

# Interleukin-6 –174 G/C promoter gene polymorphism in nasal polyposis and asthma\*

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## Summary

**Background:** Interleukin-6 (IL-6) is an inflammatory mediator linked to nasal polyposis and asthma, with a single nucleotide polymorphism –174 G/C that seems to promote an inflammatory status. We aimed to analyze the relationship between this polymorphism and asthmatic nasal polyposis patients.

**Methodology:** Cross-sectional study to investigate IL-6 - 174 G/C genotypes of 45 nasal polyposis with asthma patients, 63 nasal polyposis-only patients, 45 asthma-only patients and 81 subjects without both diseases. Aspirin intolerance and atopy were main exclusion criteria. IL-6 genotyping was performed using the PCR method with specific primers followed by restriction enzyme analysis, classifying patients in GG, GC or CC genotype.

**Results:** The GG genotype was the most frequent in all inflammatory groups. Less than 40% of controls presented with the GG genotype. There were significant differences between inflammatory groups and control group. No significant differences were seen when comparing inflammatory groups to each other, other than between nasal polyposis-only group and asthma-only group.

**Conclusion:** The IL-6 74 GG genotype was found more frequently in all inflammatory groups than in controls. This genotype could influence nasal polyposis and asthma, and seems to be more important in the latter.

**Key words:** asthma, nasal polyps, genetic polymorphism, interleukin-6, single nucleotide polymorphism

## Introduction

Chronic rhinosinusitis (CRS) is defined as inflammation of the nose and the paranasal sinuses that lasts twelve or more weeks without complete resolution of symptoms<sup>(1)</sup>. It has been typically divided into CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP), also called nasal polyposis

(NP), based on endoscopic findings<sup>(1)</sup>. Patients with NP typically present with nasal obstruction, hyposmia, and rhinorrhea, leading to substantial reduction in quality of life<sup>(2,3)</sup>. There has been emerging consensus that NP is a multifactorial disease leading to persistent inflammation that appears to be the common pathway<sup>(1)</sup>, due to the involvement of many inflammatory

mediators that that could culminate in polyp formation <sup>(4,5)</sup>.

Asthma is a chronic inflammatory disease too, characterized by bronchial hyperresponsiveness with variable airflow obstruction, which is often reversible either spontaneously or with treatment, presenting with wheezing, breathlessness, tightness of the chest and coughing <sup>(6,7)</sup>. Similar to NP, chronic inflammation appears to be the common pathway in asthma, with several inflammatory cells and mediators involved that could lead to symptoms development and maintenance <sup>(6,8)</sup>.

Many pathogenic hypotheses have been proposed to explain the link between NP and asthma <sup>(1,6)</sup>. The concept with the best supporting evidence involves production of inflammatory mediators, eosinophil precursors and T-helper (Th) lymphocytes by NP, which may lead to increased generation of eosinophils, mast cells and basophils in the bone marrow and to subsequent recruitment of cells and mediators into the lungs <sup>(6)</sup>. Confirming this relationship, it is interesting to note that NP and asthma present very similar histopathological features: both diseases are heterogeneous, where chronic inflammation plays an important role, with pronounced eosinophilic proliferation <sup>(4,6,9-11)</sup>. Moreover, both diseases have non-eosinophilic alternative phenotypes, particularly neutrophilic, which could be related to a poor response to corticosteroids <sup>(4,10,12)</sup>. Considering T-cell response patterns, both diseases are typically associated with a Th2-driven inflammation, but an alternative Th17 skewed profile could be seen, with inhibition of regulatory T (Treg) cells activity playing a role in their pathophysiological mechanisms <sup>(4,9,10,12)</sup>.

Interleukin (IL)-6 is one of several inflammatory mediators that could play a role in NP and asthma <sup>(13-15)</sup>. Classically, IL-6 has been known as one of the cytokines that drives the acute inflammatory response and innate immunity, but today it has been considered a key signal that orchestrates chronic inflammation and adaptive immunity <sup>(16)</sup>, by regulating T cell differentiation and activation, inducing Th2 cytokine production <sup>(17)</sup>. Furthermore, IL-6 induces the generation of Th17 cells together with TGF- $\beta$ , while it inhibits differentiation of Treg cells <sup>(17)</sup>. In asthma, IL-6 trans-signaling seems to play a role in controlling Th2 function, while IL-6 classic signaling controls the cell fate at the beginning of T cell differentiation toward the Th2 pathway and inhibits Treg cell differentiation in the lung <sup>(18)</sup>. In NP, increased levels of IL-6 and its soluble receptor suggest that IL-6 trans-signaling may be altered and could be partially responsible for the recruitment and retention of T cells, increasing Th2 inflammation and disabling Treg cells responses <sup>(14)</sup>.

