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Implication of BIRC5 in asthma pathogenesis

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Abstract

In the last few years, it has been recognized that the unbalanced regulation of survival and apoptosis of bronchial inflammatory cells is a key component in the development of asthma. Baculoviral IAP repeat containing 5 (BIRC5) (also known as survivin) is an important anti-apoptotic protein that has been implicated in many cancer types, and recent studies provide evidence for its role in controlling inflammatory disorders as well. Our aim was to investigate at both genetic and transcriptional levels if BIRC5 has an impact on asthma development. We found that induced sputum samples of patients with bronchial asthma contained elevated levels of BIRC5 mRNA compared with healthy subjects and its level was in correlation with sputum eosinophil percentages. Furthermore, in a case–control study examining single nucleotide polymorphisms (SNPs) in the BIRC5 regulatory regions, the minor alleles of rs8073903 and rs8073069 were found to be significantly associated with asthma and especially non-allergic asthma phenotypes, which associations were more prominent among women. Two marker haplotype analyses further strengthen the impact of these two polymorphisms on both asthma and non-allergic asthma. In the female cohort, rs1508147 was also significantly associated with increased risk of non-allergic asthma. Additionally, with linear regression analysis, we showed that rs9904341 was significantly correlated with both absolute and relative serum eosinophil levels. In conclusion, our results suggest that possibly by inhibition of the eosinophil apoptosis, BIRC5 might be an important regulator of the asthmatic processes and we provide some evidence that its effect might be affected by SNPs located in the gene regulatory regions.

Keywords: apoptosis, expression, mRNA, non-allergic asthma, SNP, survivin

Introduction

Asthma is a multifactorial disease caused and regulated by interactions of an extremely complex genetic background and a wide variety of environmental factors. Although there are numerous hypotheses for its pathogenesis, the genetic mechanisms of asthma are far from being clear.

Apoptotic processes have been widely recognized as key factors in asthma development (1). Recent studies suggest that accumulation of eosinophils in bronchial tissue is related to the dysregulation of apoptosis and eosinophil clearance (2–4). Furthermore, it has also been shown that reduced eosinophil apoptosis correlates with asthma severity (5).

Baculoviral IAP repeat containing 5 (BIRC5) (also known as survivin) is an important member of the inhibitor of apoptosis protein family. Its impact in blocking apoptosis has already been widely demonstrated in the literature (6–9). Studies investigating the molecular basis of the anti-apoptotic function of BIRC5 have shown that it not only inhibits the caspase-dependent apoptotic pathways and caspase-independent apoptotic pathways but also accelerates cell proliferation (5, 10). Under normal conditions, BIRC5 is highly expressed in fetal tissues but is barely detectable in the majority of terminally differentiated adult tissues (11). However, it has been extensively demonstrated that during tumorigenesis, survivin is dramatically over-expressed and therefore has been identified as a candidate target for cancer therapy (12).

Besides its well-known role in the development and progression of common cancers, recent studies have been suggesting that survivin may also be implicated in inflammatory processes (13, 14) and in the pathogenesis of asthma (15–17). In our previously completed whole genome gene-expression microarray analysis of ovalbumin peptide-induced mouse model of asthma (GSE11911 record number

in GEO database) (15), we found that Birc5 mRNA was expressed at elevated level after allergen exposure ($P < 0.01$, level of normalized log2 ratio: 3.38). Consistent with our findings, Tumes *et al.* (16) reported the same results. Additionally, they observed that increased Birc5 mRNA and protein levels in eosinophils recovered by bronchoalveolar lavage were in strong correlation with elevated eosinophil counts and with other inter-strain differences observed in the lung pathology following allergen challenge. Though animal studies are important sources of new insights and have already been provided numerous valuable data on asthma development, their results may not be extrapolated to humans with certainty, and therefore, human replication studies are crucial to validate their findings.

Taken these data together, we hypothesize that BIRC5 may be an important mediator in the dysregulated anti-apoptotic processes that characterize asthma development.

Therefore, the aim of our current study were to explore whether BIRC5 expression is detectable in human sputum samples and to compare its level between healthy controls and asthma patients. Furthermore, we aimed to analyze the potential relationship between genetic variations in the regulatory regions of *BIRC5* gene and asthma pathomechanism.

Methods

Subjects

For the measurement of gene expression in the airways, induced sputum was collected from 34 subjects between 19 and 61 year old, but 11 of them were excluded from the following analysis because the quantity and/or quality of sputum was too low to allow analysis.

Asthma was diagnosed by respiratory medicine specialist according to the recent Global Initiative for Asthma guidelines (http://www.ginasthma.org/). According to their lung function data, they were divided into mild ($n = 4$) and moderate to severe groups ($n = 9$). Ten patients regularly used inhaled corticosteroid (ICS): <500 μg day^{-1} beclomethasone dipropionate (BDP) or equivalent ($n = 4$), 500–1000 μg day^{-1} BDP or equivalent ($n = 4$) and >1000 μg day^{-1} BDP or equivalent ($n = 2$); while three were considered steroid naive.

