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Genetic Association Study in Nasal Polyposis

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Abstract

Background and Objectives: Nasal polyposis (NP) is a chronic inflammatory disease of the upper airways with a variable clinical course and unknown pathogenesis that often coexists with other conditions. Considering the possibility of genetic predisposition, we decided to analyze whether polymorphisms in *LTC4S, CYSLTR1, PTGDR*, and *NOS2A* were associated with NP.
Methods: The study population comprised 486 Caucasian individuals. Polyposis and aspirin intolerance were diagnosed following the recommendations of the European Position Paper on Rhinosinusitis and Nasal Polyps. Genotypes were determined using polymerase chain reaction amplification and direct sequencing.
Results: The –444A>C *LTC4S* polymorphism was significantly associated with NP and atopy (*P*=.033) and with NP and atopic asthma, (*P*=.012). In addition, a significant association was found when the (CCTTT) repetition of the *NOS2A* gene was present more than 14 times in patients with NP and asthma (*P*=.034), in patients with polyposis and intolerance to nonsteroidal anti-inflammatory drugs (*P*=.009), and in patients with the aspirin triad (*P*=.005). The *PTGDR* diplotype CCCT/CCCC (–613CC, –549CC, –441CC and –197TC) was more frequent in patients with NP (*P*=.043), NP with asthma (*P*=.013), and the aspirin triad (*P*=.041).
Conclusions: NP was associated with specific polymorphisms only when it occurred with related phenotypes. Our results suggest that this genetic background plays a more relevant role in the development of the associated clinical features of nasal polyposis than in simple polyposis.

Key words: Atopy. Asthma. *CYSLTR1. LTC4S*. Nasal polyposis. *NOS2A. PTGDR*. SNP.

Resumen

Objetivos: La poliposis nasal es una patología inflamatoria crónica de las vías respiratorias superiores cuyo curso clínico es variable, su etiopatogenia es desconocida, y frecuentemente se asocia con otras enfermedades concomitantes. Dada la posibilidad de una predisposición genética en la poliposis nasal, se decidió analizar determinados polimorfismos localizados en los genes *LTC4S, CYSLTR1, PTGDR* y *NOS2A* para ver si mostraban alguna asociación con la patología.
Métodos: En el estudio se incluyeron 486 individuos de raza blanca. La poliposis y la sensibilidad a la aspirina se diagnosticaron de acuerdo con las directrices EPOS. Los genotipos se determinaron mediante PCR y secuenciación.
Resultados: Se observó una asociación estadísticamente significativa del polimorfismo -444 A>C LTCS4 con la poliposis y atopia (p=0,033) y con poliposis y asma atópica (p=0,012). Además, se encontró una asociación significativa de la repetición (CCTTT) en el gen NOS2A cuando aparecía más de 14 veces con la poliposis con asma asociada (p=0.034), con poliposis e hipersensibilidad a los AINE (p=0.009) y con la Triada de la aspirina (p=0.005). El diplotipo CCCT/CCCC (-613CC, -549CC, -441CC and -197TC) de *PTGDR* se observó con mayor frecuencia en los pacientes con poliposis (p=0,043); poliposis con asma (p=0,013) y con triada de la aspirina (p=0,041).
Conclusiones: La poliposis nasal se asoció con los polimorfismos específicos solamente cuando se presentaba con fenotipos asociados. Nuestros resultados sugieren que esta influencia genética puede estar desempeñando un papel más relevante en el desarrollo de las características clínicas asociadas a la poliposis nasal que con la poliposis nasal simple.

Palabras clave: Atopia. Asma. *CYSLTR1. LTC4S. NOS2A*. Poliposis nasal. *PTGDR*. SNP.

Introduction

Nasal polyps are edematous semitranslucent grape-like growths originating in the ostiomeatal complex. Nasal polyposis (NP) is considered a subgroup of chronic rhinosinusitis, an inflammatory disease of the upper airways that has a variable clinical course and often coexists with asthma, aspirin intolerance, and other conditions [1].

The pathogenesis of NP remains unknown. Some studies point to an underlying genetic predisposition and report a positive family history of NP, which could account for between 14% and 52% of cases [2,3]. Few studies have evaluated the genetic aspects of NP, although some have shown a positive association with the alleles and haplotypes of the human leukocyte antigen (HLA) gene [4,5]. Other studies found no association between HLA alleles and NP [6]. In addition, a polymorphism of the gene encoding interleukin (IL) 1 α (*IL1A*) has been reported to be associated with NP [7], and a polymorphism of *IL4* (IL4 –590 C>T) was found to be protective against NP in a Korean population [8]. Differential gene expression profiles have been identified in patients with NP [9,10].