IL-6 levels could be influenced by a single-nucleotide polymorphism (SNP) at position -174 in the promoter region of the IL-6 gene, that was firstly described by Fishman et al. This SNP

presents guanosine (G) or cytosine (C) at position -174 and this variability could be functional, because it modifies transcriptional regulation and cytokine levels, thereby leading to an inflammatory phenotype <sup>(19)</sup>. In our previous study, the frequency of GG genotype was found to be higher in NP patients than in controls <sup>(2)</sup> and similar results were found in asthma patients <sup>(20,21)</sup>. Possibly, the GG genotype could raise IL-6 production and/or secretion, or the C allele could protect against NP and asthma by decreasing inflammation, then playing a role in chronic inflammation found in both diseases <sup>(2,19-21)</sup>. No studies exploring this SNP in NP with asthma were found.

Postulating that NP and asthma are related chronic inflammatory diseases, that IL-6 plays a role in both diseases, and that IL-6 -174 G/C SNP could modify inflammatory response, we designed this study to assess the relationship between IL-6 -174 G/C SNP and NP with asthma.

## Materials and methods

### Study population

The study population was formed by 45 patients with NP and asthma (NPwAsthma Group), 63 patients with NP and without asthma (NP-only Group), 45 patients with asthma and without NP (Asthma-only Group) and 81 healthy volunteers without NP and asthma (Control Group). Volunteers were selected between May 2007 and December 2011. All participants underwent an otorhinolaryngological assessment including nasal endoscopy and skin prick-test for allergies. Only NP patients underwent CT-scans. As inclusion criteria, presence of NP was clinically defined according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) 2012 <sup>(1)</sup>, with presence of two or more symptoms and the visualization of polyps in both middle meati at nasal endoscopic examination, confirmed on CT-scans; absence of NP, when neither symptoms suggesting rhinosinusitis according to EPOS 2012 <sup>(1)</sup> criteria nor middle meati alterations (like polyps, purulence, edema and/or hyperemia) were visualized by nasal endoscopic examination <sup>(2)</sup>. Presence of asthma was defined by clinical and spirometric criteria <sup>(11)</sup>; and absence of asthma, if the subject has not presented any complaints of previous or current dyspnea and/or wheezing and has not reported either prior use of bronchodilators or prior clinical follow-up due to asthma diagnosis.

Exclusion criteria included an age below 18 years, medical history or symptoms suggesting salicylic acid intolerance, cystic fibrosis or ciliary dyskinesia, presence of nasal neoplasia, and one or more positives in a skin prick-test with 10 known extracts: saline solution (negative control), histamine (positive control), *Felis domesticus*, *Aspergillus fumigates*, *Canis familiaris*, *Periplaneta americana*, *Penicillium notatum*, *Dermatophagoides pteronyssinus*, *Blomia tropicalis* and *Alternaria alternata*. A wheel

Table 1. Genotypic distribution of polymorphism.

Genotype	Groups									
	Control		NP-only		Asthma-only		NPwAsthma		Total	
	N	%	N	%	N	%	N	%	N	%
GG	31	38.27	39	61.90	37	82.22	32	71.11	139	59.40
GC	46	56.79	21	33.33	8	17.78	12	26.67	87	37.18
CC	4	4.94	3	4.76	0	0	1	2.22	8	3.42
Total	81	100	63	100	45	100	45	100	234	100

NP = Nasal polyposis; NPwAsthma = Nasal polyposis with asthma; N = Number; Fisher Exact Test All groups:  $p < 0.001^*$ ; Control vs. NP-only:  $p = 0.01^*$ ; Control vs. Asthma-only:  $p < 0.001^*$ ; Control vs. NPwAsthma:  $p < 0.001^*$ ; NPwAsthma vs. Asthma-only:  $p = 0.32$ ; NPwAsthma vs. NP-only:  $p = 0.62$ ; Asthma-only vs. NP-only:  $p = 0.04^*$ .

