

Association of the $-33C/G$ *OSF-2* and the $140A/G$ *LF* gene polymorphisms with the risk of chronic rhinosinusitis with nasal polyps in a Polish population

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Abstract Nasal polyps are strongly associated with a risk of chronic rhinosinusitis development as well as other obstruction including asthma and allergy. The following study tested the association of the $140A/G$ polymorphism of lactoferrin (*LF*) encoding gene and the $-33C/G$ polymorphism of osteoblast-specific factor-2 (*OSF-2*) encoding gene with a risk of chronic rhinosinusitis with nasal polyps in a Polish population. One hundred ninety five patients of chronic rhinosinusitis with nasal polyps as well as 200 sex, age and ethnicity matched control subjects without chronic sinusitis and nasal polyps were enrolled in this study. Among the group of patients 63 subjects were diagnosed with allergy and 65 subjects with asthma, respectively. DNA was isolated from peripheral blood lymphocytes of patients as well as controls and gene polymorphisms were analyzed by restriction fragments length polymorphism polymerase chain reaction (RFLP-PCR). We reported that the $140A/G$ *LF* (OR 4.78; 95% CI 3.07–7.24), the $-33C/G$ *OSF-2* (OR 3.48; 95% CI 2.19–5.52) and the $-33G/G$ *OSF-2* (OR 16.45; 95% CI 6.71–40.30) genotypes were associated with an increased risk of chronic rhinosinusitis with nasal polyps among analyzed group of patients. Moreover, the group of patients without allergy or asthma indicated

the association of the $-33C/G$ (OR 3.72; 95% CI 2.24–6.19 and OR 15.11; 95% CI 5.91–38.6) and $-33G/G$ (OR 3.73; 95% CI 2.24–6.19 and OR 14.07; 95% CI 5.47–36.16) genotypes of the *OSF-2* as well as $140A/G$ (OR 3.89; 95% CI 2.40–6.31 and OR 3.62; 95% CI 2.45–5.34) genotype of *OSF-2* with an increased risk of chronic rhinosinusitis with nasal polyps. Finally, it was also found that the selected group of patients with allergy or asthma indicated a very strong association of the $-33C/G$ (OR 2.40; 95% CI 1.23–4.69 and OR 2.40; 95% CI 1.23–4.69, respectively) and $-33G/G$ (OR 16.01; 95% CI 5.77–44.41 and OR 17.90; 95% CI 6.53–49.05, respectively) genotypes of the *OSF-2* as well as $140A/G$ (OR 3.22; 95% CI 1.74–6.11 and OR 3.25; 95% CI 1.75–6.04, respectively) genotypes with an increased risk of chronic rhinosinusitis with nasal polyps. Thus, our results suggest that *LF* and *OSF-2* gene polymorphisms may have deep impact on the risk of rhinosinusitis nasal polyps' formation which may also depend on asthma or allergy. Our results showed that the $140A/G$ polymorphism of *LF* gene and the $-33C/G$ polymorphism of the *OSF-2* gene may be associated with the risk of chronic rhinosinusitis with nasal polyps in a Polish population.

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Introduction

The latest years have shown a significant increase in the number of patients suffering from chronic rhinosinusitis and nasal polyps. Numerous studies indicate that this disease has an increasingly significant economic impact by reducing worker productivity and decreasing the quality of