Leukotriene C_4 synthetase, or glutathione S-transferase II (encoded by the *LTC4S* gene), is an enzyme that converts leukotriene A_4 and glutathione to create leukotriene C_4. This gene encodes an enzyme that catalyzes the first step in the biosynthesis of cysteinyl leukotrienes, potent biological compounds derived from the eicosanoid arachidonic acid. Eicosanoids have been involved in inflammatory mechanisms in NP, with the result that leukotriene levels are upregulated in nasal polyp tissue [11]. In addition, high levels of leukotriene C_4 synthase expression in the nasal polyps of patients with aspirin-intolerant asthma have been described [12]. Polymorphisms in the *LTC4S* gene have been linked to asthma [13], although conclusive evidence has not been found in the case of aspirin-sensitive asthma [14].

The inflammatory cells of nasal polyp tissue express high levels of *CYSLTR1* [15]. Cysteinyl leukotriene receptor 1 (encoded by the *CYSLTR1* gene) is a member of the G protein–coupled receptor superfamily. Pharmacologic studies show that cysteinyl leukotrienes activate at least the protein encoded by this gene and *CYSLTR2* [13], resulting in contraction and proliferation of smooth muscle, edema, eosinophil migration, and damage in the lung mucus layer.

Prostaglandins are also involved in the pathogenesis of NP [16]. Prostaglandin D_2 receptor (encoded by the *PTGDR* gene) is a G protein–coupled receptor that functions as a prostanoid DP receptor. The activity of this receptor is mediated mainly by Gs proteins that stimulate adenylate cyclase, resulting in an elevation of intracellular cyclic adenosine monophosphate and $Ca2^+$ levels. Knockout mouse models suggest that prostanoids exert both proinflammatory and anti-inflammatory effects [17], thus acting as mediators of acute inflammation and as gene expression regulators in mesenchymal and epithelial cells at the inflammatory site, actions frequently seen in immune and allergic reactions. Reduction in prostaglandin E levels and upregulation of prostaglandin E_2 and E_4 receptors has also been described in NP patients [18].

Finally, inducible nitric oxide synthase is an enzyme that is encoded in humans by genes such as *NOS2*, which is expressed in the liver and can be induced by a combination of lipopolysaccharide and specific cytokines. Nitric oxide, the molecule synthesized by *NOS2*, is a reactive free radical that acts as a biologic mediator in several processes, including neurotransmission and antimicrobial and antitumor activity. High levels of *NOS2* activity are associated with nasal polyp tissue from aspirin-sensitive asthmatics, and a variable number tandem repeat (VNTR) polymorphism in *NOS2* has been reported to be significantly associated with NP and other diseases [19-21].

Given the paucity of data from genetic studies in NP [7,22-24], we performed a case-control study to analyze polymorphisms in *LTC4S, CYSLTR1, PTGDR*, and *NOS2* as representative genes of inflammatory pathways in a population of patients with NP (with or without asthma).

Materials

Participants

The study population comprised 486 Caucasian individuals (241 patients and 245 controls). Controls met all the following criteria: *(a)* absence of symptoms or history of nasal polyps; *(b)* absence of symptoms or history of asthma; *(c)* absence of symptoms or history of other pulmonary diseases; *(d)* absence of symptoms or history of allergy; *(e)* absence of first-degree relatives with a history of asthma or atopy; and *(f)* negative skin prick test results to a battery of common aeroallergens (wheal <1 mm greater than the saline control).

Polyps were diagnosed following the criteria of the European Position Paper on Rhinosinusitis and Nasal Polyps [25]. Patients with cystic fibrosis, severe immunodeficiency, congenital mucociliary complaints, noninvasive fungal balls, invasive fungal disease, and systemic vasculitic or granulomatous diseases were excluded from the study.

Asthma was diagnosed as at least 2 symptoms consistent with asthma (cough, wheeze, and dyspnea). Bronchial hyperresponsiveness was defined as a positive bronchodilator test result or a positive methacholine challenge result in the absence of other pulmonary disorders. Lung function was measured by spirometry according to the criteria of the American Thoracic Society, and aspirin sensitivity was diagnosed based on the recommendations of the European Position Paper on Rhinosinusitis and Nasal Polyps [25].

Patients were recruited from the allergy departments of Hospital Clinic in Barcelona, Spain and University Hospital of Salamanca, in Salamanca, Spain. The ethics committees of both hospitals approved this study, and all participants gave their written informed consent.

Skin Tests

Skin prick tests were performed following the guidelines of the European Academy of Allergology and Clinical Immunology. Results were considered positive if at least 1 wheal of more than 3 mm in diameter after subtracting the negative control was observed. A battery of common

aeroallergens was used as previously described [14]. Histamine (10 mg/mL) was used as a positive control and saline as a negative control. Atopy was defined as the presence of at least 1 positive skin prick test result.

Total Serum Immunoglobulin E

Total serum immunoglobulin (Ig) E was determined using ImmunoCAP (Phadia) following the manufacturer's instructions.

