Association of the –14C/G *MET* and the –765G/C *COX-2* Gene Polymorphisms with the Risk of Chronic Rhinosinusitis with Nasal Polyps in a Polish Population

Przemysław Sitarek,¹ Hanna Zielinska-Blizniewska,² Łukasz Dzikowski,³ Jarosław Milonski,² Karolina Przybyłowska,¹ Bartosz Mucha,¹ Jurek Olszewski,² and Ireneusz Majsterek¹

Chronic rhinosinusitis with nasal polyps is strongly associated with other diseases, including asthma and allergy. The following study tested the association of the –765G/C polymorphism of cyclooxygenase-2 (*COX-2*) encoding gene and the –14C/G polymorphism of protooncogene *MET* (*MET*) 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 fragment length polymorphism–polymerase chain reaction (RFLP-PCR). Ten percent of the samples have been confirmed by a second method single-strand conformation polymorphism (SSCP)-PCR. We reported that the –765G/C *COX-2* (odds ratio [OR] 7.79; 95% confidence interval [CI] 4.88–12.4, $p < 0.001$) and the –14C/G *MET* (OR 2.83; 95% CI 1.74–4.61, $p < 0.001$) 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 –765G/C (OR 7.25; 95% CI 4.38–12.1, $p < 0.001$ and OR 7.61; 95% CI 4.47–12.6, $p < 0.001$) genotype of the *COX-2* as well as the –14C/G (OR 2.47; 95% CI 1.46–4.17, $p < 0.001$ and OR 2.59; 95% CI 1.54–4.37, $p < 0.001$) genotype of *MET* 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 –765G/C (OR 5.64; 95% CI 2.91–10.9 and OR 4.74; 95% CI 2.49–9.03, $p < 0.001$, respectively) genotype of the *COX-2* with an increased risk of chronic rhinosinusitis with nasal polyps. Thus, our results suggest that *COX-2* and *MET* gene polymorphisms may have deep impact on the risk of rhinosinusitis nasal polyp formation, which may also depend on asthma or allergy. Our results showed that the –765G/C polymorphism of *COX-2* gene and the –14C/G polymorphism of the *MET* gene may be associated with the risk of chronic rhinosinusitis with nasal polyps in a Polish population.

Introduction

THE PATHOPHYSIOLOGY of the chronic sinusitis (nasal polyps) is an inflammatory process involving the nasal mucosa and paranasal sinuses, a complex and multifactorial etiology. This is a group of conditions where the precise incidence in the population is difficult because of the variability of these disorders. Occurrence of nasal polyps in the United States is estimated at about 15.5% of the population, which places them in second place among the chronic diseases (31 million patients per year) (Collins *et al.*, 1997). The inflammatory process persists in the nasal polyps by more

than 12 weeks. About 20% of patients with this disease have nasal polyps (Settipane *et al.*, 1997). Chronic sinusitis with nasal polyps (65%–90% of cases) is often associated with the presence of eosinophilic infiltrates in the mucosa of the nose and paranasal sinuses. Mucosal eosinophilic inflammation is the ground on which easily arise, in particular, nasal polyps. Much less chronic rhinosinusitis with nasal polyps proceeds from the domination of the neutrophil infiltration in inflammatory mucosa of the nose and paranasal sinuses (Bachert *et al.*, 2003). Although the role of allergy in chronic rhinosinusitis remains controversial, it has been postulated that the swelling of the nasal mucosa in allergic rhinitis may

¹Department of Clinical Chemistry and Biochemistry, Medical University of Łódź, Łódź, Poland.

²Department of Otolaryngology and Oncology, Medical University of Łódź, Łódź, Poland.

³Department of General and Colorectal Surgery, Medical University of Łódź, Łódź, Poland.