life of people who have been affected by this disease [1, 2]. The chronic, treatment-resistant rhinosinusitis can often transform to polyps. The etiology of these objects is not fully explained. Growing polyps lead to the obstruction of the natural ostia of the sinuses. This in turn requires repeated use of antibiotics aimed at the treatment of the infections, as well as the steroid therapy designed to reduce the size of polyps. In advanced cases, the removal of the polyps and the restorage of sinus ventilation can only be possible by performing endoscopic surgery [3, 4]. The pathophysiology of nasal polyps is considered to be the ultimate manifestation of chronic inflammation of the upper respiratory tract of unknown etiology [5]. Histologically, polyps are characterized by squamous metaplasia of the respiratory epithelium, increased subepithelial fibrosis with an accumulation of extracellular matrix, associated with marked edema, dilated capillary, venous channels and eosinophile infiltrates [6]. To date, fibroblasts have been considered mainly a physical barrier but recent studies have shown their significance in the complex mechanism of polyp genesis. Airway nasal fibroblasts are major structural components of the mucosa and a rich source of cytokines and chemokines. Therefore, fibroblasts are thought to be involved in the local recruitment of inflammatory cells. Upon stimulation, nasal fibroblasts, via production of collagens and fibronectin, are known to promote extracellular matrix generation and tissue remodeling. This suggests that fibroblast play an important role in development of stromal abnormalities and thought may induce aberrations in the phenotype of epithelium [7]. Abnormal ECM components production by fibroblast in nasal polyps may depend on osteoblast-specific factor-2 (*OSF-2*) stimulation. *OSF-2* is the highly potent regulator of fibrosis and collagen deposition and its increase expression have been reported in nasal polyps [8, 9]. In the case of chronic rhinosinusitis with nasal polyps were also demonstrated excess fibroblast proliferation. The growth of fibroblasts derived from nasal polyps may inhibit by lactoferrin (*LF*), which represents one of the major host-derived antimicrobial peptides, predominantly expressed by surface epithelia in upper airway [10]. Reports on the expression and presence of the lactoferrin in nasal polyps tissue and nasal secretions are rare and have not consistently demonstrated an increase [9, 11] or decrease [12] in the lactoferrin level. Although, the results of this large study demonstrate that all subgroups of patients with chronic rhinosinusitis presented a down-regulation of lactoferrin in their nasal mucosa compared with healthy controls [13]. These findings suggest that *OSF-2* and *LF* may contribute to the pathogenesis of chronic rhinosinusitis with nasal polyps, however, their role are not fully elucidated. Therefore, this study aimed to investigate an association of the 140A/G (Lys/Arg) polymorphism of *LF* gene (rs1126478), and the

–33C/G polymorphism of *OSF-2* gene (rs 3829365) with a risk of chronic rhinosinusitis with nasal polyps in Polish population.

Materials and methods

Patients

Peripheral blood samples were obtained from 195 patients with chronic rhinosinusitis and nasal polyps (109 men and 86 women, median age 57, quartiles: 32, 74 years) treated during the study period (2009–2010) at the Department of Otolaryngology and Oncology of Medical University of Lodz in Poland. Among the group of 65 patients with chronic allergic rhinitis (sensitive to mites/Der. p., Der. f./63 subjects were diagnosed with mild controlled asthma. Symptoms of allergy in these patients have been present for 4–10 years. The patients were subjects to pharmacological treatment (inhaled steroids, antileukotrienes, antihistaminic drugs). Before blood samples collection each of the patients were clinically characterized according to physical examination, ENT examination, allergy skin test and point prick test. Two hundreds of sex, age and ethnicity matched control subjects without allergy, nasal polyps, chronic sinusitis and any other chronic inflammations within ENT diagnosed due to hearing impairment in the Department of Otolaryngology and Laryngological Oncology were enrolled in this study. All patients and controls were Caucasian. The protocol of the study was reviewed and approved by the Ethic Committee of the Medical University of Lodz in Poland.

Genotype determination

Genomic DNA was isolated from blood samples of patients and controls using the QIAamp DNA blood mini. Gene were analyzed by restriction length fragments of polymorphism PCR restriction fragments length polymorphism polymerase chain reaction (RFLP-PCR) was employed to analyze polymorphisms of *LF* and *OSF-2* gene. Each 20 µl of the PCR reaction contained 10 ng genomic DNA, 1.25 U Taq polymerase (Qiagen, Chatsworth, CA, USA) in 1 × PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 11 mM MgCl₂, 0.1% gelatin), 1.5 mM MgCl₂, 50 mM dNTPs, and 250 nM each primer. Thermal cycling conditions for the 140A/G polymorphism of *LF* gene were as follows: initial denaturation step at 95°C for 35 cycles, at 95°C for 30 s, at 56°C annealing temperature for 30 s, and at 72°C for 1 min. The final extension was performed at 72°C for 10 min. Thermal cycling conditions for the –33C/G polymorphism of *OSF-2* gene were as follows: initial denaturation step at 95°C for 5 min, 35 cycles at

94°C for 30 s, at 55°C annealing temperature for 30 s, and at 72°C for 1 min. The final extension was performed at 72°C for 7 min. The PCR was carried out in a MJ Research, INC thermal cycler, model PTC-100 (Waltham, MA, USA). The 140A/G polymorphism for *LF* was determined using the following primers (Sigma-Aldrich, St. Louis, MO, USA): sense, 5'-CTTACTCCTTGGCCCC TCTC-3'; antisense, 5'-TCTCCCTTCCATTTCAGCTTG-3'. The 239 bp PCR product was digested overnight with 5 U of the restriction enzyme *Mbo*II. The GG allele was digested into 139 and 100 bp fragments whereas the AA variant remained intact (Fig. 1). The -33C/G polymorphism was determined using the following primers:

sense, 5'-TCAGGTTGATGCAGTGTTCC-3';
antisense, 5'-CGACCCCTGATACGACTATG-3'.