Healthy controls were recruited from the staff and students of the participating Hungarian Universities. They had normal lung function and had no history of respiratory diseases.

In all subjects, lung function test (PDD-301/s, Piston Inc, Budapest, Hungary) and fractional exhaled nitric oxide (FENO) measurements (NIOX MINO, Aerocrine, Solna, Sweden) were performed, and the presence of atopy was tested by skin prick tests for common allergens. In addition, asthma control was evaluated by the Hungarian translation of the Asthma Control Test™ (ACT; QualityMetric, Inc., Lincoln, RI, USA) (18).

The healthy and asthma groups participating in sputum induction did not differ statistically regarding age, sex, smoking habit and allergic status. The group of smokers included those who reported having smoked more than 100 cigarettes during their lifetime and currently smoked either daily or occasionally and those who had quit smoking for less than 1 year before their participation in the study. The time since last cigarette was not available for each study participants, therefore analyses were not adjusted for this factor.

None of the subjects had respiratory tract infection 4 weeks prior to the study. The characteristics of this study population are shown in Table 1.

The study population involved in genotype analysis comprised 651 unrelated individuals of Hungarian (Caucasian) population. Approximately, 5% of tested subjects were probably of Gypsy origin (estimate based on state population statistics).

Asthma diagnosis was carried out according to the criteria described for the subjects above.

Atopy was defined by positive skin prick test to at least one allergen (wheal diameter 3 mm greater than saline control) and/or positive total or specific IgE levels. Total serum IgE levels and specific IgE levels to more than 100 allergens were determined by 3gAllergy blood tests in Immulite 2000 Immunoassay System (Siemens Healthcare Diagnostics; Deerfield, IL, USA). Serum IgE levels were classified as normal or high according to the following age-specific reference ranges (kU/litre): 0–1 year, <15; 1–5 year, <60; 5–10 year, <90; adult, <100. If the onset of asthma or the asthma exacerbations have been associated with an infection-related acute respiratory illness the asthma was classified as infection-induced asthma. Non-atopic patients with infection- and/or exercise-induced asthma phenotype composed the non-allergic asthma subgroup. Fifty-seven patients (18%) had no available information on asthma endophenotypes.

Blood eosinophil cell counts were measured by Coulter MAXM Analyser. A value of 1% to 6% eosinophils was considered a normal relative range and normal range of absolute eosinophil count was considered between 0.05 and 0.200 G l^{-1}.

The treatment of the patients remained unchanged before the blood was drawn. None of the asthmatic patients had experienced exacerbation or a respiratory infection for at least 4 weeks as indicated by the lack of increased symptoms.

The control children were randomly selected from outpatients from the Orthopaedic Department in the Budai

Table 1. Detailed characteristics of subjects participating in sputum analysis

Clinical and biological characteristic	Asthmatic patients, $n = 13$	Control patients, $n = 10$
Age (years) ± SD	34.6 ± 10.1	30.11 ± 4.1
Gender (male/female)	6/7	5/5
Smoking habit (yes/no)	7/6	6/4
Asthma severity		
Mild	4	0
Moderate-to-severe	9	0
Allergy (yes/no)	10/3	5/5
Sputum eosinophil %	12.18 ± 11.33	0 ± 0*
Sputum neutrophil %	25.08 ± 17.77	20.03 ± 10.01
Sputum macrophage %	61.75 ± 15.04	74.40 ± 8.39
Sputum bronchial epithelial %	1.31 ± 1.70	5.56 ± 5.25*

*indicates significant P values (≤0.05) between cases and controls; n, number of patients involved.

Children's Hospital or from the Urological Department of Heim Pal Hospital, Budapest. Children in the control group showed no symptoms of asthma and required no medication. The adult controls were healthy blood donors. About study subjects detailed questionnaires were filled out.

Detailed characteristics of subjects included in association studies are shown in Table 2.

The study was conducted according to the principles expressed in the Declaration of Helsinki and approved by the Ethics Committee of the Hungarian Medical Research Council. Written informed consent was obtained from all patients or the parents or guardians of the minors involved in the study.

Sputum induction

The participants inhaled 4.5% saline solution generated by a De Vilbiss Nebulizer (Ultra-NebTm 2000 model 200HI) for 5 min after pre-treatment with 400 μg of inhaled salbutamol. Induction was performed three times and the pulmonary function was measured each time after the sputum induction. All portions that macroscopically appeared free of salivary contamination were selected. Samples were diluted with PBS containing 0.1% dithiothreitol (Sigma, St Louis, MO, USA), portions were agitated with a vortex and placed on a bench rocker for 30 min. Samples were filtered through a 40-μm Falcon cell strainer and centrifuged at 1500 rpm for 10 min. The cell pellet was resuspended in 1 ml PBS and viability (Trypan blue exclusion method) was determined using Burker chamber. After differential cell count, cells were stocked on lysis buffer at −80°C until use.