Molecular Analysis

We analyzed 6 polymorphisms: –613C>T, –549T>C, –441C>T, and –197T>C in the promoter region of *PTGDR*, –444A>C in *LTC4S*, and 927T>C in *CYSLTR1* and 1 VNTR (CCTTT)n in the *NOS2A* promoter. For the genotyping study, genomic DNA was extracted from whole blood as previously described [14] or using the Maxwell 16 Blood DNA Purification Kit (Promega).

Polymerase chain reaction (PCR) amplifications of the corresponding fragments from *PTGDR, LTC4S*, the *NOS2A* promoter region, and the *CYSLTR1* coding region were performed in an MWG-BIOTHECH thermal cycler (Biotech) [20,26,27]. For each reaction, a pair of upstream and downstream primers was used to amplify the genomic region surrounding the single-nucleotide polymorphism (SNP) of interest. The sequences of the upstream and downstream primers used to amplify the *PTGDR* promoter region were 5′-CTCAGTTTCCTCGCCTATGC-3′ and 5′-ACCCCTGGAAGCCTACAACTGCAT-3′, respectively. To amplify the genomic region surrounding the promoter SNP of LTC4S, the primers used were 5′-CTCCATTCTGAAGCCAAA-3′ and 5′-AGACCGCCTCACCACTT-3′. For CYSLTR1, the primers used to amplify the genomic region surrounding the SNP of interest were 5′-AAATCATGTTTTGGTCTTGC-3′ and 5′-ATTTTCATTGGTTTGACTG-3′. Finally, to amplify the genomic region surrounding the (CCTTT) n polymorphism of NOS2A, the primers used were 5′-ACCCCTGGAAGCCTACAACTGCAT-3′ and 5′-GCCATCGCACCCTAGCCTGTCTCA-3′. The reaction mixtures and cycling conditions were as previously described [20,26,27]. Negative controls without genomic DNA were included in each PCR reaction to avoid contamination.

Amplicons were visualized on a 2% agarose gel using ethidium bromide. The GENECLEAN Turbo kit (Q-BIOgene) was used to clean the PCR products. The amplicons were sequenced in a 3100 Genetic Analyzer (Applied Biosystems) and the resulting chromatograms were analyzed with Chromas 2.3 (Technelysium Pty Ltd). To confirm the results of the VNTR analysis, we used an electrophoretic method with a forward-labelled 6-FAM primer. The PCR products were separated using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) with TAMURA 500 as the size standard. Allele sizes were analyzed using Peak Scanner software (Applied Biosystems).

The GenBank accession numbers for the reference genomic sequences used for *PTGDR, CYSLTR1, LTC4S*, and *NOS2A* alignments were AL355833.4, AY242130, U62025, and AF440785, respectively.

Laboratory procedures were performed following the best practice guidelines of the European Molecular Genetics Quality Network. Specific quality measures were taken in all laboratory procedures beginning with sample reception and storage. Steps were taken to minimize the risk of contamination during the DNA extraction process. The number of tube-to-tube transfers was minimized, and ready-made solutions from commercial manufacturers were routinely used. Separate pre-PCR and post-PCR areas were established to minimize the risk of contamination, and safety cabinets and pipette filter tips were used. The sequencing method for genotyping samples was chosen because of its reliability. Controls and patients were genotyped in a single batch. The analysis was performed blind with respect to case and control status. Two independent scientists performed the analysis blind with respect to case and control status for *NOS2A*.

Statistical Analysis

Allele frequency for the 927T>C *CYSLTR1* polymorphism was analyzed taking into account that males provide only 1 copy of the X chromosome. The allele and genotype frequencies of patients were compared with those of controls. For each SNP, the χ^2 test (Pearson *P* value) was used to determine the Hardy–Weinberg equilibrium.

The dichotomous variables were analyzed using the χ^2 test and Fisher exact test with contingency tables for the distribution of categorical variables. A Monte Carlo simulation and a Bonferroni correction for multiple comparisons were applied when required. Analysis of variance was used to compare continuous variables across the levels of each genotype. Immunoglobulin E levels were transformed to $\log_{10}$ values to provide a normal distribution for the statistical analysis. Linear regression was used to model the effects of multiple covariates on continuous and dichotomous outcomes. In the multivariate analysis, age and sex were included as potential covariates. A *P* value of <.05 was considered statistically significant. All statistical analyses were performed using SPSS version 12.0 (SPSS Inc) and SHEsis software [28]. Diplotypes were analyzed using SNPAnalyzer software [29]. Statistical power and false-positive report probability were also calculated when required.

Results

Allele and Genotype Analysis

The phenotypic characteristics of the sample are summarized in Table 1. Allele and genotype frequencies for the different SNPs are presented in Tables 2 to 4. Given the genetic location of the SNP in chromosome X, the sample was split into 2 subgroups (males and females) in order to study the frequency of the SNP in *CYSLTR1*.

