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Transcriptional activity of genes-encoding kinin B1 and B2 receptors and kinin-dependent genes in nasal polyps

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ABSTRACT

Purpose: The pro-inflammatory effects of kinins are mediated by two bradykinin receptors: BR1 and BR2. The aim of this study was to evaluate the expression profile of kinin receptor genes by an estimation of mRNA levels in human nasal polyps (NP) and normal mucosa (NM).

Material and Methods: BR1 and BR2-dependent genes differentially transcribed in NP were investigated using oligonucleotide microarray technology. The mRNA copy number of *BR1*, *BR2* and *TIMP1* genes was assessed by QRT-PCR. Thirty six eosinophilic (ENP), 17 neutrophilic nasal polyps (NNP) and 28 NM samples were included into the study.

Results: Among 92 genes encoding proteins involved in signal transduction via B1 and B2 kinin receptors *TIMP1* was found to be 2,63-fold higher in the NP than in NM. Increased *TIMP1* gene expression was proved by QRT-PCR (p=0,003). Moreover two genes: *FOS* and *PTGS1* presented higher (3,82- and 4,27-fold, respectively) expression in NM compared to NP tissues. In QRT-PCR analysis insignificantly higher expression of gene encoding *BR1* in ENP [2564 mRNA copies/μg RNA (22-32863)] compared with NM [1426 copies mRNA (15-27995)] was found. mRNA expression for the *BR2* in ENP [9872 copies mRNA (19-244832)] was insignificantly higher than in NM [5753 copies (46-199658)]. *BR2* mRNA was the predominant transcript in most NP and NM samples followed by *BR1* mRNA ($p<0,01$). There was a positive correlation between the expression of *BR1* and *BR2* in the ENP ($r=0,91$; $p<0,01$) and NNP ($r=0,6$; $p<0,01$).

Conclusions: We did not document any changes in the expression profile of kinin receptors in the analyzed groups, which may suggest that kinin receptors do not make an important contribution in the etiology of NP.

Key words: kinin receptors, nasal polyps, real-time QRT-PCR, oligonucleotide microarray, tissue inhibitor of matrix metalloproteinase-1 (TIMP1)

INTRODUCTION

Although the pathogenesis of nasal polyps (NP) is not fully understood, the disease is considered to be a chronic inflammation process of complex character [1]. NP formation is regulated through the release of a wide spectrum of inflammatory mediators and cytokines. The activation of the kallikrein-kininogen cascade generating the kinin peptides may be a part of this process. Kinins belong to the group of 9–11 amino acid peptides including bradykinin (BK), kallidin, T-kinin and their active metabolites, e.g. des-Arg9-kinins. They are important mediators produced in blood and tissues like nasal epithelium and are involved in both the initiation and progression of an inflammatory response. Kinins exert their biological effects through the activation of two receptors, denoted as subtypes B1 and B2. Both belong to the superfamily of seven-transmembrane G-protein coupled receptors [2]. Activation of the kinin receptors modulates release of a variety of proinflammatory mediators such as: eikozanoids, histamine, platelet-activating factor (PAF), interleucine 1 (IL-1), tumor necrosis factor (TNF), serotonin and enhanced NO synthesis [3]. In such a signal transduction pathway many genes encoding kinin-dependent proteins [i.e. prostaglandin-endoperoxide synthase (PTGS), FOS, RASs, tissue inhibitors of matrix

Table 1. **Comparison of patient data in two groups.**

		Nasal polyp patients	Control group	p
mean age [y]		51,2 ± 15,2	36,4 ± 16,8	0,0006*
sex	females	21	9	0,507#
	males	32	21	

*U Mann-Whitney test; # χ^2 test

metalloproteinase (TIMPs) and others] could be involved. The expression of B1 receptors (BR1) is tightly regulated and physiologically absent or at very low level in normal tissue. In response to pathophysiological processes like tissue inflammation or damage, BR1 expression can be unregulated [4-7]. Thus, B1 receptors may be candidate as therapeutic targets upstream in the cascade in airway inflammation. In contrast, kinin B2 receptor (BR2) is expressed constitutively in many cell types under normal conditions.

The induction of bradykinin receptors has been strongly linked to inflammatory pathologies including chronic bronchial asthma or allergic rhinitis [8]. Although it is quite well-recognized that kinin receptors play an important role in the process of human airway inflammation [9], their potential role in nasal polyp formation is unknown.

The aim of the current study was to compare the expression profile of kinin receptors on the mRNA levels in human nasal polyps and normal controls. Oligonucleotide microarray technology was used to investigate the transcriptional activity of kinin-dependent genes in the nasal polyps.