RNA isolation and gene-expression measurement

RNA was isolated successfully from induced sputum samples of 13 patients and 10 control subjects with the Qiagen Mini RNeasy Kit (Qiagen, Maryland, USA). RNA was transcribed to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR was performed for *BIRC5* and β-actin using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems). β-Actin was used as an endogenous control and all results were normalized to it.

DNA isolation and genotyping

Genomic DNA was isolated from whole blood samples using iPrep PureLink gDNA Blood Kit on iPrep Purification Instrument (Invitrogen, Carlsbad, CA, USA).

A total of six single nucleotide polymorphisms (SNPs) were genotyped using the Sequenom iPLEX Gold MassARRAY technology at the McGill University and Génome Québec Innovation Centre, Montréal (Québec), Canada.

Statistical analysis and bioinformatics

For sputum analysis, normalized gene-expression levels were compared by Mann–Whitney *U* test or Kruskal–Wallis test, when appropriate. Contingency tables were analyzed by Fisher's exact test. Correlation studies were performed by Spearman non-parametric test. Differences were considered to be significant if $P < 0.05$. Allele frequencies between groups of case and control subjects were estimated by allele counting and tested for deviation from Hardy–Weinberg equilibrium (HWE) by the software program DeFinetti (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). For the significant deviation threshold, we used $P = 0.01$ value.

SNP data were analyzed using MedCalc and SPSS v17 (SPSS Inc., Chicago, IL, USA) softwares. Logistic regression analyses adjusted for age and gender were used to evaluate the association between *BIRC5* genotypes and asthma, its intermediate phenotypes and the discretized (normal/high) IgE level (see at Subjects). Linear regression models adjusted for age and gender were used to analyze the effect of the genetic background on dependent scale variables, such as absolute and relative eosinophil levels. Confidence intervals (CIs) were calculated at the 95% level. Multiple comparisons were corrected for using the Bonferroni method and alpha levels of $P < 0.01$ were considered to be significant. Odds ratios (ORs) for haplotypes were counted by

Table 2. Detailed characteristics of subjects participating in SNP analysis

Clinical and biological characteristic	Asthma subjects, *n* = 307	Control subjects, *n* = 344
Age (years) ± SD	10.55 ± 4.74	21.77 ± 13.88*
Gender (male/female)	200/107	173/171*
Endophenotypes		
Atopic asthma, *n* (%)	191 (62.21%)	—
Exercise-induced asthma, *n* (%)	63 (20.52%)	—
Infection-induced asthma, *n* (%)	88 (28.66%)	—
Non-allergic asthma, *n* (%)	59 (19.22%)	—
Asthma with associated diseases, n (%) (allergic rhinitis or allergic conjuctivitis)	110 (35.83%)	—
Absolute eosinophil count (mean ± SD, G/L)[a]	0.37 ± 0.27	0.25 ± 0.21*
Number of patients with normal or high absolute eosinophil count[a]	33/78	34/21
Relative eosinophil level (mean ± SD, %)[a]	5.46 ± 4.21	3.70 ± 2.58*
Number of patients with normal or high relative eosinophil level[a]	68/49	53/5
IgE (mean ± SD, kU/l)[a]	288.06 ± 317.10	83.46 ± 165.84*
Number of patients with normal or high IgE level (normal/high)[a]	43/73	70/20*

[a]Data are available in a limited data set only.
*indicates significant *P*-values (≤0.05) between cases and controls. *n*, number of patients involved.

MedCalc 10.0.2 (MedCalc Software, Mariakerke, Belgium) software. Figures were made by using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com).

Results

Sputum analyses

Differences in BIRC5 mRNA levels between adult subjects with and without asthma were tested on 13 asthmatic and 10 control samples. RNA isolation and reverse transcription were successful from all sputum samples.

As shown in Fig. 1(A), the mean gene expression level of BIRC5 was significantly higher in induced sputum derived from asthmatic patients in comparison to healthy controls (P = 0.03). When studying the asthma severity subgroups defined by GINA guidelines, we found no differences between the mild and moderate-to-severe asthma groups.

There was a significant relationship between sputum eosinophil percentages and BIRC5 mRNA levels (P = 0.02, r = 0.468, Fig. 1B). Gender, allergic status or smoking habits had no effect on BIRC5 mRNA expression (data not shown).

It is noteworthy, that consistent with previous publications, eosinophil ratios detected in sputum samples were significantly increased with increasing asthma severity, as shown in Fig. 1(C). However, sputum neutrophil level was not correlated with asthma severity (P = 0.75, data not shown).