restrict ventilation and obstruct the sinus ostia, leading to mucus retention and an infection (Fokkens *et al.*, 2005). Inflammation associated with allergic rhinitis and rhinosinusitis can reduce the physical size of the nasal passages by inducing vasodilatation, increasing blood flow and vascular permeability. Nasal polyps are found in about 36%–72% of patients with intolerance to nonsteroidal antiinflammatory drugs (aspirin triad: asthma, intolerance to nonsteroidal antiinflammatory drugs, and nasal polyps), 13% of patients with nonallergic asthma, 5% of people with allergic asthma, 20%–50% of patients with cystic fibrosis, and 85% of people with allergic fungal sinusitis. Nasal polyps also occur in patients with primary ciliary dyskinesia syndrome (Kartagener and Young syndrome) as well as in one-third of patients with nasal polyps, in which also occurs asthma or allergy, where asthma is associated with hypersensitivity to aspirin (Hamad *et al.*, 2004). Previous studies show that the chronic sinusitis, asthma, allergy, and nasal polyp formation may be associated with a specific expression of such factors as *COX-2* and *MET*. *COX-2* is induced during inflammation, and takes an active part in the synthesis of proinflammatory prostanooids. In patients with intolerance to aspirin, there are large amounts of eosinophils from both peripheral blood and lavage fluid from the nose, sinuses, and bronchial tree. However, the number of lymphocytes in these samples did not deviate from the norm. Additionally, recent studies indicate the ability of the *MET* receptor to induce the expression of the prostaglandin synthesis of *COX-2* (Lee *et al.*, 2004). Searches for genetic determinants of chronic sinusitis may contribute to the efficient selection of high-risk patients who are likely to develop nasal polyps. Finally, these studies are able to explore the still unexplained descent. Therefore, this study aimed to investigate an association of the –765G/C polymorphism of *COX-2* gene (rs20417) and the –14C/G polymorphism of *MET* gene (rs78116323) 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 and 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 subjected to pharmacological treatment (inhaled steroids, antileukotrienes, and antihistaminic drugs). Before blood sample collection, each of the patients was clinically characterized according to physical examination, ear, nose, throat (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 was analyzed by restriction fragment length polymorphism–polymerase chain reaction (RFLP-PCR) was employed to analyze polymorphisms of *COX-2* and *MET* genes. Each 20 μ L of the PCR reaction contained 10 ng genomic DNA, 1.25 U Taq polymerase (Qiagen, Chatsworth, CA) in 1 $\times$ PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 11 mM MgCl₂, and 0.1% gelatin), 1.5 mM MgCl₂, 50 mM dNTPs, and 250 nM each primer. Thermal cycling conditions for the –765G/C polymorphism of *COX-2* gene were as follows: initial denaturation step at 94°C for 35 cycles, at 94°C for 30 s, at 56°C annealing temperature for 30 s, and at 72°C for 30 s. The final extension was performed at 72°C for 5 min. Thermal cycling conditions for the –14C/G polymorphism of *MET* gene were as follows: initial denaturation step at 95°C for 6 min, 35 cycles at 95°C for 30 s, at 57°C annealing temperature for 30 s, and at 72°C for 1 min. The final extension was performed at 72°C for 8 min. The PCR was carried out in an MJ Research, Inc., thermal cycler, model PTC-100 (Waltham, MA). The –765G/C polymorphism of *COX-2* was determined using the following primers (Sigma–Aldrich, St. Louis, MO): sense, 5'-CATTAAGTATT TACAGGGTAACTGCTT-3'; antisense, 5'-TGCAGCACA TACATACATAGCTTTT-3'. Primers were designed in the Primer3plus program (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). The 238-bp PCR product was digested overnight with 5 U of the restriction enzyme *Ssi*I. The GG allele was digested into 168- and 70-bp fragments whereas the CC variant remained intact (Fig. 1). The –14C/G polymorphism was determined using the following primers: sense, 5'-GAGCGCCTCAGTCTGGTC-3; antisense,

FIG. 1. Representative restriction fragment length polymorphism–polymerase chain reaction (RFLP-PCR) analysis of the –765G/C polymorphism of the *COX-2* gene. Lane M: DNA marker 50 bp; lanes 1, 3, and 9: the G/G homozygote is not cleaved by *Ssi*I and remains a single 238-bp band; lanes 2, 5, and 6: the C/C homozygote is cleaved by *Ssi*I and yields a 168-bp band; lanes 4, 7, and 8: the G/C heterozygote contains both bands (168 and 70 bp) following restriction digestion. Digested PCR product was electrophoresed on 8% polyacrylamide gel containing ethidium bromide. *COX-2* RFLP-PCR band sizes are indicated on the right side of the panel.

FIG. 2. Representative RFLP-PCR analysis of the $-14C/G$ polymorphism of the *MET* gene. Lane M: DNA marker 100 bp; lanes 1, 4, and 9: the C/C homozygote is not cleaved by *Bsa*I and remains a single 290-bp band; lanes 3, 5, and 8: the G/G homozygote is cleaved by *Bsa*I and yields a 220-bp band; lanes 2, 6, and 7: the C/G heterozygote contains both bands (220 and 67 bp) following restriction digestion. Digested PCR product was electrophoresed on 8% polyacrylamide gel containing ethidium bromide. *MET* RFLP-PCR band sizes are indicated on the right side of the panel.

5'-CGCCTCCTCTCAGCAAGTC-3'. The 290-bp product was digested overnight with 5 U of the restriction enzyme *Bsa*II. The GG allele was digested into 220- and 67-bp fragments and the CC also remained intact (Fig. 2). Restriction fragments were separated on an 8% polyacrylamide gel and visualized by ethidium bromide staining. Blinded replicate samples were used for quality control of PCR-RFLP method. More than 10% of the samples were repeated by a second method single-strand conformation polymorphism (SSCP)-PCR, and the results were 100% concordant.

SSCP-PCR method

More than 10% of the samples were repeated by a second method SSCP-PCR, and the results were 100% concordant. The electrophoretic analysis was carried out on PhastSystem using PhastGel® Homogeneous 12.5 polyacrylamide gels and PhastGel Native Buffer Strips. Samples were applied using PhastGel Sample Applicator 8/1 μ L. The resulting PCR products were diluted in Elga water at 1/4 and mixed with an equal volume (2 μ L) of denaturing solution (99% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). The DNA samples were denatured at 95°C for 5 min followed by rapid cooling on ice. Running conditions were performed as follows: Prerun 100 V, 7 mA, 1.0 W, 18°C, and 10 Vh. Separation was performed under the following conditions: 200 V, 10 mA, 2.5 W, 18°C, and 75 Vh. The gels were stained using PhastGel DNA Silver Staining Kit according to the instructions (Figs. 3 and 4).

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. To calculate the probability, Pearson correction was used, and if the expected

FIG. 3. PCR-SSCP analysis of the *COX-2* gene. Mutated factors: 1, 2, 4, 6, and 7 (homozygous); normal factors: 3 and 5 (heterozygous).

cell values were less than 5, then Fisher's exact test was used. A p -value of <0.05 was taken as statistically significant. 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) was used to perform analyses.