The 228 bp product was digested overnight with 5 U of the restriction enzyme *Esr*I. The CC allele was digested into 143 and 85 bp fragments and the GG also remained intact (Fig. 2). Restriction fragments were separated on a 8% polyacrylamide gel and visualized by ethidium bromide staining. More than 10% of the samples were repeated, and the results were 100% concordant.

Data analysis

The allele frequencies were estimated by gene counting and genotypes were scored. The χ^2 test was used to compare the observed numbers of genotypes with those expected for a population in the Hardy–Weinberg equilibrium and to test the significance of the differences of observed alleles and genotypes between groups. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. In order to calculate the probability Pearson

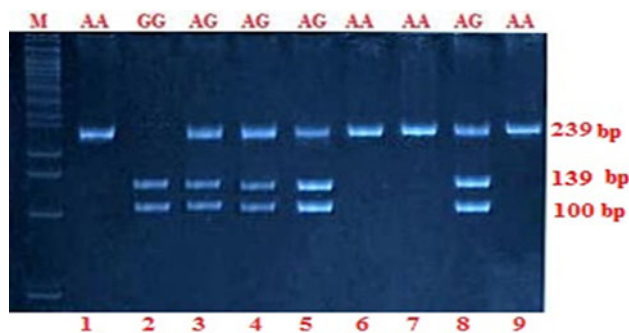


Fig. 1 Representative electrophoresis of restriction fragments length polymorphism-polymerase chain reaction (RLFP-PCR) analysis of the 140A/G polymorphism of the *LF* gene. Lane M DNA marker 50 bp, lanes 1,6,7,9 the A/A homozygote is not cleaved by *Mbo*II and remains a single 239-bp band; lane 2 the G/G homozygote is cleaved by *Mbo*II and yields a 139-bp; lanes 3,4,5,8 the A/G heterozygote contains both bands (139 and 100 bp) following restriction digestion. Digested PCR product were separated on 8% polyacrylamide gel

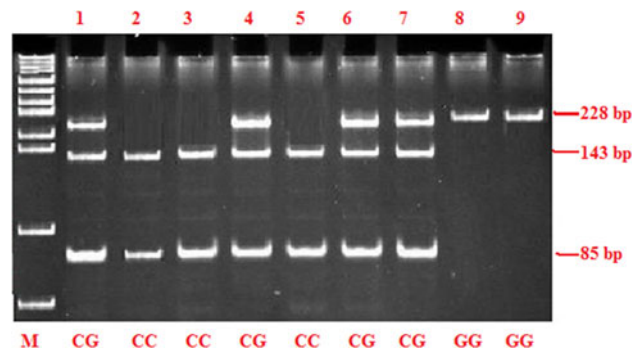


Fig. 2 Representative electrophoresis of restriction fragments length polymorphism-polymerase chain reaction (RLFP-PCR) analysis of the -33C/G polymorphism of the *OSF-2* gene. Lane M DNA marker 50 bp, lanes 8,9 the G/G homozygote is not cleaved by *Esr*I and remains a single 228-bp band; lane 2 the C/C homozygote is cleaved by *Esr*I and yields a 143-bp; lanes 1,4,6,7 the C/G heterozygote contains both bands (143 and 85 bp) following restriction digestion. Digested PCR product were separated on 8% polyacrylamide gel

correction was used, and if the expected cell values were less than 5 Fisher's exact test was used. A *P*-value of <0.05 was taken as statistically significant. The *t*-test (for normal distribution) or Mann–Whitney test (for non-normal distribution) was used to compare each parameter between two groups. An analysis of variance test was used to identify parameters that would make significant differences between more than two groups; Scheffe's test was then used to assess the significance of difference in each identified parameter between any two groups. STATISTICA 6.0 software (Statsoft, Tulsa, OK, USA) was used to perform analyses.