3 mm larger than the negative control was considered a positive response <sup>(2)</sup>.

### Study design

This study was approved by the university's ethics committee under protocol number CEP0068/10. All subjects signed informed consent. This was a cross-sectional study with four groups: one Control Group and three inflammatory groups, called NPwAsthma Group, NP-only Group and Asthma-only Group. After inclusion and exclusion criteria had been observed, venous blood samples from all participants were collected for genomic DNA extraction. DNA was extracted, amplified by polymerase chain reaction (PCR), digested by restriction enzyme NlaIII and separated by electrophoresis, revealing the specific genotypes. All participants were classified in -174GG, -174GC or -174CC genotype and statistical analysis was performed to compare groups.

### DNA genotyping

Genomic DNA from all participants was extracted from venous blood samples using a commercial extraction kit (Amersham Biosciences, Buckinghamshire, UK) as described previously <sup>(2)</sup>. IL-6 genotyping was performed as described by Fishman et al. <sup>(19)</sup> using the PCR with forward primer 5'-ATGCCAAGTGCT-GAGTCACTA-3' and reverse primer 5'-GGAAAATCCCACATTT-GATA-3', amplifying a 226-bp DNA fragment that contained the -174 position. The PCR product was digested with NlaIII (New England Biolabs Inc., Beverly, MA, USA) <sup>(2,19)</sup>. When the -174 position had the C allele, the 226-bp PCR product was cleaved into two fragments of 117 and 109-bp. No cleavage occurred when the G allele was present. Digestion products were separated by electrophoresis through 2% agarose gel (Invitrogen Life Technologies, Carlsbad, CA, USA) and visualized with ethidium bromide. Participants were classified in GG, GC and CC.

### Statistical analysis

Age distributions normality in both groups were analysed

by Kolmogorov-Smirnov (KS) test. Sex and age homogeneity among groups were assessed by chi-squared ( $\chi^2$ ) test and analysis of variance (ANOVA), respectively. Hardy-Weinberg equilibrium analysis was performed using  $p^2 + 2pq + q^2 = 1$ , where p is the G allele frequency and q is the C allele frequency. Finally, genotype and allele distributions in groups were compared using the  $\chi^2$  test or Fisher exact test if necessary. The odds ratio (OR) with a 95% confidence interval was calculated if possible. For all tests, the p-values of 0.05 were considered significant.

### Results

Two hundred and thirty four subjects were included in this study: 81 in Control Group, 63 in NP-only Group, 45 in Asthma-only Group and 45 in NPwAsthma Group. Age had a normal distribution without significant differences among groups (ANOVA:  $p=0.11$ ). All groups were homogeneous considering gender distribution, but the Asthma-only Group presented strong female predominance ( $\chi^2$  test:  $p<0.05$ ). Genotype frequencies of all groups were in Hardy-Weinberg equilibrium.

Considering genotype distribution, the GG genotype was more frequent in the inflammatory groups, whereas the Control Group showed GC genotype predominance, with a significant difference among groups, as presented in Table 1. Comparing only the inflammatory groups, no significant differences were observed, but between NP-only Group and Asthma-only Group, there was a greater frequency of the GG genotype in the latter. Due to the difference found between NP-only and Asthma-only Groups, comparisons between subjects with and without asthma (regardless of NP status) and between subjects with and without NP (regardless of asthma status) were conducted, as shown in Tables 2 and 3. There was significant difference in genotype distribution between asthmatics vs. nonasthmatics subjects, but no difference was seen between subjects with NP vs. without NP.

The CC genotype was rare in this study, not reaching a 5%

Table 2. Genotypic distribution - with and without asthma.

Genotype	Groups					
	Without Asthma		With Asthma		Total	
	N	%	N	%	N	%
GG	70	48.61	69	76.67	139	59.40
GC	67	46.53	20	22.22	87	37.18
CC	7	4.86	1	1.11	8	3.42
Total	144	100	90	100	234	100

Fisher Exact Test: Without Asthma vs. With Asthma:  $p < 0.001^*$

Table 3. Genotypic distribution - with and without NP .