MATERIAL AND METHODS

Subjects

Fifty-three patients (21 females and 32 males) with nasal polyps treated surgically at the Department of Otolaryngology, Wroclaw Medical University were included in the study. All the subjects met the diagnostic criteria for chronic rhinosinusitis as established by the Task Force on Rhinosinusitis (AAO-HNS) [10]. The patients' ages ranged from 12 to 82 years (mean 51,2 ± 15,2 years). The extent of the disease was assessed by CT and also endoscopically. Patients had been free of any medication for at least 4 weeks before surgery and had bilateral polyps in the nasal cavities on endoscopic examination. The presence of comorbidity or smoking history was also excluded. The control group consisted of 28 healthy persons (9 females and 19 males). The patients' ages ranged from 17 to 76 years (mean 36,4 ± 16,8 years) *(Tab. 1)*. Absence of NP was assessed by clinical history, endoscopic examination and imaging. Prick tests were performed to rule out the existence of allergy. A history of other diseases was also excluded. Control tissue samples were taken from unchanged nasal mucosa of the lower turbinate during septoplasty.

For DNA microarray analysis 21 patients with NP (14 males and 7 females; mean age 50,82 ± 16,37 years) from the above group were included; the control samples were taken from 8 healthy people (6 males and 2 females; mean age 32,43 ± 13,29 years).

Nasal polyp specimens and control mucosae were immediately frozen in liquid nitrogen, and stored at -70°C pending further investigations. A part of each sample was fixed in 10% buffered neutral formalin, processed routinely, and embedded in paraffin wax for subsequent immunohistochemical examination. The study was approved by the Local Ethical Committee of Wroclaw Medical University.

Histological Examination

Serial sections of paraffin-embedded samples were stained with hematoxylin-eosin to visualize inflammatory cells and to exclude other pathologies. Five visual fields per section were examined to calculate the mean percentage of eosinophils out of the total inflammatory cells. NPs were classified as eosinophilic when eosinophils comprised more than 8.0% of inflammatory cells, and as neutrophilic when eosinophils comprised less than 8.0% of the inflammatory cells. Non-eosinophilic polyps show characteristic counterparts including profound glandular hypertrophy, fibrosis, and the presence of lymphocytes, neutrophils, and plasma cells.

According to this criterion 36 (68%) cases were classified as eosinophilic and 17 (32%) as neutrophilic. Eleven patients with eosinophilc and 6 with neutrophilc NP were subjected to microarray analysis.

RNA extraction

Total RNA was extracted from frozen tissue specimens (about 40 mg) with the use of TRIzol® reagent (Invitrogen, Carlsbad, CA) according to the producer's protocol. Tissues were homogenized in Trizol using a Tissues Homogenizer Polytron® (Kinematyka AG, Switzerland). All RNA extracts were treated with DNA-se I and cleaned with a Mini-spin column using an Rneasy Mini Total RNA Purification kit (Qiagen, Valencia, CA).

The RNA extracts were qualitatively checked by electrophoresis in 1,0% agarose gel stained with ethidium bromide. RNA concentration was determined on the basis of absorbance values at a wavelength of 260 nm using a GeneQuant pro (Biochrom, Cambridge, UK).

Microarray procedure

Gene expression profiles were determined using commercially available oligonucleotide microarrays HG-U133A (Affymetrix, Santa Clara, CA) according to the manufacturer's recommendations. Each gene chip contains 22238 probe sets

Table 2. **Characteristics of primers used for amplification.**

Gene	GenBank	Sequence of primers	Length of amplicon (bp)	TM (ºC)
BR1	NM_000710	Forward 5'CTGCACAGAGTGCTGCCAACATT3'	150	82
		Reverse: 5'ACACCAGATCAGAGGCTGCCAGG3'		
BR2	NM_000623	Forward:5'CACGGTGCTAGTCCTGGTTGTGCT3'	313	84
		Reverse: 5'AGGTCCGCAGTGTGCCCATG 3'		
TIMP1	NM_003254	Forward:5'GCCATGGAGAGTGTCTGCGGATACTTCC3'	118	86
		Reverse: 5'GCCACAAAACTGCAGGTAGTGATGT3'		

bp – base pair; TM - temperature of melting

that correspond to more than 18400 transcripts and 14500 well-characterized human genes. About 8 μg of total RNA were used for the cDNA synthesis using SuperScript Choice System (Gibco BRL Life Technologies). In the next step, cDNA were used as a template to produce biotin-labeled cRNA using BioArray HighYield RNA Transcript Labeling Kit (Enzo Life Sciences). cRNA was purified on Rneasy Mini Kit columns (Qiagen). Next, the biotin-labeled cRNA were fragmented using Sample Cleanup Module (Qiagen) and hybridized with the HG-U133A microarray (Affymetrix). The hybridized cRNA probes to oligonucleotide arrays were stained with streptavidin-phycoerythrin conjugate and were scanned using GeneArray Scanner G2500A. The scanned data were processed for signal values using Microarray Suite 5.0 software (Affymetrix). The results obtained were normalized using RMAExpress software.