Results

The genotypes of patients and controls were scored according to 140A/G polymorphism of the *LF* gene, and -33C/G polymorphism of the *OSF-2* gene. All distributions in patients and control group did not differ significantly from those predicted by the Hardy–Weinberg equilibrium. The genotype distributions and allele frequencies of analyzed polymorphisms for *LF* gene and *OSF-2* gene in patients and controls are displayed in Table 1. It was shown an increased risk of chronic rhinosinusitis with nasal polyps occurrence in association with the A/G genotype of the *LF* gene (OR 4.78; 95% CI 3.07–7.24) and the C/G and G/G genotypes (OR 3.48; 95% CI 2.19–5.52; OR 16.45; 95% CI 6.71–40.30, respectively) of *OSF-2*. It was also observed the differences in the frequency of the G allele between the group of patients and controls for the 140A/G *LF* gene polymorphism (OR 4.27; 95% CI 2.99–6.11) and G allele between the group of patients and controls for the -33C/G *OSF-2* gene polymorphism (OR 4.56; 95% CI 3.24–6.41).

Table 1 Distribution of genotypes, frequency of alleles of the $-33C/G$ *OSF-2* and the $140A/G$ *LF* gene polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in rhinosinusitis with nose polyps patients and controls

Genotype or allele	Patients ($n = 195$)		Controls ($n = 200$)		OR (95% CI)	P value
	Number	Frequency	Number	Frequency		
140 A/G						
A/A	66	0.33	149	0.74	1.00 Ref.	
A/G	108	0.55	51	0.25	4.78 (3.07–7.24)	<0.001
G/G	21	0.1	0	0	–	
A	240	0.61	349	0.87	1.00 Ref.	
G	150	0.38	51	0.12	4.27 (2.99–6.11)	<0.001
$-33C/G$						
C/C	70	0.34	147	0.73	1.00 Ref.	
C/G	78	0.4	47	0.23	3.48 (2.19–5.52)	<0.001
G/G	47	0.24	6	0.03	16.45 (6.71–40.30)	<0.001
C	218	0.55	341	0.85	1.00 Ref.	
G	172	0.44	59	0.14	4.56 (3.24–6.41)	<0.001

CI confidence interval

The genotype distributions and allele frequencies of analyzed polymorphisms for *LF* gene and *OSF-2* gene in patients without allergy or asthma and group of healthy controls are displayed in Tables 2 and 3. The selected group of patients without allergy or asthma indicated the association of the $-33C/G$ (OR 3.87; 95% CI 2.39–6.26 and OR 3.89; 95% CI 2.40–6.31, respectively) and $-33G/G$ (OR 15.11; 95% CI 5.91–38.6 and OR 14.07; 95% CI 5.47–36.16, respectively) genotypes of the *OSF-2* as wells as $140A/G$ (OR 3.87; 95% CI 2.39–6.26 and OR 3.89; 95% CI 2.40–6.31) genotypes show an increased risk of chronic rhinosinusitis with nasal polyps. It was also observed the differences in the frequency of the G allele between the group of patients without allergy or asthma and group of healthy controls for the $140A/G$ *LF* gene polymorphism (OR 3.59; 95% CI 2.44–5.30 and OR 3.62; 95% CI 2.45–5.34, respectively). It was also observed the differences in the frequency of the C allele between the group of patients without allergy or asthma and group of healthy controls for the allele of the $-33C/G$ *OSF-2* (OR 4.39; 95% CI 3.03–6.34 and OR 4.23; 95% CI 2.92–6.13, respectively).

The genotype distributions and allele frequencies of analyzed polymorphisms for *LF* gene and *OSF-2* gene in patients with allergy or asthma and group of healthy controls are displayed in Tables 4 and 5. The selected group of patients with allergy or asthma indicated the association of the $-33C/G$ (OR 2.40; 95% CI 1.23–4.69 and OR 2.40; 95% CI 1.23–4.69, respectively) and $-33G/G$ (OR 16.01; 95% CI 5.77–44.41 and OR 17.90; 95% CI 6.53–49.05, respectively) genotypes of the *OSF-2* as wells as $140A/G$ (OR 3.22; 95% CI 1.74–6.11 and OR 3.25; 95% CI 1.75–6.04, respectively) genotypes with an increased risk of chronic rhinosinusitis with nasal polyps. It was also observed the differences in the frequency of the G allele between the group of patients with allergy or asthma and group of healthy controls for the $140A/G$ *LF* gene polymorphism (OR 4.21; 95% CI 2.64–6.69 and

OR 4.13; 95% 2.61–6.56, respectively). It was also observed the differences in the frequency of the G allele between the group of patients with allergy or asthma and group of healthy controls for the $-33C/G$ *OSF-2* polymorphism (OR 4.33; 95% CI 2.76–6.78 and OR 4.65 95% CI 2.99–7.24, respectively).