Results

The genotypes of patients and controls were scored according to $-765G/C$ polymorphism of the *COX-2* gene and $-14C/G$ polymorphism of the *MET* 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 *COX-2* gene and *MET* gene in patients and controls are displayed in Table 1. It was shown that there is an increased risk of chronic rhinosinusitis with nasal polyp occurrence in association with the G/C genotype of the *COX-2* gene (OR 7.79; 95% CI 4.88–12.4, $p<0.001$) and the C/G genotype (OR 2.83; 95% CI 1.74–4.61, $p<0.001$, respectively) of the *MET* gene. It was also observed that there are differences in the frequency of the C allele between the group of patients and controls for the $-765G/C$ *COX-2*

FIG. 4. PCR-SSCP analysis of the *MET* gene. Mutated factors: 1, 2, 4, 5, and 7 (homozygous); normal factors: 3 and 6 (heterozygous).

TABLE 1. DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE -765G/C OF THE COX-2 GENE, AND THE -14C/G POLYMORPHISM OF THE MET GENE AND ODDS RATIO WITH 95% CONFIDENCE INTERVAL IN PATIENTS WITH CHRONIC RHINOSINUSITIS AND NOSE POLYPS AND CONTROLS

Genotype or allele	Patients (n=195)		Controls (n=200)		OR (95% CI)	p-Value
	Number	Frequency	Number	Frequency		
-765G/C						
G/G	56	0.28	158	0.79	1.00 Ref.	
G/C	116	0.59	42	0.21	7.79 (4.88–12.4)	$p < 0.001$
C/C	23	0.11	0	0	–	
G	228	0.58	358	0.88	1.00 Ref.	
C	162	0.41	42	0.10	6.05 (4.15–8.83)	$p < 0.001$
-14C/G						
C/C	98	0.50	165	0.82	1.00 Ref.	
C/G	59	0.30	35	0.17	2.83 (1.74–4.61)	$p < 0.001$
G/G	38	0.19	0	0	–	
C	255	0.65	365	0.91	1.00 Ref.	
G	135	0.34	35	0.08	5.52 (3.68–8.27)	$p < 0.001$

CI, confidence interval; OR, odds ratio.

Bold values indicate an increased risk of chronic rhinosinusitis with nasal polyps.

gene polymorphism (OR 6.05; 95% CI 4.15–8.83, $p < 0.001$) and the G allele between the group of patients and controls for the -14C/G MET gene polymorphism (OR 5.52; 95% CI 3.68–8.27, $p < 0.001$).

The genotype distributions and allele frequencies of analyzed polymorphisms for COX-2 gene and MET 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 -765C/G (OR 7.25; 95% CI 4.38–12.1, $p < 0.001$ and OR 7.61; 4.57–12.6, $p < 0.001$, respectively) genotypes of the COX-2 as well as -14C/G (OR 2.47; 95% CI 1.46–4.17, $p < 0.001$ and OR 2.59; 95% CI 1.54–4.37, $p < 0.001$) genotypes with an increased risk of chronic rhinosinusitis with nasal polyps. It was also observed that there are differences in the frequency of the C allele between the group of patients without allergy or asthma and group of healthy controls for the -765G/C COX-2 gene polymorphism (OR 5.11; 95% CI 3.41–7.67, $p < 0.001$

and OR 5.24; 95% CI 3.49–7.86, $p < 0.001$, respectively). It was also observed that there are differences in the frequency of the G allele between the group of patients without allergy or asthma and group of healthy controls for the allele of the -14C/G MET (OR 3.09; 95% CI 1.97–4.96, $p < 0.001$ and OR 3.19; 95% CI 2.03–5.01, $p < 0.001$, respectively).

The genotype distributions and allele frequencies of analyzed polymorphisms for COX-2 gene and MET 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 -765C/G (OR 5.64; 95% CI 2.91–10.9, $p < 0.001$ and OR 4.74; 95% CI 2.49–9.03, $p < 0.001$, respectively) genotypes of the COX-2 with an increased risk of chronic rhinosinusitis with nasal polyps. It was also observed that there are differences in the frequency of the C allele between the group of patients with allergy or asthma and group of healthy controls for the -765G/C COX-2 gene polymorphism (OR 6.81; 95%

TABLE 2. DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE -765G/C OF THE COX-2 GENE, AND THE -14C/G POLYMORPHISM OF THE MET GENE AND ODDS RATIO WITH 95% CONFIDENCE INTERVAL IN RHINOSINUSITIS PATIENTS WITH NOSE POLYPS WITHOUT ALLERGY AND CONTROLS

Genotype or allele	Patients (n=132)		Controls (n=200)		OR (95% CI)	p-Value
	Number	Frequency	Number	Frequency		
-765G/C						
GG	42	0.31	158	0.79	1.00 Ref.	
C/G	81	0.61	42	0.21	7.25 (4.38–12.1)	$p < 0.001$
CC	9	0.06	0	0	–	
G	165	0.62	358	0.88	1.00 Ref.	
C	99	0.37	42	0.10	5.11 (3.41–7.67)	$p < 0.001$
-14C/G						
C/C	80	0.61	165	0.82	1.00 Ref.	
C/G	42	0.32	35	0.17	2.47 (1.46–4.17)	$p < 0.001$
G/G	9	0.06	0	0	–	
C	202	0.77	365	0.91	1.00 Ref.	
G	60	0.22	35	0.08	3.09 (1.97–4.96)	$p < 0.001$

Bold values indicate an increased risk of chronic rhinosinusitis with nasal polyps.