Real-time quantitative RT-PCR

Transcriptional activity of *BR1*, *BR2* and *TIMP1* genes was identified by the use of real-time QRT-PCR technique with a SYBR Green I chemistry (SYBR Green Quantitect RT-PCR Kit, Qiagen, Valencia, CA, USA). Analysis was carried out using an Opticon™ DNA Engine Sequence Detector (MJ Research, USA). Primers for *BR1*, *BR2* and *TIMP1* were designed using Primer Express 1.0 software (PE Applied Biosystems, USA) *(Tab. 2)*. Oligonucleotide primers specific for mRNAs *BR1* and *BR2* were described previously [11]. The thermal conditions for one-step RT-PCR were as follows: 50˚C for 30 min for reverse transcription and 95˚C for 15 min, 50 cycles at 94˚C for 15 s, 60˚C for 30 s and 72˚C for 30 s. After completion of the cycle process, the samples were subjected to temperature ramp from 60 to 95˚C at a rate of 0,2˚C/s with continuous fluorescence monitoring from melting curve analysis. Glyceraldehydre-3-phosphate dehydrogenase (GAPDH), and β-actin were used as internal controls in each single QRT-PCR for all samples. All samples were analyzed in triplicate. Standards, from 400 to 8000 copies cDNA β-actin (TaqMan® DNA Template Reagents Kit, PE Applied Biosystems) were run alongside the samples to generate a standard curve. β-actin and GAPDH were used to monitor RT-PCR reaction and to exclude the presence of its inhibitors. The specifics of RT-PCR reaction was confirmed by determining characteristic temperature of melting for each amplimer. The RT-PCR products were separated on 6% polyacrylamide (PAA) gels and visualized with silver salts.

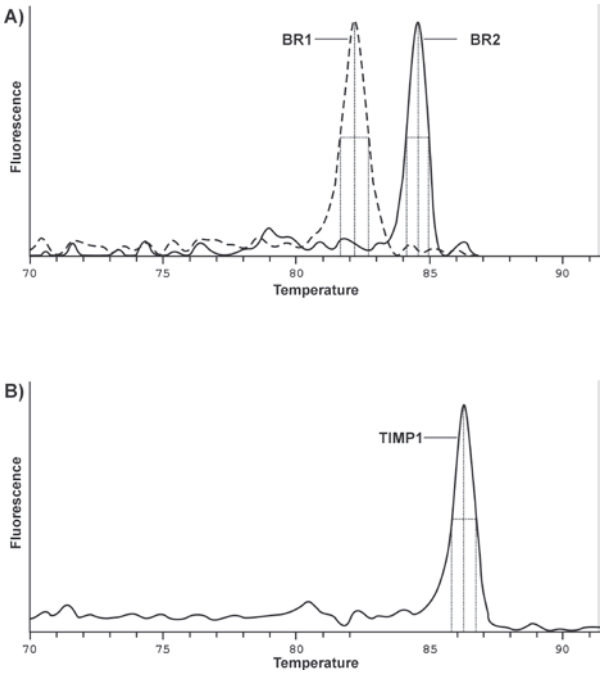
Statistical analysis

Statistical analysis was performed with Statistica 6.0 software. The Mann-Whitney U and χ^2 tests were used in the comparison of age and sex between patients and the control groups. The regression analysis and the t test for the two unpaired group were used in microarray data analysis. The differences between polyps and normal mucosa tissues in the expression of examined genes, detected by real-time RT-PCR, were analyzed using the Wilcoxon and Mann-Whitney U tests. The Spearman rank correlation test was used to assess the relationship between mRNAs *BR1* and *BR2* level. The final results of mRNA copy number per μg of total RNA were expressed as medians and range. The significance level was assumed for $p<0,05$.

RESULTS

In the first stage of the study, *BR1* and *BR2* mRNAs expression by real-time QRT-PCR were determined. For each RT-PCR product, a single peak was performed by melting curve analysis at the expected temperatures *(Fig. 1)*. Additionally, the specificity of RT-PCR for the target genes was confirmed on the basis of PAA electrophoresis, which revealed the presence of single products of the predicted length *(Fig. 2)*. The *BR1* and *BR2* mRNAs were detected in all cases of both the study and the control group. The transcriptional activity of examined genes in NP and in controls was presented in *Tab.3*. *BR2* mRNA was the predominant transcript in most NP and normal mucosa samples followed by *BR1* mRNA ($p<0,01$; Wilcoxon test). A lower expression of *BR1* was detected in the control group than in NP including eosinophilic nasal polyps, but these differences were not statistically significant ($p>0,05$; Mann-Whitney U test). B1 receptor gene expression in NNP was similar to control subjects ($p=0,570$). Expression of mRNA *BR2* in NP and normal mucosa at a comparable level was found ($p>0,05$). Transcriptional activity of the *BR2* gene in ENP was higher than in the controls, but not statistically significant ($p=0,423$).