NOS2A was evaluated according to the number of repeats of (CCTTT)n, which ranged from 7 to 16 repeats, with 12 repeats being the most frequent allele in our population. The distribution of the VNTR in the study population is shown in Figure 1. The distribution of the *NOS2A* polymorphism between controls and patients with NP and between controls

Table 1. Characteristics of the Study Population[a]

	N	Age, y	Male, %	Female, %	IgE, $\log_{10}$	Positive SPT Result
Controls	245	48 (19)	37.1	62.9	1.55 (0.57)	0
NP	241	54 (15)	51.5	48.5	1.96 (0.54)	30.7
Asthma	145	56 (15)	39.3	60.7	2.00 (0.56)	32.4
NSAIDi	81	56 (15)	38.3	61.7	1.97 (0.57)	27.1
Aspirin triad	75	57 (14)	36	64	2 (0.53)	20

Abbreviations: Ig, immunoglobulin; NP, nasal polyposis; NSAIDi, nonteroidal anti-inflammatory drug intolerance; SPT, skin prick test.
[a]Data are presented as mean (SD) unless otherwise indicated.

Table 2. Allele and Genotype Frequency for Single-Nucleotide Polymorphisms in *PTGDR* According to the Different Subgroups

			Controls n=245	NP n=241	NP and Asthma n=144	NP and NSAIDi n=81	Aspirin Triad n=75
PTGDR–613 C>T	Genotype	CC	0.84	0.83	0.84	0.80	0.80
		CT	0.16	0.16	0.15	0.20	0.20
		TT	0.00	0.01	0.01	–	–
	Allele	C	0.92	0.91	0.91	0.90	0.90
		T	0.08	0.09	0.09	0.10	0.10
TGDR–549 T>C	Genotype	TT	0.23	0.20	0.20	0.27	0.25
		TC	0.51	0.50	0.51	0.46	0.49
		CC	0.26	0.30	0.29	0.27	0.26
	Allele	T	0.49	0.45	0.45	0.50	0.49
		C	0.51	0.55	0.55	0.50	0.51
PTGDR–441 C>T	Genotype	CC	0.60	0.66	0.66	0.63	0.65
		CT	0.35	0.30	0.30	0.30	0.29
		TT	0.05	0.04	0.04	0.07	0.06
	Allele	C	0.78	0.81	0.81	0.78	0.79
		T	0.22	0.19	0.19	0.22	0.21
PTGDR–197 C>T	Genotype	TT	0.80	0.75	0.74	0.78	0.76
		CT	0.16	0.22	0.25	0.22	0.24
		CC	0.04	0.03	0.01	–	–
	Allele[a]	T	0.88	0.86	0.87	0.89	0.88
		C	0.12	0.14	0.13	0.11	0.12

Abbreviations: NP, nasal polyposis; NSAIDi, nonsteroidal anti-inflammatory drug intolerance.
[a]Genotypic and allelic distributions for the SNPs were in Hardy-Weinberg equilibrium ($P>.05$) except for *PTGDR* –197T>C in controls ($P=.0007$). A second round of genotyping was performed to verify this finding.

Table 3. Allele and Genotype Frequency for the Single-Nucleotide Polymorphism in *LTC4S* According to the Different Subgroups

LTC4S–444 A>C		Genotype			Allele		HWE
	N	AA	AC	CC	A	C	*P* Value
Controls	245	0.59	0.33	0.08	0.76	0.24	.15
Polyposis	241	0.51	0.41	0.08	0.71	0.29	.97
Polyposis and asthma	145	0.48	0.43	0.09	0.69	0.31	.94
Aspirin triad	75	0.51	0.42	0.07	0.72	0.28	.71
Polyposis and atopy	74	0.41	0.49[a]	0.10	0.66	0.34[b]	.49

HWE, Hardy-Weinberg equilibrium
[a]OR, 2.62; 95% CI, 1.33-5.16; $P=.047$.
[b]OR, 0.61; 95% CI, 0.39-0.96; $P=.033$.

Table 4. Allele and Genotype Frequency for the Single-Nucleotide Polymorphism in *CYSLTR1* According to Sex

CYSLTR1		Female					Male	
		Genotype			Allele		Allele	
927 T>C	N (female/male)	TT	TC	CC	T	C	T	C
Controls	155/90	0.65	0.26	0.09	0.78	0.22	0.80	0.20
NP	117/90	0.65	0.26	0.09	0.78	0.22	0.81	0.19
NP and asthma	88/56	0.72	0.20	0.08	0.82	0.18	0.81	0.19
Aspirin triad	48/27	0.70	0.23	0.07	0.82	0.18	0.92	0.08

Abbreviations: NP, nasal polyposis.