Gene–gene combined interactions analysis was displayed in Table 6. There was observed a significant association between the combined genotype A/A–C/G (OR 3.20; 95% CI 1.68–6.09), the A/A–G/G genotype (OR 21.04; 95% CI 7.46–59.31), the A/G–C/C genotype (OR 3.85; 95% CI 2.10–7.07), the A/G–C/G (OR 8.85; 95% CI 4.19–18.70) of the *LF* gene and *OSF-2* gene polymorphisms in chronic rhinosinusitis with nasal polyps. These genotype combinations may increase the risk of chronic rhinosinusitis with nasal polyps in Polish population.

The genotype distributions and allele frequencies of analyzed polymorphisms for *LF* gene and *OSF-2* gene in patients with adjustment for age, gender, and allergy or asthma and group of healthy controls are displayed in Table 7. The group of patients with age, gender, asthma or allergy indicated a very strong association of the $-33C/G$ (OR 3.39; 95% CI 2.13–5.44 and OR 3.54; 95% CI 2.08–6.02 and OR 3.55; 95% CI 2.10–6.03, respectively) and $-33G/G$ (OR 20.48; 95% CI 7.64–65.91 and OR 18.61; 95% CI 6.50–53.02 and OR 19.78; 95% CI 6.99–56.05, respectively), genotypes of the *OSF-2* as wells as $140A/G$ (OR 3.63; 95% CI 2.34–5.64 and OR 3.44; 95% CI 1.93–5.09 and OR 3.11; 95% CI 1.92–5.02, respectively).

Discussion

Nasal polyps are strongly associated with a risk of chronic rhinosinusitis development as well as other obstruction

Table 2 Distribution of genotypes, frequency of alleles of the $-33C/G$ *OSF-2* and the 140 *A/G* *LF* gene polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in rhinosinusitis patients with nose polyps without allergy and controls

Genotype or allele	Patients (<i>n</i> = 132)		Controls (<i>n</i> = 200)		OR (95% CI)	<i>P</i> value
	Number	Frequency	Number	Frequency		
140A/G						
A/A	52	0.39	149	0.74	1.00 Ref.	
A/G	69	0.52	51	0.25	3.87 (2.39–6.26)	<0.001
G/G	11	0.08	0	0	–	
A	173	0.65	349	0.87	1.00 Ref.	
G	91	0.34	51	0.12	3.59 (2.44–5.30)	<0.001
$-33C/G$						
C/C	47	0.35	147	0.73	1.00 Ref.	
C/G	56	0.42	47	0.23	3.72 (2.24–6.19)	<0.001
G/G	29	0.21	6	0.03	15.11 (5.91–38.6)	<0.001
C	150	0.56	341	0.85	1.00 Ref.	
G	114	0.43	59	0.14	4.39 (3.03–6.34)	<0.001

CI confidence interval

Table 3 Distribution of genotypes, frequency of alleles of alleles of the $-33C/G$ *OSF-2* and the 140A/G *LF* gene polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in rhinosinusitis patients with nose polyps without asthma and controls

Genotype or allele	Patients (<i>n</i> = 130)		Controls (<i>n</i> = 200)		OR (95% CI)	<i>P</i> value
	Number	Frequency	Number	Frequency		
140A/G						
A/A	51	0.39	149	0.74	1.00 Ref.	
A/G	68	0.52	51	0.25	3.89 (2.40–6.31)	<0.001
G/G	11	0.08	0	0	–	
A	170	0.65	349	0.87	1.00 Ref.	
G	90	0.34	51	0.12	3.62 (2.45–5.34)	<0.001
$-33C/G$						
C/C	47	0.36	147	0.73	1.00 Ref.	
C/G	56	0.43	47	0.23	3.73 (2.24–6.19)	<0.001
G/G	27	0.2	6	0.03	14.07 (5.47–36.16)	<0.001
C	150	0.57	341	0.85	1.00 Ref.	
G	110	0.42	59	0.14	4.23 (2.92–6.13)	<0.001