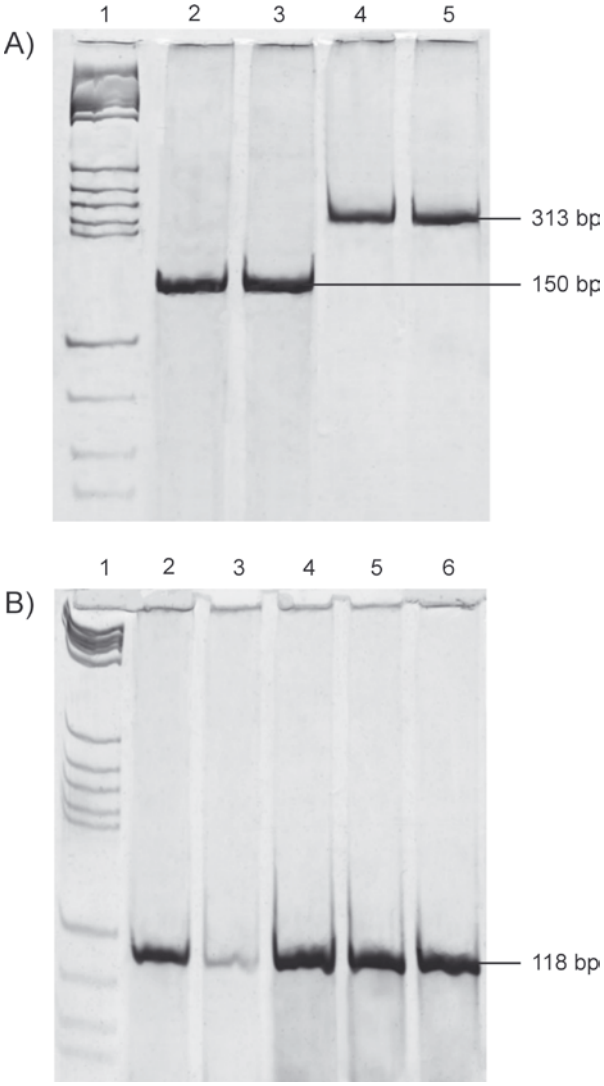
Figure 1. **Melting curve analysis of the RT-PCR products. A) BR1 (82°C), BR2 (84°C) amplimers and B) TIMP1 (86°C) amplimer.**



A positive correlation between the *BR1* and *BR2* mRNA expression in the ENP (r=0,91; p<0,01) and NNP (r=0,6; p<0,01) was identified.

In the next step of the research, genes showing various expressions in NPs in comparison with normal nasal tissues were identified by DNA microarray analysis. On the basis of the Affymetrix database (http://www.affymetrix.com/analysis/index.affx) 92 genes encoding proteins involved in signal transduction via B1 and B2 kinin receptors were chosen. Genes were considered as potentially differencing if p<0,05 and there was at least a 2-fold change in the mean expression levels between NPs and the control tissues [12,13]. Three genes were identified as having at least a 2-fold difference in expression between the groups *(Fig. 3)*. Gene encoding tissue inhibitor of matrix metalloproteinase-1 (TIMP1) was found to be expressed 2,63-fold higher in the NP compared with the control subjects. Two genes: *FOS* and *PTGS1* (Prostaglandin-endoperoxide synthase-1) presented higher (3,82- and

Figure 2. **RT-PCR products on 6% PAA electrophoreses A) lane 1 – marker of size pBR 322/Hae III, lanes 2, 3 – BR1 (150 bp); lanes 4, 5 – BR2 (313 bp); B) lane 1 – marker of size pBR 322/Hae III; lanes 2-6 – TIMP1 (118 bp).**



4,27-fold, respectively) expression in the controls compared with NP tissues. Gene encoding kallikrein 1 (*KLK1)* had also a higher (2,44-fold), but not significantly expression in the controls *(Tab. 4)*.

Table 3. **Quantitative comparison of examined genes of mRNA copy number per 1 µg of total RNA between nasal polyp tissue and normal nasal mucosa (median; range).**

	BR1	*BR2*	*TIMP1*
Nasal polyps	2374 (22-32863)	5946 (19-214987)	23161 (2124-299072)
Eosinophilic nasal polyps	2564 (22-32863)	9872 (19-244832)	23161 (2124-233684)
Neutrophilic nasal polyps	1874 (75-8045)	5119 (90-214987)	28772 (2846-299072)
Control group	1426 (15-27995)	5753 (46-199658)	7675 (1536-111076)

BR1 - kinin B1 receptor; BR2 - kinin B2 receptor; TIMP1 - Tissue inhibitor of metalloproteinase 1.
BR1 Polyps vs. control p=0,236; ENP vs. control p=0,202; NNP vs control p=0,57; ENP vs NNP p=0,302
BR2 Polyps vs. control p=0,443; ENP vs. control p=0,423; NNP vs control p=0,6851; ENP vs NNP p=0,466
TIMP1 Polyps vs. control p=0,003; ENP vs. control p=0,009; NNP vs control p=0,027; ENP vs NNP p=0,693

Figure 3. **Expression levels of genes described as BR1 and BR2 dependent in nasal polyps and controls. The diagonal line is the line of identity. *TIMP1* had a significantly higher expression in NP. *FOS* and *PTGS1* genes had significantly higher expression in controls. *KLK1* gene had a not significantly higher expression in controls.**