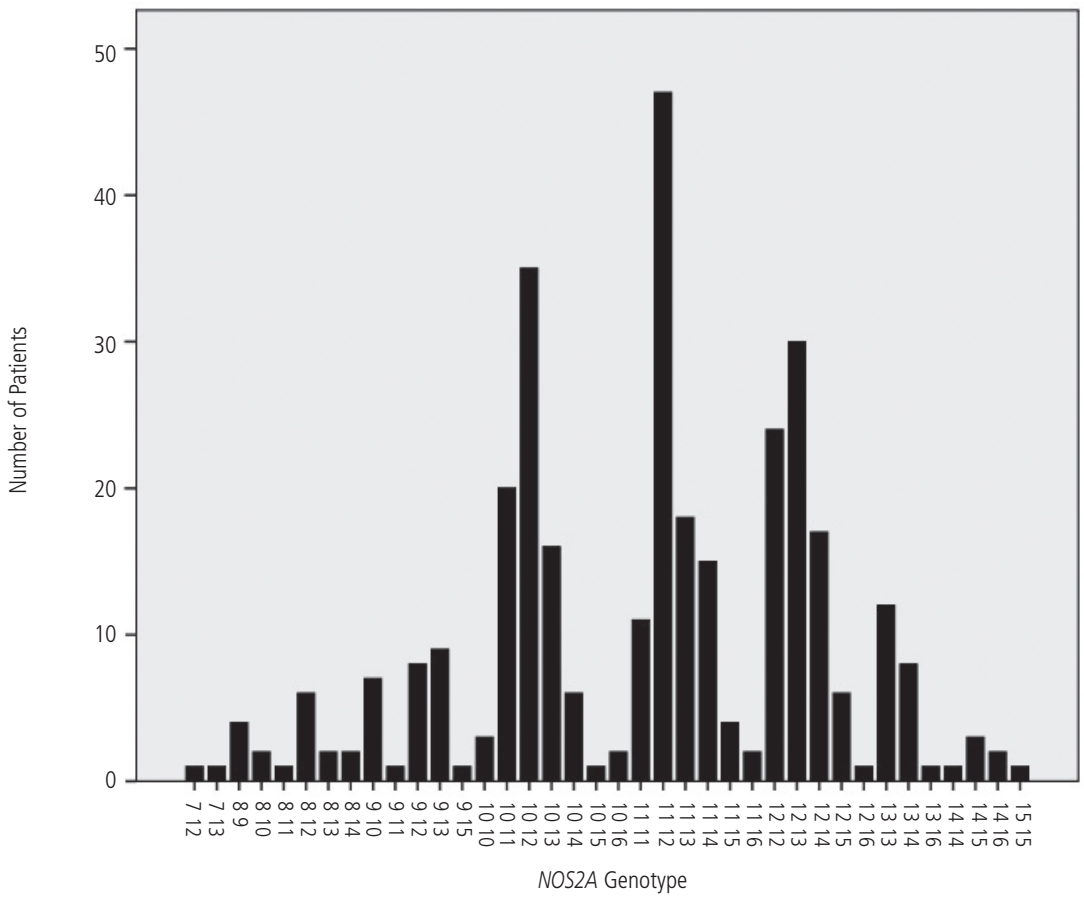

Figure 1. Overall distribution of (CCTTT)n repeats from *NOS2A* in the study population.

and aspirin-intolerant patients is shown in Figure 2 and Figure 3, respectively.

The *P* values for the allelic distributions of the (CCTTT) *NOS2A* repeat cluster are shown in Table 5. An association was observed for NP and atopy, and especially for NP and atopic asthma, when this VNTR was repeated more than 13 times (OR, 2.16; 95% CI, 1.12-4.16; *P*=.018). However, a significant association was found with more than 14 repeats in patients with aspirin intolerance (OR, 3.68; 95% CI, 1.31-10.36; *P*=.009) and in patients with the aspirin triad (OR, 0.25; 95% CI, 0.09-0.72; *P*=.005). Allele frequencies for the different repeats in the patient groups and in the healthy controls are presented in Table 6. Thresholds of repeat numbers 13 to 16 are represented and the results are expressed as less or more than the corresponding threshold.

The *LTC4S* –444A>C SNP was significantly associated with NP and atopy (*P*=.033) and with NP and atopic asthma (*P*=.012). The C allele appeared more frequently in patients with NP and atopy (OR 0.61; 95% CI, 0.39-0.96). The AC and CC genotypes shared the same association in these patients