CI confidence interval

including asthma and allergy. It is suggested that *OSF-2* and *LF* might contribute to the pathogenesis of chronic rhinosinusitis with nasal polyps, however, their role are not fully elucidated. There is only little data concerning *OSF-2* expression in association with nasal polyps. Recently, a increase in *OSF-2* expression was demonstrated in chronic rhinosinusitis with polyp formation [9]. According to literature data, *OSF-2* contributes in fibroblast activation and selectively synergizes with basic fibroblast growth factor (FGF-2) to enhance syndecan-4 and osteopontin gene expression [14]. Engagement of syndecan-4 and osteopontin is essential for focal adhesion formation via certain integrins and for fibroblast proliferation and migration in response to FGF-2 stimulation [15]. This transformation of fibroblasts to proliferating, excessively matrix-producing cells is a critical feature of progressive tissue fibrosis [16]. This suggests that the deregulation of *OSF-2* expression may play a role in a risk of chronic rhinosinusitis with polyp formation. Furthermore, Stankovic et. al analyzed

gene expression profiling of nasal polyps associated with chronic sinusitis and aspirin-sensitive asthma by microarrays and has shown the increased expression of the particular genes in the tissue of nasal polyps in comparison to normal sinonasal mucosa. These results suggest that $-33C/G$ *OSF-2* and the 140A/G *LF* gene polymorphisms may play an important role in the pathogenesis of polyps associated with chronic rhinosinusitis and therefore may serve as attractive targets for novel medical therapies for this disease [24]. Our studies are the first one which reports the analysis of an association between the $-33C/G$ polymorphism of *OSF-2* gene with the nasal polyps development in patients with chronic rhinosinusitis. The $-33C/G$ polymorphism is located in the promoter region of *OSF-2* gene which may result in its expression level variation. We have shown that the $-33C/G$ (OR 3.48) and the $-33G/G$ (OR 16.45) genotypes of the *OSF-2* gene are strongly associated with an increased risk of the chronic rhinosinusitis with nasal polyps in the Polish patients.

Table 4 Distribution of genotypes, frequency of alleles of the $-33C/G$ *OSF-2* and the 140A/G *LF* gene polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in rhinosinusitis patients with nose polyps with allergy and controls

Genotype or allele	Patients ($n = 63$)		Controls ($n = 200$)		OR (95% CI)	<i>P</i> value
	Number	Frequency	Number	Frequency		
140A/G						
A/A	25	0.39	149	0.74	1.00 Ref.	
A/G	28	0.44	51	0.25	3.22 (1.74–6.11)	<0.001
G/G	10	0.15	0	0	–	
A	78	0.61	349	0.87	1.00 Ref.	
G	48	0.38	51	0.12	4.21 (2.64–6.69)	<0.001
$-33C/G$						
C/C	26	0.41	147	0.73	1.00 Ref.	
C/G	20	0.31	47	0.23	2.40 (1.23–4.69)	0.014
G/G	17	0.26	6	0.03	16.01 (5.77–44.41)	<0.001
C	72	0.57	341	0.85	1.00 Ref.	
G	54	0.42	59	0.14	4.33 (2.76–6.78)	<0.001

CI confidence interval

Table 5 Distribution of genotypes, frequency of alleles of alleles of the $-33C/G$ *OSF-2* and the 140A/G *LF* gene polymorphisms and odds ratio (OR) with 95% confidence interval (95% CI) in rhinosinusitis patients with nose polyps with asthma and controls

Genotype or allele	Patients ($n = 65$)		Controls ($n = 200$)		OR (95% CI)	<i>P</i> value
	Number	Frequency	Number	Frequency		
140A/G						
A/A	26	0.4	149	0.74	1.00 Ref.	
A/G	29	0.44	51	0.25	3.25 (1.75–6.04)	<0.001
G/G	10	0.15	0	0	–	
A	81	0.62	349	0.87	1.00 Ref.	
G	49	0.37	51	0.12	4.13 (2.61–6.56)	<0.001
$-33C/G$						
C/C	26	0.4	147	0.73	1.00 Ref.	
C/G	20	0.3	47	0.23	2.40 (1.23–4.69)	0.014
G/G	19	0.29	6	0.03	17.90 (6.53–49.05)	<0.001
C	72	0.55	341	0.85	1.00 Ref.	
G	58	0.44	59	0.14	4.65 (2.99–7.24)	<0.001