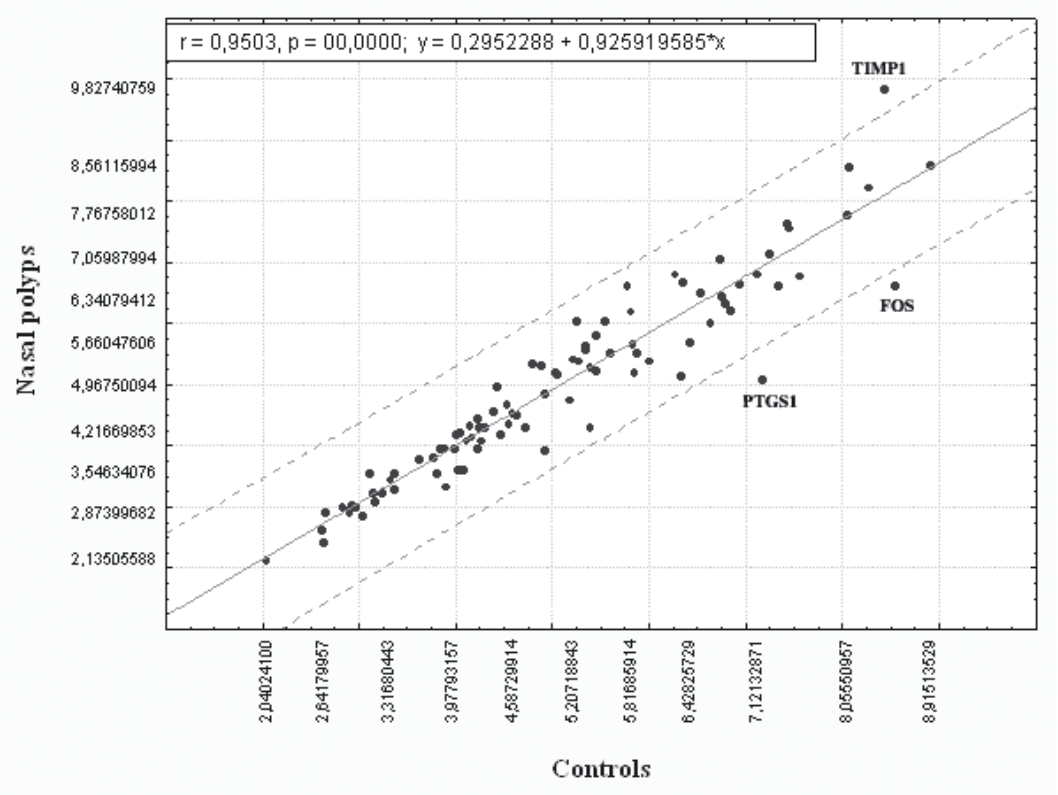
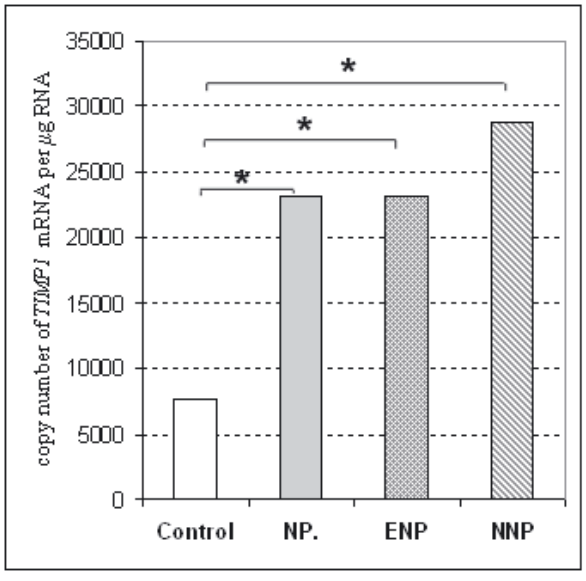


Figure 4. **Quantitative comparison of TIMP1 mRNA copy number between the control group and nasal polyps (median; * p<0,05).**



Subsequently, transcriptional activity of *TIMP1* gene was confirmed by real-time QRT-PCR. The specificity of RT-PCR was experimentally proved *(Fig. 1, 2)*. Expression of *TIMP1* mRNA was statistically higher in NPs than in normal mucosa (p=0,003) *(Tab. 1)*. Comparative analysis of the copy number of TIMP1 mRNA revealed a higher expression in both NNP (p=0,027) and ENP (p=0,009) than in normal nasal tissues *(Fig. 4)*.

DISCUSSION

Kinin receptor genes expression profile was revealed to change in various pathological states. However, the role of kinin B1 receptors in humans is still controversial [14]. Here we present the observation that kinin B1 and B2 receptor expression can be not significantly different in NPs compared to the control group. Especially among eosinophilic NPs the mRNA copy number for *BR1* and *BR2* was revealed to be higher than in healthy mucosae.