TABLE 3. DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE -765G/C OF THE COX-2 GENE, AND THE -14C/G POLYMORPHISM OF THE MET GENE AND ODDS RATIO WITH 95% CONFIDENCE INTERVAL IN RHINOSINUSITIS PATIENTS WITH NOSE POLYPS WITHOUT ASTHMA AND CONTROLS

Genotype or allele	Patients (n=130)		Controls (n=200)		OR (95% CI)	p-Value
	Number	Frequency	Number	Frequency		
-765G/C						
GG	40	0.30	158	0.79	1.00 Ref.	
C/G	81	0.62	42	0.21	7.61 (4.57–12.6)	$p < 0.001$
CC	9	0.06	0	0	–	
G	161	0.61	358	0.88	1.00 Ref.	
C	99	0.38	42	0.10	5.24 (3.49–7.86)	$p < 0.001$
-14C/G						
C/C	78	0.60	165	0.82	1.00 Ref.	
C/G	43	0.33	35	0.17	2.59 (1.54–4.37)	$p < 0.001$
G/G	9	0.06	0	0	–	
C	199	0.76	365	0.91	1.00 Ref.	
G	61	0.23	35	0.08	3.19 (2.03–5.01)	$p < 0.001$

Bold values indicate an increased risk of chronic rhinosinusitis with nasal polyps.

CI 4.20–10.9, $p < 0.001$ and OR 6.25; 95% 3.89–10.2, $p < 0.001$, respectively). It was also observed that there are differences in the frequency of the G allele between the group of patients with allergy or asthma and group of healthy controls for the -14C/G MET polymorphism (OR 9.48; 95% CI 5.79–15.5, $p < 0.001$ and OR 8.93; 95% CI 5.48–14.5, $p < 0.001$, respectively). Gene–gene combined interaction analysis was displayed in Table 6. A significant association was observed between the combined genotypes G/C-C/G (OR 2.53; 95% CI 1.20–5.33, $p = 0.021$) and G/C-G/G (OR 4.07; 95% CI 1.97–8.40, $p < 0.001$) of the COX-2 gene and MET gene polymorphisms in chronic rhinosinusitis with nasal polyps. These genotype combinations may increase the risk of chronic rhinosinusitis with nasal polyps in Polish population.

Discussion

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

struction, including asthma and allergy. It is suggested that COX-2 and MET might contribute to the pathogenesis of chronic rhinosinusitis with nasal polyps; however, their role is not fully elucidated. The functional variant polymorphism of -765G/C in the COX-2 gene has been described (Vane *et al.*, 1998; Fritsche *et al.*, 2001; Huuskonen *et al.*, 2008; Hoff *et al.*, 2009) and related with several pathologies, such as colorectal cancer risk (Brown *et al.*, 2005), breast cancer (Langsenlehner *et al.*, 2006; Shen *et al.*, 2006), coronary artery disease (Linton *et al.*, 2004), head and neck cancer (Peters *et al.*, 2009), prostate cancer (Shahedi *et al.*, 2006), and lung cancer (Yuan *et al.*, 2005; Grimminger *et al.*, 2009). It was demonstrated by Papafili *et al.* that -765C allele had lower COX-2 promoter activity than the -765G allele (Ishibashi *et al.*, 1998). Kristinsson *et al.* reported an association of the presence of the COX-2-765G/C genotype and a higher risk of esophageal adenocarcinoma and squamous cell carcinoma (Rho *et al.*, 2006). Sitarz *et al.* (2008) showed that the COX-2-765G allele promoter

TABLE 4. DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE -765G/C OF THE COX-2 GENE, AND THE -14C/G POLYMORPHISM OF THE MET GENE AND ODDS RATIO WITH 95% CONFIDENCE INTERVAL IN PATIENTS WITH NOSE POLYPS WITH ALLERGY AND CONTROLS

Genotype or allele	Patients (n=63)		Controls (n=200)		OR (95% CI)	p-Value
	Number	Frequency	Number	Frequency		
-765G/C						
GG	20	0.31	158	0.79	1.00 Ref.	
C/G	30	0.47	42	0.21	5.64 (2.91–10.9)	$p < 0.001$
CC	13	0.20	0	0	–	
G	70	0.55	358	0.88	1.00 Ref.	
C	56	0.44	42	0.10	6.81 (4.20–10.9)	$p < 0.001$
-14C/G						
C/C	27	0.42	165	0.82	1.00 Ref.	
C/G	12	0.19	35	0.17	2.09 (0.96–4.53)	$p < 0.001$
G/G	24	0.38	0	0	–	
C	66	0.52	365	0.91	1.00 Ref.	
G	60	0.47	35	0.08	9.48 (5.79–15.5)	$p < 0.001$

Bold values indicate an increased risk of chronic rhinosinusitis with nasal polyps.

TABLE 5. DISTRIBUTION OF GENOTYPES AND FREQUENCY OF ALLELES OF THE -765G/C OF THE COX-2 GENE, AND THE -14C/G POLYMORPHISM OF THE MET GENE AND ODDS RATIO WITH 95% CONFIDENCE INTERVAL IN RHINOSINUSITIS PATIENTS WITH NOSE POLYPS AND ASTHMA AND CONTROLS

Genotype or allele	Patients (n=65)		Controls (n=200)		OR (95% CI)	p-Value
	Number	Frequency	Number	Frequency		
-765G/C						
GG	23	0.35	158	0.79	1.00 Ref.	
C/G	29	0.44	42	0.21	4.74 (2.49–9.03)	$p < 0.001$
CC	13	0.20	0	0	–	
G	75	0.57	358	0.88	1.00 Ref.	
C	55	0.42	42	0.10	6.25 (3.89–10.2)	$p < 0.001$
-14C/G						
C/C	29	0.44	165	0.82	1.00 Ref.	
C/G	12	0.18	35	0.17	1.95 (0.90–4.19)	$p < 0.001$
G/G	24	0.36	0	0	–	
C	70	0.53	365	0.91	1.00 Ref.	
G	60	0.46	35	0.08	8.93 (5.48–14.5)	$p < 0.001$