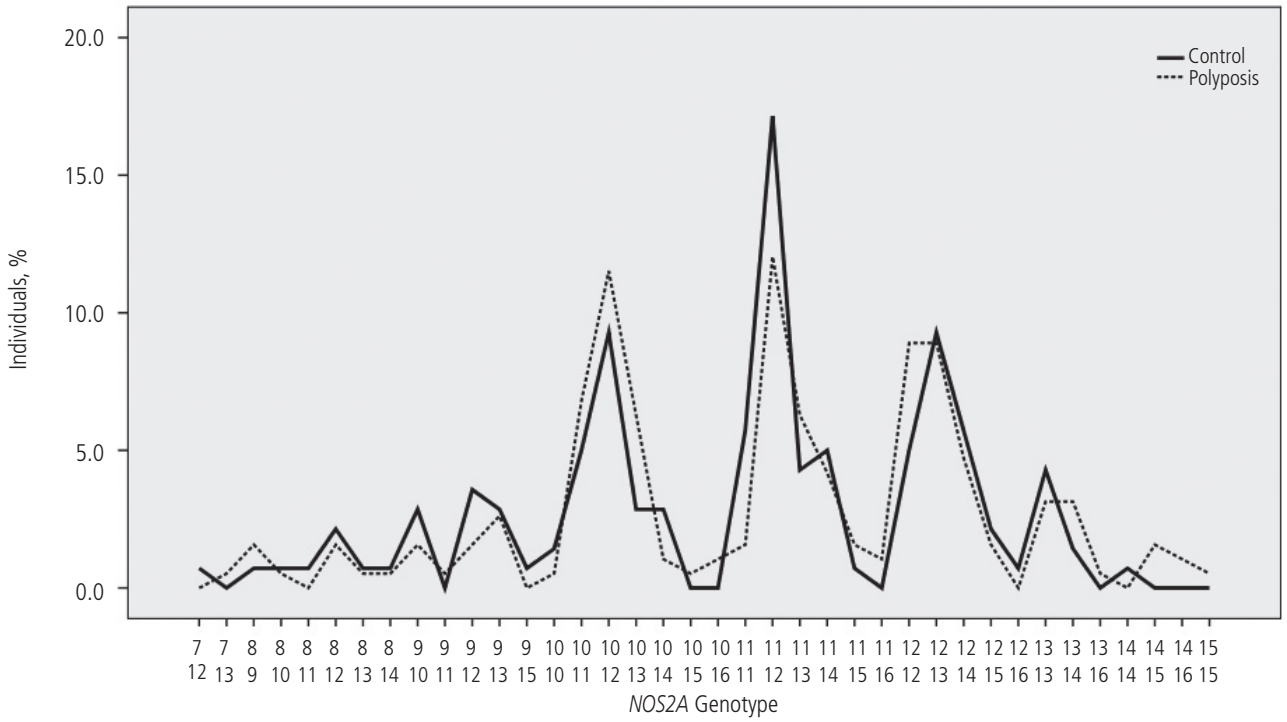

Figure 2. Distribution of *NOS2A* polymorphism between controls and patients with nasal polyposis.