In the control group both types of BK receptors were detected which contrasts with previous observations as the BR1 is generally absent or at a very low level in healthy tissues. Christiansen et al [8] observed mRNA of B1 receptor only in 18% of healthy nasal epithelia. They also demonstrated significantly higher expression of *BR1* in allergic rhinitis subjects than in normal individuals. Similar to our data, they did not prove a substantial difference in *BR2* expression between the groups examined. Due to the BR1 multicellular location and mode of persistent signaling mechanism, the receptor is likely to exert a strategic role particularly on

Table 4. **Characteristics of genes encoding kinin B1 and B2 receptors and kinin-dependent genes showing expression in normal mucosa and nasal polyps.**

Probe set	Gene symbol	Gene name	SLR	Fold change	p[a]
201666_at	**TIMP1**	**Tissue inhibitor of metallopeptidase 1**	**1,3956**	**2,63 ↑**	**0,0001**
213187_x_at	FTL	ferritin, light polypeptide	0,7702	1,71	0,0008
203388_at	ARRB2	arrestin, beta 2	0,7138	1,64	0,0001
200696_s_at	GSN	gelsolin (amyloidosis, Finnish type)	0,7054	1,63	0,0067
213217_at	ADCY2	adenylate cyclase 2 (brain)	0,5147	1,43	0,0949
209432_s_at	CREB3	cAMP responsive element binding protein 3	0,4928	1,41	0,0059
204748_at	PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	0,4786	1,39	0,0686
204498_s_at	ADCY9	adenylate cyclase 9	0,4387	1,36	0,0025
212647_at	RRAS	related RAS viral (r-ras) oncogene homolog	0,3863	1,31	0,0540
204613_at	PLCG2	phospholipase C, gamma 2 (phosphatidylinositol-specific)	0,3858	1,31	0,0463
205075_at	SERPINF2	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2	0,3739	1,30	0,0396
206631_at	PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa	0,3406	1,27	0,0597
202087_s_at	CTSL	cathepsin L1	0,3322	1,26	0,1129
201469_s_at	SHC1	SHC (Src homology 2 domain containing) transforming protein 1	0,3181	1,25	0,2154
201750_s_at	ECE1	endothelin converting enzyme 1	0,2985	1,23	0,0075
201895_at	ARAF	v-raf murine sarcoma	0,2859	1,22	0,0411
204392_at	CAMK1	calcium/calmodulin-dependent protein kinase I	0,2825	1,22	0,0623
206429_at	F2RL1	coagulation factor II (thrombin) receptor-like 1	0,2760	1,21	0,1492
201040_at	GNAI2	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	0,2687	1,20	0,0445
203175_at	RHOG	ras homolog gene family, member G (rho G)	0,2611	1,20	0,1469
215075_s_at	GRB2	growth factor receptor-bound protein 2	0,2530	1,19	0,1309
206917_at	GNA13	guanine nucleotide binding protein (G protein), alpha 13	0,2183	1,16	0,0504
209195_s_at	ADCY6	adenylate cyclase 6	0,2154	1,16	0,0778
209320_at	ADCY3	adenylate cyclase 3	0,2047	1,15	0,3148
206541_at	KLKB1	kallikrein B, plasma (Fletcher factor) 1	0,1884	1,14	0,1326
210892_s_at	GTF2I	general transcription factor II, i	0,1739	1,13	0,0432
206248_at	PRKCE	protein kinase C, epsilon	0,1737	1,13	0,2109
200605_s_at	PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	0,1706	1,13	0,7830
213874_at	SERPINA4	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	0,1577	1,12	0,1737
217620_s_at	PIK3CB	phosphoinositide-3-kinase, catalytic, beta polypeptide	0,1549	1,11	0,1269
213245_at	ADCY1	adenylate cyclase 1 (brain)	0,1545	1,11	0,1356
211551_at	EGFR	epidermal growth factor receptor	0,1499	1,11	0,1940
217561_at	CALCA	calcitonin-related polypeptide alpha	0,1343	1,10	0,0804
209855_s_at	KLK2	kallikrein-related peptidase 2	0,1096	1,08	0,4924
215195_at	PRKCA	protein kinase C, alpha	0,1038	1,07	0,3662
211426_x_at	GNAQ	guanine nucleotide binding protein (G protein), q polypeptide	0,0879	1,06	0,5467
204897_at	PTGER4	prostaglandin E receptor 4 (subtype EP4)	0,0865	1,06	0,7878
201189_s_at	ITPR3	inositol 1,4,5-triphosphate receptor, type 3	0,0856	1,06	0,6345
204935_at	PTPN2	protein tyrosine phosphatase, non-receptor type 2	0,0853	1,06	0,0957
206044_s_at	BRAF	v-raf murine sarcoma viral oncogene homolog B1	0,0849	1,06	0,4013
207562_at	DGKQ	diacylglycerol kinase, theta 110kDa	0,0755	1,05	0,6278

Table 4. **Characteristics of genes encoding kinin B1 and B2 receptors and kinin-dependent genes showing expression in normal mucosa and nasal polyps. (continued)**