Genotype	Groups					
	Without NP		With NP		Total	
	N	%	N	%	N	%
GG	68	53.97	71	65.74	139	59.40
GC	54	42.86	33	30.56	87	37.18
CC	4	3.17	4	3.70	8	3.42
Total	144	100	90	100	234	100

Fisher Exact Test: Without NP vs. With NP:  $p = 0.14$ ; NP = Nasal Polyposis

frequency in each group. Considering the low prevalence of the CC genotype in all groups and the GG genotype predominance in the inflammatory groups compared to the Control Group, we decided to compare subjects with at least one C allele, now called non-GG genotype, to subjects with GG genotype, as shown in Table 4. In this situation, comparison of each inflammatory group to the Control Group was significantly different, with OR as high as 7.46 for Asthma-only Group vs. Control Group, suggesting that subjects with the GG genotype have higher risk of developing asthma and/or NP.

## Discussion

Fishman et al., in the first report of the IL-6 -174 G/C SNP, observed that the CC genotype could protect against systemic-onset juvenile chronic arthritis, while the G allele seemed to have proinflammatory status<sup>(19)</sup>. After that, several diseases were related to this SNP<sup>(22-31)</sup>, but the allele or genotype responsible for the proinflammatory phenotype was not always the G allele or the GG genotype<sup>(2)</sup>. Terry et al., studying two different cell lines (HeLa and ECV304), observed that polymorphisms could really influence IL-6 transcription and that this influence would vary according to the cell type. So, the allele or the genotype related to inflammatory status could vary depending on the cell

type<sup>(32)</sup>.

The present study found predominance of the GG genotype in inflammatory groups and GC genotype in controls, suggesting that GG genotype could promote a "more inflammatory" phenotype in airways' diseases. Other studies have already found a GG genotype predominance in asthma-only<sup>(20,21)</sup> and in NP-only<sup>(2)</sup>, but this is the first report relating this SNP to NP with asthma. Although NP and asthma are not exactly the same tissue, both diseases affect the respiratory epithelium, so we expected that they could present the same results, like postulated by Terry et al.<sup>(32)</sup>.

The CC genotype was rare in this study, as seen in other reports on asthma<sup>(20,21,33,34)</sup> and NP<sup>(2)</sup>. Probably, due to its low frequency, the CC genotype should not play a role in the pathophysiology of NP and asthma. Furthermore, it would be reasonable to believe that the presence of at least one C allele (non-GG genotypes) could play a role in both diseases reducing inflammation, whereas the GG genotype seemed to increase inflammatory phenotype. This theory seemed to be valid in the Brazilian population, since both our previous<sup>(2)</sup> and the current study in NP showed non-GG genotype predominance in controls and GG

Table 4. Adjusted genotypic distribution of polymorphism..

Genotype	Groups									
	Control		NP-only		Asthma-only		NPwAsthma		Total	
	N	%	N	%	N	%	N	%	N	%
GG	31	38,27	39	61.90	37	82.22	32	71.11	139	59.40
Non-GG	50	61.73	24	38.10	8	17.78	13	28.89	95	40.60
Total	81	100	63	100	45	100	45	100	234	100

NP = Nasal polyposis; NPwAsthma = Nasal polyposis with asthma; N = Number; 95% CI= 95% confidence interval.

	Chi-squared test	Odds ratio	95% CI
All groups:	p < 0.001*		
Control vs. NP-only:	p < 0.01*	2.62	1.33 to 5.16
Control vs. Asthma:	p < 0.00001*	7.46	3.08 to 18.09
Control vs. NPwAsthma:	p < 0.001*	3.97	1.81 to 8.70
NPwAsthma vs. Asthma-only:	p = 0.21	0.53	0.19 to 1.44
NPwAsthma vs. NP-only:	p = 0.32	1.51	0.67 to 3.44
Asthma-only vs. NP-only:	p = 0.02*	2.85	1.14 to 7.13

genotype predominance in NP. However, there has no clear genotype distribution pattern of this SNP in asthma been reported. Only Trajkov et al. <sup>(21)</sup> found the same pattern of non-GG genotype predominance in controls and GG genotype predominance in asthmatics. Settin et al. <sup>(20)</sup> and Mahdavian et al. <sup>(33)</sup> reported non-GG genotype predominance in controls and asthmatics, although only Settin et al. <sup>(20)</sup> found a difference between groups (GG genotype was more frequent in asthmatics than in controls). On the other hand, Daneshmandi et al. <sup>(34)</sup> showed GG genotype predominance in controls and asthmatics, without difference between groups. These contradictory results were explained by Ivanova et al., which showed differences in genotype distribution of the IL-6 –174 G/C SNP depending on the ethnic characteristics of the study population <sup>(35)</sup>.

