%)	77.1	67.8	0.270

P-values calculated by chi-square test in 2 × 2 table except * by Mann–Whitney *U*-test.

Table 3. Genotype and allele frequencies of *IL1A* (+4845G > T) and *IL1B* (–511C > T) polymorphism in asthmatics with and without nasal polyposis

	Nasal polyposis		<i>P</i> -value
	Yes (<i>n</i> = 35)	No (<i>n</i> = 208)	
<i>IL-1A</i> (+4548)			
Genotype			
GG	68.6	40.4	0.005
GT	22.9	51.4	
TT	8.6	8.2	
Allele frequency			
Allele G	80.0	66.1	0.021*
Allele T	20.0	33.9	
Carriers of allele T			
Carrier	31.4	59.6	0.002*
<i>IL-1B</i> (–511)			
Genotype			
CC	37.1	42.3	0.848
CT	45.7	41.8	
TT	17.1	15.9	
Allele frequency			
Allele C	60.0	63.2	0.606*
Allele T	40.0	36.8	
Carriers of allele T			
Carrier	62.9	57.7	0.566*

P-values were calculated using the chi-square test in 3 × 2 and *2 × 2 tables.

The distribution of the *IL1A* genotype differed significantly between asthmatics with and without NP (Table 3). This was mainly because of the increased number of allele G homozygotes among subjects with NP. The risk of NP was markedly increased in subjects with common G/G genotype (OR = 2.73; 95%CI = 1.40–5.32). Moreover, when surgical removal of nasal polyps was used as additional criterion for NP, the association with *IL1A* genotype stayed highly significant (*P* = 0.010 for genotype frequencies, *P* = 0.023 for allele frequencies and *P* = 0.003 for allele two carrier status) and the OR for subjects with G/G genotype was further increased to 3.51 (1.47–8.38). In the case of *IL1B* the genotype distribution was similar in subjects with and without NP (Table 3).

Discussion

Our study was conducted in a population-based sample of adult asthmatics to establish whether the *IL1A* and *IL1B* genotypes are associated with NP. Recently, we demonstrated an association between asthma and *IL1B* genotype in men (18) and between atopy and *IL1A* genotype in nonasthmatics (12). Moreover, *IL1A* (–889C > T) gene polymorphism was associated with recurrent acute otitis media in nonallergic children (19). Interestingly, we found no association between *IL1B* (–511C > T) polymorphism and NP, whereas in the case of *IL1A* (+4845G > T) polymorphism a clear association was seen. These findings suggest that the IL-1 gene family

plays an important role in individual liability to diseases affecting mucosal surfaces, but the genes involved in different phenotypes are not the same.

Interleukin-1 seems to play an important role in the pathogenesis of NP. The development of NP has been held to be the result of stimulation of the epithelium by aerodynamic changes, allowing irritants to metabolically or physically alter or injure the surface epithelium (10). The second phase would thereafter be related to activity of tumour necrosis factor- α and IL-1 β . The message and the product of these two cytokines are found in the epithelium and endothelium of the nasal polyps (10). The most important function of these two cytokines consists in enhancing the expression of adhesion molecules on endothelial cells. This results in an increased transendothelial migration of eosinophils into the tissues (2), which has been regarded as the third phase in the development of NP (10).

The IL-1 α and IL-1 β molecules are structurally related and share a similar profile of functions by binding to the same receptors with different affinity. Interleukin-1 α remains primarily cell-associated and is found largely in the cytosol and on the plasma membrane of cells, whereas IL-1 β is the main secreted form of IL-1 (7). The biological significance of *IL1A* exon 5 polymorphism has not been conclusively established. The single G-to-T base exchange polymorphism in exon 5 at +4845 of the *IL1A* gene causes an amino acid substitution of alanine to serine (16). The change in the amino acid sequence of IL-1 α may alter protein conformation, thus resulting in a difference in biological functions, receptor affinity or half-life of this protein. It is also possible that the +4845 allele associations described here are mediated by the other adjacent genes linked to the +4845 site, or by a combined effect of the +4845 alleles and the other alleles embedded in the IL-1 gene complex.

Nasal polyposis and asthma appear to have a cumulative negative effect on the quality of life (3). In our study population, NP was associated with increased asthma symptoms and usage of anti-inflammatory medication. It is thus justifiable to consider asthma with NP as a clinically important subgroup of asthmatics. However, it should be kept in mind that aspirin hypersensitivity and chronic rhinosinusitis are also common disorders, which overlap significantly with NP (20, 21). Particularly, the triad of NP, aspirin intolerance and asthma has long been recognized as a distinct phenotype of asthma (22). In our study population there were 26 subjects with a history of aspirin-related asthma symptoms, but only seven of them also had NP. When we compared these asthmatics with those without aspirin-related symptoms we found no differences in *IL1A/IL1B* genotypes (data not shown). We therefore consider it unlikely that our findings could be related to aspirin hypersensitivity.

In a recent population-based study by Hedman and coworkers, the overall prevalence of NP in Finland was 4.3% (13). The prevalence was 16.5% in subjects with

doctor-diagnosed asthma and 3.7% in subjects without. This is in concordance with the occurrence of NP found in this study (14.3%). Moreover, from the results of the aforementioned study it can be calculated that approximately 20% of Finnish adults with NP also have doctor-diagnosed asthma. Another disease comorbid with NP is cystic fibrosis (CF) and it has been estimated that 6–48% of CF patients suffer from nasal polyps (2, 23). In contrast to tissue eosinophilia found in 80–90% of all cases with NP, in CF patients the inflammation in the nasal mucosa is neutrophil-dominated (2). The eosinophilic inflammation type should be even more common in our study cohort because one special feature of the Finnish population is a very low prevalence of CF. Only one in 80 Finns carries the CF gene (24), while in the

USA, e.g. one in 25 Caucasians is a carrier of a gene mutation (25).

In summary, this is to our knowledge the first study to produce evidence of a specific gene locus associated with NP in asthmatic patients. Moreover, the findings indicate the potential role of IL-1 α as an inflammatory modulator in the pathogenesis of this disease.

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