206054_at	KNG1	kininogen 1	0,0727	1,05	0,4151
216234_s_at	PRKACA	protein kinase, cAMP-dependent, catalytic, alpha	0,0689	1,05	0,5890
212559_at	PRKAR1B	protein kinase, cAMP-dependent, regulatory, type I, beta	0,0588	1,04	0,7203
206330_s_at	SHC3	SHC (Src homology 2 domain containing) transforming protein 3	0,0411	1,03	0,6381
217271_at	GNA11	guanine nucleotide binding protein (G protein), alpha 11 (Gq class)	0,0379	1,03	0,7485
209749_s_at	ACE	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	0,0272	1,02	0,7912
208351_s_at	MAPK1	mitogen-activated protein kinase 1	0,0232	1,02	0,8870
210850_s_at	ELK1	ELK1, member of ETS oncogene family	0,0052	1,00	0,9718
215813_s_at	**PTGS1**	**prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)**	**-2,0937**	**4,27 ↓**	**0,0001**
209189_at	**FOS**	**v-fos FBJ murine osteosarcoma viral oncogene homolog**	**-1,9317**	**3,82 ↓**	**0,0174**
216699_s_at	KLK1	kallikrein 1	-1,2869	2,44	0,0800
203680_at	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta	-0,9070	1,88	0,0045
201244_s_at	RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	-0,6178	1,53	0,0011
201494_at	PRCP	prolylcarboxypeptidase (angiotensinase C)	-0,5819	1,50	0,0005
202187_s_at	PPP2R5A	protein phosphatase 2, regulatory subunit B', alpha isoform	-0,5707	1,49	P<0,0001
214157_at	GNAS	GNAS complex locus	-0,5360	1,45	0,0279
217971_at	MAPKSP1	MAPK scaffold protein 1	-0,5130	1,43	0,1102
211323_s_at	ITPR1	inositol 1,4,5-triphosphate receptor, type 1	-0,4436	1,36	0,0015
209576_at	GNAI1	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	-0,4375	1,35	0,0479
202450_s_at	CTSK	cathepsin K	-0,4367	1,35	0,1599
204271_s_at	EDNRB	endothelin receptor type B	-0,4200	1,34	0,0002
202741_at	PRKACB	protein kinase, cAMP-dependent, catalytic, beta	-0,3809	1,30	0,1573
204313_s_at	CREB1	cAMP responsive element binding protein 1	-0,2994	1,23	0,1801
213052_at	PRKAR2A	protein kinase, cAMP-dependent, regulatory, type II, alpha	-0,2925	1,22	0,0648
222103_at	ATF1	activating transcription factor 1	-0,2484	1,19	0,1973
212983_at	HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	-0,2319	1,17	0,3665
202401_s_at	SRF	serum response factor (c-fos serum response element-binding transcription factor)	-0,2147	1,16	0,0886
202670_at	MAP2K1	mitogen-activated protein kinase kinase 1	-0,2039	1,15	0,3604
205803_s_at	TRPC1	transient receptor potential cation channel, subfamily C, member 1	-0,1893	1,14	0,2468
205870_at	BDKRB2	bradykinin receptor B2	-0,1431	1,10	0,4899
205357_s_at	AGTR1	angiotensin II receptor, type 1	-0,1330	1,10	0,0172
213384_x_at	PLCB3	phospholipase C, beta 3 (phosphatidylinositol-specific)	-0,1327	1,10	0,3680
201465_s_at	JUN	jun oncogene	-0,1189	1,09	0,7371
206811_at	ADCY8	adenylate cyclase 8 (brain)	-0,1176	1,08	0,3284
210145_at	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)	-0,1065	1,08	0,6638
215938_s_at	PLA2G6	phospholipase A2, group VI (cytosolic, calcium-independent)	-0,1025	1,07	0,4286
221284_s_at	SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	-0,0547	1,04	0,4780
217808_s_at	MAPKAP1	mitogen-activated protein kinase associated protein 1	-0,0374	1,03	0,7577
207510_at	BDKRB1	bradykinin receptor B1	-0,0337	1,02	0,7478
202424_at	MAP2K2	mitogen-activated protein kinase kinase 2	-0,0321	1,02	0,8433
211925_s_at	PLCB1	phospholipase C, beta 1 (phosphoinositide-specific)	-0,0267	1,02	0,4523
204920_at	CPS1	carbamoyl-phosphate synthetase 1, mitochondrial	-0,0256	1,02	0,8465
209643_s_at	PLD2	phospholipase D2	-0,0244	1,02	0,8451
205962_at	PAK2	p21 protein (Cdc42/Rac)-activated kinase 2	-0,0234	1,02	0,7745

Table 4. **Characteristics of genes encoding kinin B1 and B2 receptors and kinin-dependent genes showing expression in normal mucosa and nasal polyps. (continued)**

212777_at	SOS1	son of sevenless homolog 1	-0,0221	1,02	0,9094
211446_at	RGSL1	regulator of G-protein signaling like 1	-0,0206	1,01	0,8581
210388_at	PLCB2	phospholipase C, beta 2	-0,0160	1,01	0,8974
203235_at	THOP1	thimet oligopeptidase 1	-0,0141	1,01	0,9333
207119_at	PRKG1	protein kinase, cGMP-dependent, type I	-0,0124	1,01	0,8735
204010_s_at	KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	-0,0034	1,00	0,9575
202647_s_at	NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	-0,0028	1,00	0,9849

SLR – signal log ratio between patient and control expression levels. Base 2 is used as the log scale, therefore a SLR of 1 indicates a 2-fold increase in the transcript level and 1 indicates a 2-fold decrease.
[a] t-test; ↑ significantly higher expression in nasal polyps than in the control group; ↓ significantly lower expression in nasal polyps than in the control group

inflammation with an immune etiology [15]. However, such an etiology rather does not underlie the cause of NPs. What is more, it has been reported in several studies that NP subjects present a lower incidence of allergic rhinitis [16].