Besides population differences, another issue that hampers NP and asthma research is a pathophysiological heterogeneity <sup>(1,2,6)</sup>, because this SNP could play an essential role in a certain mechanism, but it is not necessary true in another mechanism <sup>(32)</sup>. This study established several criteria of exclusion, trying to restrict this SNP study to one inflammatory pathway <sup>(2)</sup>, because we could not assure if this SNP would be more related to aspirin sensitivity or to atopy, for instance. This study's population showed age distribution homogeneity, but not gender's. Nevertheless, considering that this SNP occurs in chromosome 7, an autosomal chromosome, possibly a gender variance would not affect this SNP's genotype distribution <sup>(36)</sup>.

NP – asthma association seems to produce more inflammation than each single disease since clinical outcomes, endoscopic findings and postoperative prognosis are worse in NP with asthma than in NP-only <sup>(1,37)</sup>. Histopathological features in NP with asthma include an increased number of activated eosinophils and myofibroblasts when compared to NP-only <sup>(38)</sup>. Further-

more, IL-6 levels in nasal secretion are higher in NP with asthma than in NP-only <sup>(39)</sup>. Based on these findings, we expected to find a higher proportion of the GG genotype in the NPwAsthma Group, but our data showed 82.22% of the GG genotype in Asthma-only Group, 71.11% in NPwAsthma Group and 61.90% in NP-only Group. Although there was no statistical difference between Asthma-only and NPwAsthma Groups, and between NPwAsthma and NP-only Groups, a comparison of Asthma-only and NP-only Groups showed a significant difference in genotype distribution. Moreover, a comparison of subjects with and without asthma, regardless of NP's status, revealed a statistical difference with GG genotype predominance in asthmatics, but no difference was found when comparing subjects with and without NP, regardless of the asthma status. So, according to our data, the IL-6 –174 G/C SNP seemed to be more relevant in asthma development than in polyp formation. However, even if this SNP could play a greater role in asthma than in NP, we still expected higher frequencies of the inflammatory genotype (GG) in NPwAsthma Group, since this group put together inflammatory mechanisms of asthma and NP.

This inconsistency of our data could be explained by selection bias. Firstly, asthma's severity has not been stratified, so Asthma-only Group patients could have more severe asthma than those in the NPwAsthma Group. Increased sputum IL-6 levels has already been related to asthma's severity <sup>(40)</sup>, then, the Asthma-only Group could have higher levels of IL-6 than the NPwAsthma Group. Considering a possible relationship between GG genotype and increased levels of IL-6 <sup>(19)</sup>, the Asthma-only Group could present higher frequencies of this genotype than the other. Lastly, in almost 70% of patients with NP – asthma association, asthma's symptoms precede polyps' formation over 9 to 13 years <sup>(1)</sup>. In cross-sectional studies, it is possible that a part of the Asthma-only patients could develop NP few years later, configuring another selection bias.

Regardless of which disease could be more related to the IL-6 -174 G/C SNP, the major finding of the current study was to first associate GG genotype to NP with asthma, and to corroborate the association between this genotype and each disease singly, suggesting a possible role of this SNP in NP and asthma's pathophysiology. Functional studies, relating genotype and local production of IL-6 in polyps and bronchial mucosa, are the next step to elucidate the real role of this SNP.

Concluding, in the IL-6 -174 G/C SNP study, we observed GG genotype more frequently in all inflammatory groups than in controls. This genotype could influence nasal polyposis and asthma, and seemed to be more important in the latter.

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### Authorship contribution

EMK: Conception and design; acquisition, analysis, and interpretation of data; drafting the article and final approval of the version to be published. CMC-K: Acquisition, analysis, and interpretation of data. ERH: Conception and design; acquisition, analysis, and interpretation of data. JAM-N: Acquisition, analysis, and interpretation of data. LCG: Drafting the article and revising it critically for intellectual content. IDS: Concept and design; and revising it critically for intellectual content and final approval of the version to be published. LLW: Concept and design; and revising it critically for intellectual content and final approval of the version to be published.

### Conflict of interest

The authors state that there is no conflict of interest regarding this manuscript.

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