CI confidence interval

Table 6 The combined genotype and allele distribution and odds ratios (OR) of the $-33C/G$ *OSF-2* and the 140 A/G *LF* gene polymorphisms in rhinosinusitis patients with nose polyps and controls

Polymorphism genotype combination	Patients ($n = 195$) Number	Controls ($n = 200$) Number	OR (95% CI)	<i>P</i> value
140A/G –33C/G				
A/A–C/C	29	109	1.00 Ref.	
A/A–C/G	29	34	3.20 (1.68–6.09)	<0.001
A/A–G/G	28	5	21.04 (7.46–59.31)	<0.001
A/G–C/C	39	38	3.85 (2.10–7.07)	<0.001
A/G–C/G	33	14	8.85 (4.19–18.70)	<0.001
A/G–G/G	20	0	–	
G/G–C/C	4	0	–	
G/G–C/G	8	0	–	
G/G–G/G	5	0	–	

CI confidence interval

Despite lactoferrin abundance in airway secretions and its presumed importance as a first line defense against a broad spectrum of invading pathogens [17], the previous

study demonstrated that patients with chronic rhinosinusitis presented lower expression of lactoferrin at both the mRNA and protein level [12, 13]. Given its diverse

Table 7 Distribution of genotypes, frequency of alleles of the −33C/G *OSF-2* and the 140A/G *LF* gene polymorphisms and odds ratio (OR) with adjustment for age, gender, asthma or allergy in rhinosinusitis with nose polyps patients and controls

Genotype or allele	Patients (<i>n</i> = 195)		Controls (<i>n</i> = 200)		Crude	Adjusted ^a	Adjusted ^b	Adjusted ^c
	Number	Frequency	Number	Frequency	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
140A/G								
A/A	66	0.33	149	0.74	1.00 Ref	1.00 Ref	1.00 Ref	1.00 Ref
A/G	108	0.55	51	0.25	4.78 (3.07–7.24) <i>P</i> < 0.001	3.63 (2.34–5.64) <i>P</i> < 0.001	3.44 (1.93–5.09) <i>P</i> < 0.001	3.11 (1.92–5.02) <i>P</i> < 0.001
G/G	21	0.1	0	0	–	–	–	–
A	240	0.61	349	0.87	1.00 Ref	1.00 Ref	1.00 Ref	1.00 Ref
G	150	0.38	51	0.12	4.27 (2.99–6.11) <i>P</i> < 0.001	3.18 (2.06–4.90) <i>P</i> < 0.001	3.05 (1.88–4.93) <i>P</i> < 0.001	3.06 (1.90–4.95) <i>P</i> < 0.001
−33C/G								
C/C	70	0.34	147	0.73	1.00 Ref	1.00 Ref	1.00 Ref	1.00 Ref
C/G	78	0.3	47	0.23	3.48 (2.19–5.52) <i>P</i> < 0.001	3.39 (2.13–5.44) <i>P</i> < 0.001	3.54 (2.08–6.02) <i>P</i> < 0.001	3.55 (2.10–6.03) <i>P</i> < 0.001
G/G	47	0.24	6	0.03	16.45 (6.71–40.30) <i>P</i> < 0.001	20.48 (7.64–54.91) <i>P</i> < 0.001	18.61 (6.50–53.02) <i>P</i> < 0.001	19.78 (6.99–56.05) <i>P</i> < 0.001
C	218	0.55	341	0.85	1.00 Ref	1.00 Ref	1.00 Ref	1.00 Ref
G	172	0.44	59	0.14	4.56 (3.24–6.41) <i>P</i> < 0.001	2.10 (1.35–3.29) <i>P</i> = 0.001	2.32 (1.41–3.81) <i>P</i> < 0.001	2.27 (1.38–3.71) <i>P</i> = 0.001

Data in boldface is statistically significant, Ref. denote reference group i.e. group relative which ORs were calculated