Bradykinin receptors mediate the opening of the tight junctions between endothelial cells causing increased vascular permeability, plasma exudation and edema. The BR1 is involved in most of the cardinal signs of inflammation [17]. It is induced and overexpressed in response to bacterial endotoxins and cytokines such as IL-1β, IL-2, IL-8, TNF-α and INF-γ [4]. Activation of BR1 has been demonstrated to stimulate cell proliferation and induce IL-1β and TNF-α releasing [18-20]. Moreover, inflammation cytokines like INF-γ, IL-1β and TNF-α may cause up-regulation of *BR2* mRNA and protein in cells [21,22]. These findings imply that a wide spectrum of cytokines connected with NP formation may inflict BK receptor expression and inversely.

Prolonged tissue inflammation is accompanied by an increased amount of active metabolites (des-Arg9-bradykinin and des-2 Arg10-kallidin), which induce *BR1* expression [23]. In the main, a higher expression of BR1 seems to occur only during the chronic stages of inflammation, whereas BR2 is most likely seen in the acute phase of inflammation [24]. Within the group of NPs, kinin B1 receptor mRNA level increased more visibly than in the control subjects what complies with previous observations.

Trifilieff et al. [25] suggest that the B2 receptor is the major kinin receptor involved in airway responses to kinins. Icatibant (HOE-140), a BR2 antagonist, removed hyperresponsiveness to histamine and reduced nasal eosinophilia induced by Ag in patients with allergic rhinitis [26]. Christiansen et al. [8] hypothesize that the kinin system contributes to the development of airway inflammation initially through the BR2, with subsequent up-regulation of the BR1. It could explain our data as we observed a higher level of mRNA *BR2* than *BR1* in most NPs and normal mucosae.

In ENP and NNP tissues a statistically significant correlation of both receptors was observed. This could be explained in part by coexpression of genes encoding B1 and B2 kinin receptors in nasal tissues.

It has been shown in recent studies that activation of the BK receptors leads to a number of intracellular events. Through the intracellular network of secondary transmitters, receptors of kinin B modulate various effectors. To gain further insight into the cellular mechanism following kinin B receptors induction, BR1 and BR2 dependent genes were investigated using oligonucleotide microarray technology. Microarrays are a high-throughput method, which allow the simultaneous evaluation of the expression of tens of thousands of genes. Moreover, this tool permits the identification of the new biological disease markers or drug targets and also pathogenetic mechanisms. Among genes 92 encoding proteins which take part in kinin signaling pathways only *TIMP1* was revealed to be statistically more highly expressed in NP tissues compared to the controls. Also Liu et al. by the use of DNA microarrays determined *TIMP1* as up-regulated gene in NPs compared to normal and sphenoid sinus mucosa [13]. The elevated TIMP1 level has been found previously in chronic rhinosinusitis and NPs [27]. Such observation confirms that the MMP/TIMP balance is, in principle, a critical factor in regulating the breakdown of connective tissues and non-matrix substrates by MMPs. Recent studies, however, have shown that MMP9 deficiency can promote airway inflammation [28]. TIMP1 as specific inhibitor was shown to have growth promoting activity in several cell types [29]. Excess of TIMP1 is supposed to be responsible for fibrosis airway in asthma [30].

Prostaglandin-endoperoxide synthase-1 (PTGS-1) catalyzes the synthesis of prostaglandins G2 and H2. Moreover, it serves as the precursor for a number of important prostanoids including prostaglandin E2. *PTGS-1* is constructively expressed and relatively unaffected by inflammatory mediators. It has been documented that the *PTGS-1* is expressed in normal mucosa of upper respiratory tract and its expression is not subjected to change in NPs [31]. Our findings support the thesis that PTGS-1 may possibly play some role in chronic rhinosinusitis. However, the degree of commitment PTGS-1 in inflammatory reactions depends on the inflammatory stimulus and kind of tissue.

Activation of kinin B receptors leads to activation of *FOS* gene and finally to the formation of AP-1. AP-1 transcription factor participates in the induction of a wide variety of proinflammatory proteins, receptors and mediators. *c-FOS* mRNA has been previously detected in both NPs and normal nasal epithelium [32]. Our data agrees with earlier observations that fibroblasts, endothelium and inflammatory cells of NP show low *c-FOS* protein expression. This may suggest that *c-FOS*/*AP-1* mediated processes are more important in the epithelium. The production of *FOS* by epithelial cells indicates a more integral role and mirrors sophisticated relations.

CONCLUSIONS

The current report for the first time demonstrates the expression pattern of kinin receptors genes and also kinin-dependent genes in nasal polyps. We did not identify changes in the expression profile of kinin receptors in analyzed groups, which suggests that kinin receptors may not make an important contribution to the etiology of nasal polyps. The potential role of genes encoding proteins involved in transduction signal via B1, B2 kinin receptors in inflammation processes and NP formation needs further investigation.

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