Bold values indicate an increased risk of chronic rhinosinusitis with nasal polyps.

polymorphism is significantly associated with gastric cancer (Horton *et al.*, 1999). Other reports have suggested a significant genetic association between variants in COX-2 and prostate cancer risk in South African men (Fernandez-Morata *et al.*, 2000). Increased genetic risk between COX-2 variants and prostate cancer has been shown in African American (Mullol *et al.*, 2002; Roca-Ferrer *et al.*, 2006) and Nigerian men (Mullol *et al.*, 2002). The other study that was found by Xing *et al.* (2008) showed that the COX-2-765G/C polymorphism is not associated with an increased risk of colorectal cancer (CRC), whereas -765GG genotype appears to be related to an increased risk in the presence of smoking and higher body mass index. In contrast, Tan *et al.* obtained contradictory positive results that the increased risk for CRC was associated with the COX-2-765G/C genotype in Chinese population (Coste *et al.*, 1996). Moreover, Zhang *et al.* (2005) reported that the -765GC genotype was associated with an increased risk of esophageal squamous cell carcinoma and CRC in Chinese population (Weidner *et al.*, 1993). Xing *et al.* (2008) showed that the COX-2-765G/

C allele promoter polymorphism is significantly associated with colorectal cancer risk (Bachert *et al.*, 2003). In addition, the -765GC and 765CC genotypes correlate with decreased risk of myocardial infarct and stroke and decreased COX-2 expression in atherosclerotic plaques compared with -765GG (Coste *et al.*, 2000). In a Dutch study, Brosens *et al.* (2005) reported that the polymorphism -765G/C of COX-2 gene was significantly higher in the normal-appearing mucosa of patients with familial adenomatous polyposis (Peterson *et al.*, 1988). However, Huuskonen *et al.* (2008) demonstrated that the functional C variant of the -765G/C polymorphism in the human COX-2 gene associates with the severity of coronary atherosclerosis measured at the coronary artery level in Finnish population (Bachert *et al.*, 2003). Little data evidenced an association between nose polyps and this polymorphism. Our study has demonstrated that G/C variant of the COX-2-765G/C polymorphism may increase the risk of nose polyps.

Recently, an increase in COX-2 expression was demonstrated in chronic rhinosinusitis with polyp formation

TABLE 6. THE COMBINED GENOTYPE AND ALLELE DISTRIBUTION AND ODDS RATIOS OF THE -765G/C POLYMORPHISM OF THE COX-2 GENE AND THE -14C/G POLYMORPHISM OF THE MET GENE IN PATIENTS WITH CHRONIC RHINOSINUSITIS AND NOSE POLYPS AND CONTROLS

<i>Polymorphism</i>	<i>Patients (n=195)</i>	<i>Controls (n=200)</i>	OR (95% CI)	<i>p-Value</i>
<i>Genotype combination</i>	<i>Number</i>	<i>Number</i>		
−765G/C−14C/G				
G/G-G/G	31	65	1.00 Ref.	<i>p</i> =0.021
G/G-C/G	23	19	2.53 (1.20–5.33)	
G/G-C/C	11	0		
C/C-C/C	6	0	–	
C/C-C/G	5	0	–	
C/C-G/G	7	0	–	
G/C-C/C	13	0	–	
G/C-C/G	33	17	4.07 (1.97–8.40)	<i>p</i> <0.001
G/C-G/G	66	99	1.39 (0.82–2.37)	<i>p</i> =0.267

Bold values indicate an increased risk of chronic rhinosinusitis with nasal polyps.

(Mullol *et al.*, 2002; Rho *et al.*, 2006; Roca-Ferrer *et al.*, 2006). According to literature data, a key phenomenon of this specific type of chronic inflammatory disease in nasal respiratory mucosa is remarkable edema. Vascular permeability/vascular endothelial growth factor (VPF/VEGF) plays an important role in inducing angiogenesis and modulating capillary permeability. The markedly increased expression in nasal polyps, as opposed to healthy nasal mucosa, suggests that VPF/VEGF may play a significant role in both the formation of nasal polyps and in the induction of heavy tissue edema. This finding is discussed with respect to the differential expression of cyclooxygenase isoenzyme-2 (COX-2) in nasal polyps; where in the group of patients the expression of COX-2 in nasal mucosa was significantly stronger than in specimens of healthy nasal mucosa of controls (Gosepath *et al.*, 2005). COX-2 is expressed in nose polyps, and as prostanoids are known to control chemotaxis of inflammatory cells, vascular tone, vascular permeability, and mucous secretion (all hallmarks of nose polyps) (Horton *et al.*, 1999; Fernandez-Morata *et al.*, 2000), their possible involvement in the pathogenesis of nose polyps has long been studied. As COX-2 is presumed to be induced under pro-inflammatory conditions, most studies focused on the role of this enzyme in the pathogenesis of nasal polyposis. This suggests that the deregulation of COX-2 expression may play a role in the risk of chronic rhinosinusitis with polyp formation. Our study is the first one that reports the analysis of an association between the -765G/C polymorphism of COX-2 gene with the nasal polyp development in patients with chronic rhinosinusitis. The -765G/C polymorphism is located in the promoter region of COX-2 gene, which may result in its expression level variation. We have shown that the -765G/C (OR 7.79) genotype of the COX-2 gene is strongly associated with an increased risk of the chronic rhinosinusitis with nasal polyps in the Polish patients. We did not find an association between the -765G/C polymorphism of the COX-2 gene and a prevalence of chronic rhinosinusitis with nasal polyps in the group of patients with allergy or asthma; however, the selected group of patients without allergy or asthma still indicated association of the -765G/C genotypes of the COX-2 gene with an increased risk of the chronic rhinosinusitis with nasal polyps. This indicates that COX-2 may have an influence on rhinosinusitis nasal polyps' formation that is not depending on asthma or allergy. The expression of various growth factors in nasal polyps has been examined, and several of these have been implicated in the pathogenesis, formation, and growth of polyps (Coste *et al.*, 1996; Ishibashi *et al.*, 1998; Petruson *et al.*, 1988; Coste *et al.*, 2000). Hepatocyte growth factor (HGF), also known as scatter factor, is a pleiotropic polypeptide growth factor with several biological activities, including epithelial cell proliferation, stimulation of cell motility, mitogenesis, morphogenesis, angiogenesis, and cellular invasiveness (Weidner *et al.*, 1993; Balkovetz *et al.*, 1999). In addition, HGF plays a key role in directing the integrated phases of wound repair, which consists of inflammation, epithelialization, formation of granulation tissue, and tissue remodeling (Wilson *et al.*, 1999; Cowin *et al.*, 2001; Toyoda *et al.*, 2001). These diverse biological effects of HGF are mediated via interaction with the transmembrane tyrosine kinase receptor *MET*. HGF and its receptor *MET* have been identified in various neoplastic tissue types