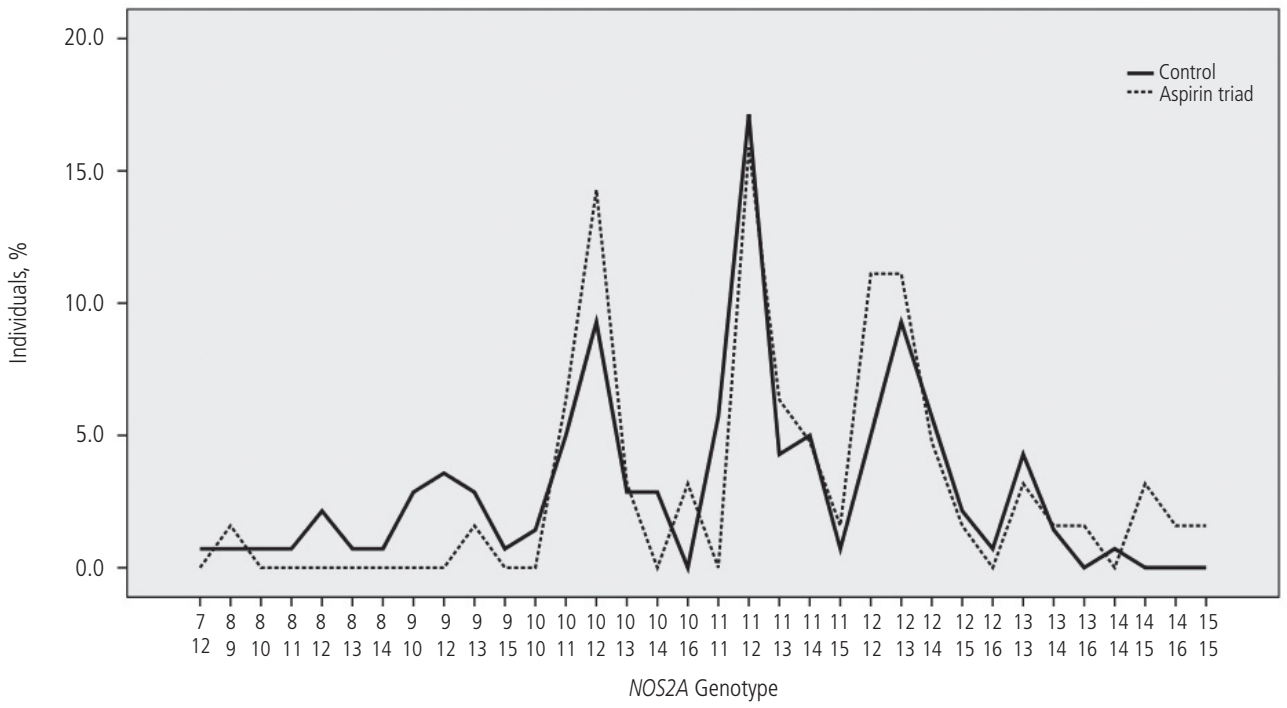

Figure 3. Distribution of *NOS2A* polymorphism between and controls and patients with the aspirin triad.

Table 5. *P* Values for Allele Distributions of the *NOS2A* Variable Number Tandem Repeat (CCTTT) According to the Different Subgroups Compared to the Control Group

(CCTTT)n Repeats	NP	NP and Asthma	NP and NSAIDi	NP and Aspirin Triad	NP and Atopy	NP and Atopic Asthma
Number of alleles	382	246	134	126	114	80
13	.167	.311	.175	.208	.175	.263
14	.209	.138	.127	.119	.051	.018
15	.059	.034	.009	.005	.044	.018
16	.086	.038	.022	.017	.148	.063

Abbreviations: NP, nasal polyposis; NSAIDi, nonsteroidal anti-inflammatory drug intolerance.

Table 6. Allele Frequencies for the Different Repeats in Patients and in Healthy Controls

	Control		NP		NP and Asthma		NP and NSAIDi		Aspirin Triad		NP and Atopy	
Th[a]	Less than	More than	Less than	More than	Less than	More than	Less than	More than	Less than	More than	Less than	More than
13	0.74	0.26	0.69	0.31	0.70	0.30	0.68	0.32	0.68	0.32	0.68	0.32
14	0.89	0.11	0.86	0.14	0.85	0.15	0.84	0.16	0.83	0.17	0.82	0.18[g]
15	0.98	0.02	0.95	0.05	0.94	0.06[b]	0.92	0.08	0.92	0.08[e]	0.94	0.06[h]
16	0.99	0.01	0.98	0.02	0.97	0.03[c]	0.97	0.03[d]	0.97	0.03[f]	0.98	0.02

Abbreviations: NP, nasal polyposis; NSAIDi, nonsteroidal anti-inflammatory drug intolerance.
[a]Thresholds (Th) of the repeat numbers are represented in the first column, and results are expressed as less or more than the corresponding threshold.
[b]OR, 0.36; 95% CI, 0.14-0.96; *P*=.034.
[c]OR, 0.14; 95% CI, 0.02-1.00; *P*=.038.
[d]OR, 0.12; 95% CI, 0.01-1.00; *P*=.021.
[e]OR, 0.25; 95% CI, 0.09-0.71; *P*=.005.
[f]OR, 0.11; 95% CI, 0.01-0.97; *P*=.017.
[g]OR, 0.55; 95% CI, 0.30-1.00; *P*=.05.

Table 7. Haplotype and Diplotype Frequencies for *PTGDR* Polymorphisms

		Control n=235	NP n=190	Aspirin Triad n=59	NP and Asthma n=117	NP and Atopy n=58
Haplotype	CCCC	0.113	0.136	0.110	0.127	0.069
	CCCT	0.313	0.325	0.288	0.335	0.362
	CTCT	0.260	0.262	0.297	0.267	0.293
	CTTT	0.221	0.188	0.203	0.186	0.164
	TCCT	0.081	0.089	0.102	0.081	0.112
Diplotype		Control n=141	NP n=142	NP and Aspirin Triad n=51	NP and Asthma n=90	NP and Atopy n=39
	CCCT CCCC	0.038	0.079[a]	0.119[b]	0.103[c]	0.034
	CTCT TCCT	0.034	0.058	0.102	0.068	0.069
	CCCT CTCT	0.199	0.189	0.186	0.171	0.276

Abbreviation: NP, nasal polyposis.
[a]OR, 2.44; 95% CI, 1.03 5.78; *P*=.043.
[b]OR, 3.16; 95% CI, 1.05 9.49; *P*=.041.
[c]OR, 3.17; 95% CI, 1.27 7.88; *P*=.013.

(OR, 2.62; 95% CI, 1.33-5.16; *P*=.047). This association was confirmed by binary logistic regression adjusted for age and sex (*P*=.018), particularly in the AC genotype, where the risk of having NP and atopy was 2.62 (95% CI, 1.33-5.16) (Table 3).

The *PTGDR* –197T>C SNP was associated with NP and asthma (*P*=.048); this association was confirmed when NP patients with asthma were compared with NP patients without asthma (*P*=.043). However, this association was not confirmed by logistic regression.