^a Adjusted for age, gender in rhinosinusitis with nose polyps

^b Adjusted for age, gender, asthma in rhinosinusitis with nose polyps

^c Adjusted for age, gender, allergy in rhinosinusitis with nose polyps

antipathogenic activity, it also appears plausible that a decrease in the synthesis and production of lactoferrin in certain patients may also predispose them to chronic infections such as chronic rhinosinusitis [18]. It was also found that lactoferrin might inhibit proliferation of nasal polyps fibroblast by compete with bFGF for the cellular receptor [10, 19]. This indicated that lactoferrin may counteract to rhinosinusitis nasal polyps formation. This hypothesis is confirmed by the finding of lower levels of lactoferrin in patients with chronic rhinosinusitis and nasal polyps as compared to those without polyps [13]. However, there are no literature data concerning an association between polymorphisms of the *LF* gene and nasal polyps formation. Latest studies have revealed that the functional polymorphism of the 140A/G of *LF* gene might result in amino acid exchange (Lys > Arg) which affected protein function [20]. In our study we observed a statistically significant difference between patients of rhinosinusitis with nasal polyps and healthy controls in Polish population. Our study has demonstrated that the 140A/G genotype of *LF* may increase the risk of chronic rhinosinusitis with nasal polyps (OR 4.78). The high frequency of the G allele in patients group suggests that it may be involved into the

increase of their susceptibility to chronic rhinosinusitis with nasal polyps, which was verified in this study (OR 4.27).

Lactoferrin has been shown to reduce allergen-induced increases in cellular reactive oxygen species and to reduce the accumulation of eosinophils in the airways and subepithelium of intranasally challenged [21]. Moreover, lactoferrin inhibits eotaxin stimulated eosinophil migration [22]. It was also shown that orally administered *LF* protein decreased the eosinophil content in the circulating blood [23]. In our study we analyzed the correlation of the 140A/G polymorphism of *LF* gene as well as −33C/G polymorphism of *OSF-2* gene with a prevalence of allergies and asthma in patients with chronic rhinosinusitis and the risk of nasal polyps development. We evaluated, that the group of patients without allergy or asthma, still indicated the association of the −33C/G (OR 3.72 and 3.73) and −33G/G (OR 15.11 and 14.07) genotypes of the *OSF-2* as wells as 140A/G (OR 3.87 and 3.89) genotype of *OSF-2* with an increased risk of chronic rhinosinusitis with nasal polyps. The most interestingly, it was found that the selected group of patients with allergy or asthma indicated even stronger association of the −33G/G homozygote (OR 16.01 and

17.90) as well as the 140A/G heterozygote (OR 3.22 and 3.25) with an increased risk of chronic rhinosinusitis with nasal polyps than patients without allergy or asthma. Thus, our results suggest that *LF* and *OSF-2* gene polymorphisms may have deep impact on the risk of rhinosinusitis nasal polyps' formation which may also depend on asthma or allergy. We also analyzed the adjustment for multiple comparisons of the 140A/G polymorphism of *LF* gene as well as -33C/G polymorphism of *OSF-2* gene with age, gender and asthma or allergy in patients with chronic rhinosinusitis and the risk of nasal polyps development. We showed, that the patients with adjustment for age, gender and allergy or asthma, indicated the association of the -33C/G (OR 3.39, 3.54 and 3.55, respectively) and -33G/G (OR 20.48, 18.61 and 19.78, respectively), genotypes of the *OSF-2* as well as 140A/G (OR 3.63, OR 3.44 and 3.11, respectively) genotypes with an increased risk of chronic rhinosinusitis with nasal polyps.

Finally, we indicated that the combined genotypes of *LF* gene and *OSF-2* may influence the risk of chronic rhinosinusitis with nasal polyps. We found that the genotype interactions of the A/A-C/G (OR 3.20), the A/A-G/G (OR 21.04), the A/G-C/C (OR 3.85) and the A/G-G/G (OR 8.85) may increase the risk of chronic rhinosinusitis with nasal polyps. The results obtained in our work indicate that the 140A/G polymorphism of the *LF* gene and the -33C/G polymorphism of the *OSF-2* gene may be associated with the risk of chronic rhinosinusitis with nasal polyps in Polish population. Further in vitro studies are required to better understand the mechanism of nasal polyps formation in patients with chronic rhinosinusitis and the role of *LF* and *OSF-2* gene polymorphisms in their pathogenesis.

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Conflict of interest The authors have declared that no conflict of interest exists.

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