(Weidner *et al.*, 1993; Balkovetz *et al.*, 1999). In this respect, HGF and c-MET may play a role in the development of nasal polyps. Further, it has been hypothesized that HGF might play an important role in regulating the regeneration of the nasal epithelia (Puchelle *et al.*, 2000). Therefore, it is assumed that HGF and *MET* levels in normal nasal mucosa can be modulated by injury and by factors associated with wound healing. However, there are no literature data concerning an association between the -14C/G polymorphism of the *MET* gene and nasal polyp formation. The biological effect of this polymorphism is yet to be elucidated and will be important to investigate. The -14C/G polymorphism is located in the promoter region of the *MET* gene and could affect transcription efficiency, leading to altered product levels, which could influence the biological function of HGF dependent on interaction with the receptor *MET*. 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 -14C/G genotype of *MET* may increase the risk of chronic rhinosinusitis with nasal polyps (OR 2.83). The high frequency of the G allele in the patient group suggests that it may be involved in the increase of their susceptibility to chronic rhinosinusitis with nasal polyps, which was verified in this study (OR 5.52).

In our study we analyzed the correlation of the -765G/C polymorphism of COX-2 gene as well as -14C/G polymorphism of *MET* gene with a prevalence of allergies and asthma in patients with chronic rhinosinusitis and the risk of nasal polyp development. We evaluated that the group of patients without allergy or asthma still indicated the association of the -765G/C (OR 7.6, $p < 0.001$ and OR 7.25, $p < 0.001$) genotype of the COX-2 as well as -14C/G (OR 2.47, $p < 0.001$ and OR 2.59, $p < 0.001$) genotype of *MET* with an increased risk of chronic rhinosinusitis with nasal polyps. Most interestingly, it was found that the selected group of patients with allergy or asthma indicated stronger association of the -765G/C heterozygote (OR 5.64, $p < 0.001$ and OR 4.74, $p < 0.001$) with an increased risk of chronic rhinosinusitis with nasal polyps than patients without allergy or asthma. Thus, our results suggest that COX-2 and *MET* gene polymorphisms may have deep impact on the risk of rhinosinusitis nasal polyps' formation, which may also depend on asthma or allergy.

Finally, we indicated that the combined genotypes of COX-2 gene and *MET* may influence the risk of chronic rhinosinusitis with nasal polyps. We found that the genotype interactions of the G/C-C/G (OR 2.53, $p = 0.021$), and the G/C-G/G (OR 4.07, $p < 0.001$) may increase the risk of chronic rhinosinusitis with nasal polyps. The results obtained in our work indicate that the -765G/C polymorphism of the COX-2 gene and the -14C/G polymorphism of the *MET* 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 polyp formation in patients with chronic rhinosinusitis and the role of COX-2 and *MET* gene polymorphisms in their pathogenesis.

Conclusions

The present study showed that the -765G/C polymorphism of COX-2 gene and the -14C/G polymorphism of

MET gene may result in an increased risk of developing nose polyps in chronic rhinosinusitis in the Polish population. We have shown that the combined genotypes of the *COX-2* and *MET* genes can modulate the risk of nose polyps in chronic rhinosinusitis in the Polish population. In accordance with our knowledge, this is the first report showing an association between *COX-2* and *MET* gene polymorphisms with susceptibility to nose polyps in chronic rhinosinusitis among the Polish population. To clarify the pathogenesis of nose polyps in chronic rhinosinusitis, further studies are required.

Acknowledgments

This work was supported by the grant N N402 422138 from Polish Ministry of Science and Higher Education as well as Higher Education and Young Scientists grants No. 502-03/7-124-04/502-54-020 and No. 502/03/5-120-02/502-54-012 from Medical University of Lodz.

Disclosure Statement

The authors have declared that no conflict of interest exists.