Haplotype and Diplotype Analysis

Table 7 shows the most representative haplotype combinations and their frequencies. The diplotype CCCT/CCCC (–613CC, –549CC, –441CC, and –197TC) of *PTGDR* was more frequent in patients with NP (OR, 2.44; 95% CI, 1.03-5.78; *P*=.043), NP with asthma (OR, 3.17; 95% CI, 1.27-7.88; *P*=.013), and NP with the aspirin triad (OR, 3.16; 95% CI, 1.05-9.49; *P*=.041).

Gene-Gene Interactions

Analysis of the interactions between the different SNPs revealed that the CTCT_C haplotype combination (*PTGDR* positions –613, –549, –441, and –197 and *LTC4S* position –444) was associated with NP (OR, 2.13; 95% CI, 1.06-4.28; *P*=.035) and NP with asthma (OR, 2.11; 95% CI, 1.02-4.37; *P*=.045). An increase in the diplotype combination CTCT_C/CCCT_A (–613CC, –549TC, –441CC, –197TT of *PTGDR* and –444AC of *LTC4S*) was detected in patients with NP and atopic asthma (*P*=.02). This association was confirmed by adjusted logistic regression (OR, 10.14; 95% CI, 1.93-53.31; *P*=.006).

Discussion

Although the pathogenesis of NP is unknown, some studies have reported familial aggregation and others have described an association with various SNPs [2,3,7,8]. In the present study, we evaluated several of the SNPs implicated in inflammatory pathways in patients with NP. We found no association between simple polyposis and the SNPs investigated. However, we did find a statistically significant association between NP and specific phenotypes. NP is an inflammatory disease with several underlying pathogenic mechanisms, as occurs in asthma. Thus, the clinical setting is different in NP patients with or without asthma and with or without atopy, even if differences in cytokine expression cannot be found [30]. The association between *PTGDR* variants such as the unique –197T>C polymorphism or the SNP (CCCT/CCCC) diplotype and NP with asthma could result from the previously described relationship between this gene and asthma [26,27,31]. In addition, the association between the –444A>C SNP and NP with atopy also seems to result from the atopic component in these patients. We also analyzed the genetic interactions or combinations of polymorphisms that might better explain the different clinical presentations of NP. Interestingly, we detected an association between the CTCT *PTGDR* haplotype and NP and asthma only when it co-occurred with the *LTC4S* SNP. As is the case in other diseases [26,32], most identified polymorphisms seem to have a slight effect on multifunctional diseases, and, when they co-occur, have a larger functional effect than individual variants. In this sense, the analysis of isolated *PTGDR* SNPs showed limited statistical power; however, association with the *LCT4* SNP showed a statistical power higher than 70% for a 0.05 alfa error and a false positive report probability of 8.7% for an a priori probability of 0.01. Therefore, a *P* value <.05 is not sufficient to indicate a positive association.

Regarding the (CCTTT) repeats in the *NOS2A* promoter, our results confirm the assumption that the number of repeats is associated with NP [20]. Previously, Kawaguchi et al [33] demonstrated that the (CCTTT)n polymorphism in the *NOS2* gene was associated with its transcriptional activity in inflammatory diseases, depending on the length of the repeats. Thus, in this study, when the (CCTTT)n polymorphism in the promoter region of *NOS2A* had more than 13 repeats, it seemed to be associated with the atopy phenotype, and when the (CCTTT)n VNTR had more than 15 repeats, it was associated with nonatopic asthma. These findings reinforce the role of polymorphic sequences (VNTRs) in complex multigenic traits. VNTRs control genes by influencing their transcriptional activity, resulting in phenotypes with functional differences that pave the way for pathogenesis. Nitric oxide (NO) is an important neurotransmitter and mediator of the inflammatory process, increasing transudation of serum from the vessels and glandular secretion, which leads to the edema that is typical of NP [34]. NOS2A is the main NO-producing enzyme in inflammation [35], synthesizing mostly total NO in the paranasal sinuses. Aberrant *NOS2A* gene expression is critical for the development of NP, and could depend on the number of (CCTTT) repeats [20]. We confirmed a relationship between the (CCTTT)n polymorphism in the promoter region of the *NOS2A* gene and intolerance to nonsteroidal anti-inflammatory drugs and the aspirin triad. In addition, previous studies found an association between increased *NOS2A* expression and NP [19,36]; however, these studies did not take into account the number of (CCTTT) repetitions detected in the promoter region of *NOS2A* or other polymorphisms.

In summary, we found a relationship between the (CCTTT)n polymorphism in the promoter region of *NOS2A* and NP. Although we did not detect any association between the polymorphisms of *PTGDR, LTC4S*, and *CYSLTR* that we investigated, our results highlight the importance of gene-gene interactions in genetic association studies, as we observed epistatic effects. In addition, our results show that the relevant role played by genetic features in NP could make it possible to identify patients with additional diseases.

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