References

- Bachert, C., van Zele, T., Gevaert, P., De Schrijver, L., and Van Cauwenberge, P. (2003). Superantigens and nasal polyps. *Curr Allergy Asthma Rep* 3, 523–531.
- Balkovetz, D.F., and Lipschutz, J.H. (1999). Hepatocyte growth factor and the kidney: it is not just for the liver. *Int Rev Cytol* 186, 225–260.
- Brosens, L.A., Iacobuzio-Donahue, C.A., Keller, J.J., Hustinx, S.R., Carvalho, R., Morsink, F.H., *et al.* (2005). Increased cyclooxygenase-2 expression in duodenal compared with colonic tissues in familial adenomatous polyposis and relationship to the -765G → C *COX-2* polymorphism. *Clin Cancer Res* 11, 4090–4096.
- Brown, J.R., and DuBois, R.N. (2005). *COX-2*: a molecular target for colorectal cancer prevention. *J Clin Oncol* 23, 2840–2855.
- Collins, J.G. (1997). Prevalence of selected chronic conditions: United States, 1990–1992. *Vital Health Stat* 194, 1–89.
- Coste, A., Brugel, L., and Maitre, B. (2000). Inflammatory cells as well as epithelial cells in nasal polyps express vascular endothelial growth factor. *Eur Respir J* 15, 367–372.
- Coste, A., Wang, Q.P., and Roudot-Thoraval, F. (1996). Epithelial cell proliferation in nasal polyps could be up-regulated by platelet-derived growth factor. *Laryngoscope* 106, 578–583.
- Cowin, A.J., Kallincos, N., Hatzirodos, N., Robertson, J.G., Pickering, K.J., Couper, J., *et al.* (2001). Hepatocyte growth factor and macrophage-stimulating protein are upregulated during excisional wound repair in rats. *Cell Tissue Res* 306, 239–250.
- Fernandez-Morata, J.C., Mullol, J., Fuentes, M., Pujols, L., Roca-Ferrer, J., Perez, M., *et al.* (2000). Regulation of cyclooxygenase-1 and -2 expression in human nasal mucosa. Effects of cytokines and dexamethasone. *Clin Exp Allergy* 30, 1275–1284.
- Fokkens, W., Lund, V., Bachert, C., Clement, P., Hellings, P., Holmstrom, M., *et al.* (2005). EAACI position paper on rhinosinusitis and nasal polyps executive summary. *Allergy* 60, 583–601.
- Fritsche, E., Baek, S.J., King, L.M., Zeldin, D.C., Eling, T.E., and Bell, D.A. (2001). Functional characterization of cyclooxygenase-2 polymorphisms. *J Pharmacol Exp Ther* 299, 468–476.
- Gosepath, J., Brieger, J., Lehr, H.A., and Mann, W.J. (2005). Expression, localization, and significance of vascular permeability/vascular endothelial growth factor in nasal polyps. *Am J Rhinol* 19, 7–13.
- Grimminger, P.P., Stohlmacher, J., Vallbohmer, D., Schneider, P.M., Holscher, A.H., Metzger, R., *et al.* (2009). Prognostic Significance and Clinicopathological Associations of *COX-2* SNP in Patients with Nonsmall Cell Lung Cancer. *J Oncol* 2009, 139590.
- Hamad, A.M., Sutcliffe, A.M., and Knox, A.J. (2004). Aspirin-induced asthma: clinical aspects, pathogenesis and management. *Drugs* 64, 2417–2432.
- Hoff, J.H., te Morsche, R.H., Roelofs, H.M., van der Logt, E.M., Nagengast, F.M., and Peters, W.H. (2009). *COX-2* polymorphisms -765G → C and -1195A → G and colorectal cancer risk. *World J Gastroenterol* 15, 4561–4565.
- Horton, J.K., Williams, A.S., Smith-Phillips, Z., Martin, R.C., and O'Beirne G. (1999). Intracellular measurement of prostaglandin E2: effect of anti-inflammatory drugs on cyclooxygenase activity and prostanoid expression. *Anal Biochem* 271, 18–28.
- Huuskonen, K.H., Kunnas, T.A., Tanner, M.M., Mikkelsen, J., Ilveskoski, E., Karhunen, P.J., *et al.* (2008). *COX-2* gene promoter polymorphism and coronary artery disease in middle-aged men: the Helsinki sudden death study. *Mediators Inflamm* 2008, 289453.
- Ishibashi, T., Tanaka, T., Nibu, K., Ishimoto, S., and Kaga, K. (1998). Keratinocyte growth factor and its receptor messenger RNA expression in nasal mucosa and nasal polyps. *Ann Otol Rhinol Laryngol* 107, 885–890.
- Kristinsson, J.O., van Westerveld, P., te Morsche, R.H., Roelofs, H.M., Wobbes, T., Witteman, B.J., *et al.* (2009). Cyclooxygenase-2 polymorphisms and the risk of esophageal adenoma or squamous cell carcinoma. *World J Gastroenterol* 15, 3493–3497.
- Langsenlehner, U., Yazdani-Biuki, B., Eder, T., Renner, W., Wascher, T.C., Paulweber, B., *et al.* (2006). The cyclooxygenase-2 (PTGS2) 8473T > C polymorphism is associated with breast cancer risk. *Clin Cancer Res* 12, 1392–1394.
- Liu, Z., Kim, J., Sypek, J.P., Wang, I.M., Horton, H., Oppenheim, F.G., Bochner, B.S. (2004). Gene expression profiles in human nasal polyps tissues studied by means of DNA microarray. *J Allergy Clin Immunol* 114, 783–790.
- Linton, M.F., and Fazio, S. (2004). Cyclooxygenase-2 and inflammation in atherosclerosis. *Curr Opin Pharmacol* 4, 116–123.
- Mullol, J., Fernández-Morata, J.C., Roca-Ferrer, J., Pujols, L., Xaubet, A., Benitez, P., and Picado, C. (2002). Cyclooxygenase 1 and cyclooxygenase 2 expression is abnormally regulated in human nasal polyps. *J Allergy Clin Immunol* 109, 824–830.
- Papafili, A., Hill, M.R., Brull, D.J., McNulty, R.J., Marshall, R.P., Humphries, S.E., *et al.* (2002). Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol* 22, 1631–1636.
- Peters, W.H., Lacko, M., Te Morsche, R.H., Voogd, A.C., Oude Ophuis, M.B., and Mann, J.J. (2009). *COX-2* polymorphisms and the risk for head and neck cancer in white patients. *Head Neck* 31, 938–943.
- Petruson, B., Hansson, H.A., and Petruson, K. (1988). Insulin-like growth factor I is a possible pathogenic mechanism in nasal polyps. *Acta Otolaryngol* 106, 156–160.

- Puchelle, E. (2000) Airway epithelium wound repair and regeneration after injury. *Acta Otorhinolaryngol Belg* **54**, 263–270.
- Rho, H.S., Lee, S.H., Lee, H.M., Jung, H.H., Choi, J., Park, M.K., *et al.* (2006). Overexpression of hepatocyte growth factor and its receptor c-Met in nasal polyps. *Arch Otolaryngol Head Neck Surg* **132**, 985–989.
- Roca-Ferrer, J., Pujols, L., Gartner, S., Moreno, A., Pumarola, F., Mullol, J., *et al.* (2006). Upregulation of COX-1 and COX-2 in nasal polyps in cystic fibrosis. *Thorax* **61**, 592–596.
- Settipane, G.A., Lund, V.J., Bernstein, J.M., and Tos, M. (1997). Epidemiology of nasal polyps. In: *Nasal Polyps: Epidemiology, Pathogenesis and Treatment*. G.A. Settipane, V.J. Lund, J.M. Bernstein, and M. Tos, eds. (OceanSide Publications, Inc., Providence, Rhode Island), pp. 17–24.
- Shahedi, K., Lindstrom, S., Zheng, S.L., Wiklund, F., Adolfsson, J., Sun, J., *et al.* (2006). Genetic variation in the COX-2 gene and the association with prostate cancer risk. *Int J Cancer* **119**, 668–672.
- Shen, J., Gammon, M.D., Terry, M.B., Teitelbaum, S.L., Neugut, A.I., and Santella, R.M. (2006). Genetic polymorphisms in the cyclooxygenase-2 gene, use of nonsteroidal anti-inflammatory drugs, and breast cancer risk. *Breast Cancer Res* **8**, R71.
- Sitarz, R., Leguit, R.J., de Leng, W.W., Polak, M., Morsink, F.M., Bakker, O., *et al.* (2008). The COX-2 promoter polymorphism –765 G>C is associated with early-onset, conventional and stump gastric cancers. *Mod Pathol* **21**, 685–690.
- Tan, W., Wu, J., Zhang, X., Guo, Y., Liu, J., Sun, T., *et al.* (2007). Associations of functional polymorphisms in cyclooxygenase-2 and platelet 12-lipoxygenase with risk of occurrence and advanced disease status of colorectal cancer. *Carcinogenesis* **28**, 1197–1201.
- Toyoda, M., Takayama, H., Horiguchi, N., Otsuka, T., Fukusato, T., Merlino, G., *et al.* (2001). Overexpression of hepatocyte growth factor/scatter factor promotes vascularization and granulation tissue formation *in vivo*. *FEBS Lett* **509**, 95–100.
- Vane, J.R., Bakhle, Y.S., and Botting, R.M. (1998). Cyclooxygenases 1 and 2. *Annu Rev Pharmacol Toxicol* **38**, 97–120.
- Weidner, K.M., Hartmann, G., Sachs, M., and Birchmeier, W. (1993). Properties and functions of scatter factor/hepatocyte growth factor and its receptor c-Met. *Am J Respir Cell Mol Biol* **8**, 229–237.
- Wilson, S.E., Liang, Q., and Kim, W.J. (1999). Lacrimal gland HGF, KGF, and EGF mRNA levels increase after corneal epithelial wounding. *Invest Ophthalmol Vis Sci* **40**, 2185–2190.
- Xing, L.L., Wang, Z.N., Jiang, L., Zhang, Y., Xu, Y.Y., Li, J., *et al.* (2008). Cyclooxygenase 2 polymorphism and colorectal cancer: –765G>C variant modifies risk associated with smoking and body mass index. *World J Gastroenterol* **14**, 1785–1789.
- Yuan, A., Yu, C.J., Shun, C.T., Luh, K.T., Kuo, S.H., Lee, Y.C., *et al.* (2005). Total cyclooxygenase-2 mRNA levels correlate with vascular endothelial growth factor mRNA levels, tumor angiogenesis and prognosis in non-small cell lung cancer patients. *Int J Cancer* **115**, 545–555.
- Zhang, X., Miao, X., Tan, W., Ning, B., Liu, Z., Hong, Y., *et al.* (2005). Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. *Gastroenterology* **129**, 565–576.

Address correspondence to:

Ireneusz Majsterek, Ph.D.

Department of Clinical Chemistry and Biochemistry

Medical University of Lodz

Hallera 1

Lodz 90-647

Poland

E-mail: ireneusz.majsterek@umed.lodz.pl

Received for publication September 19, 2011; received in revised form February 7, 2012; accepted February 7, 2012.