When analyzing the relationship between BIRC5 and clinical characteristics of asthmatic patients, we found no correlation between BIRC5 mRNA expression levels and FENO, ICS dose or ACT total scores. However, there was a tendency for negative correlation between BIRC5 mRNA levels and ICS dose.

There was a significant correlation between FENO levels and both eosinophil and neutrophil percentages in induced sputum (P = 0.006, r = 0.742 and P = 0.048, r = −0.58, respectively). In addition, we found a significant negative relationship between ACT score and eosinophil percentages (P = 0.048, r = −0.55). There was no correlation between the amount of ICS used and sputum eosinophil level (P = 0.1). Results are presented in Fig. 2.

SNP association studies

Gender and age were found to be significant confounder in our statistical models and were included as covariates. Description of the studied SNPs is summarized in Table 3. Out of the six genotyped SNPs, rs3764384 was excluded from further analyses due to significant deviation from the HWE.

Results of logistic and linear regression analyses adjusted for age and gender are shown in Table 4. We found that the minor alleles of rs8073903 and rs8073069 were significantly associated with increased risk of asthma, though rs8073069 did not meet Bonferroni-adjusted criterion for significance (OR = 1.458, 95% CI = 1.126–1.889, P = 0.004 and OR = 1.393, 95% CI = 1.061–1.830, P = 0.017, respectively). In addition, these associations were more evident when only women were studied and in this cohort rs8073069 reached the Bonferroni-corrected 0.01 significance threshold (OR = 1.887, 95% CI = 1.238–2.878, P = 0.003 for rs8073069 and

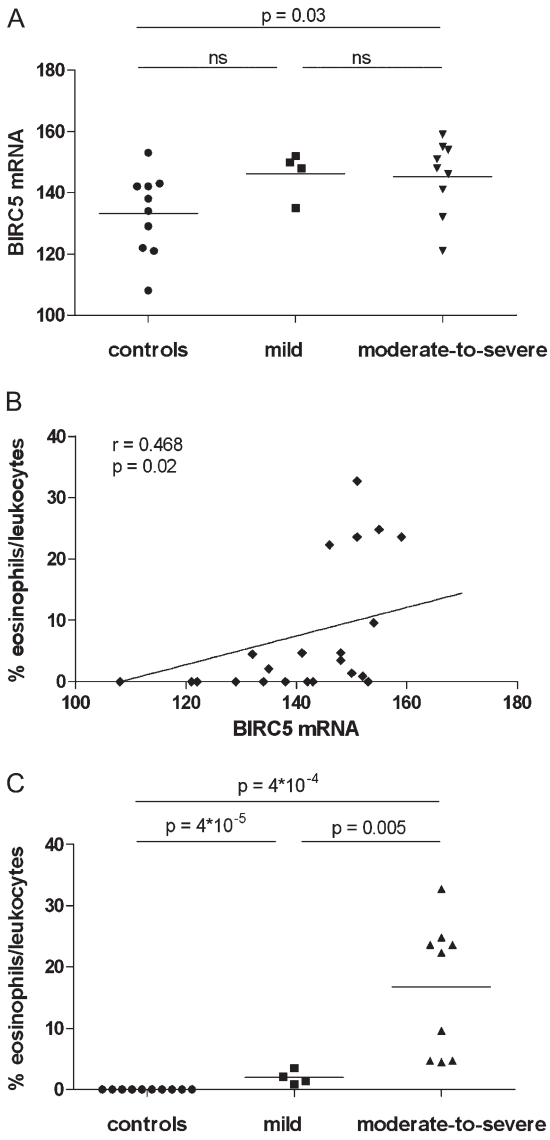

Fig. 1. Relationships between BIRC5 mRNA and eosinophil levels and asthma severity. mRNA levels are expressed as the ratio of BIRC5 cDNA copy numbers and β-actin cDNA copy numbers multiplied by 100. Patients were grouped by asthma severity determined according to the GINA criteria. Comparisons were performed by the Mann–Whitney U test and Kruskal–Wallis test (A, C). The median is represented by a horizontal line. Correlation between BIRC5 mRNA and sputum eosinophil percentages were studied by the Spearman non-parametric test (B).

OR = 1.872, 95% CI = 1.232–2.843, P = 0.003 for rs8073903). Furthermore, rs1508147 was also slightly associated with asthma among women (OR = 1.683, 95% CI = 1.096–2.585, P = 0.017).

In respect of asthma endophenotypes, the minor alleles of rs8073069 and rs8073903 were associated with and showed significantly higher predisposing effect for the development of non-allergic asthma (OR = 2.010, 95% CI = 1.336–3.024, P = 0.001 for rs8073069 and OR = 1.622, 95% CI = 1.072–2.454, P = 0.022 for rs8073903). Moreover, consistent with the results observed between asthma cases and controls,

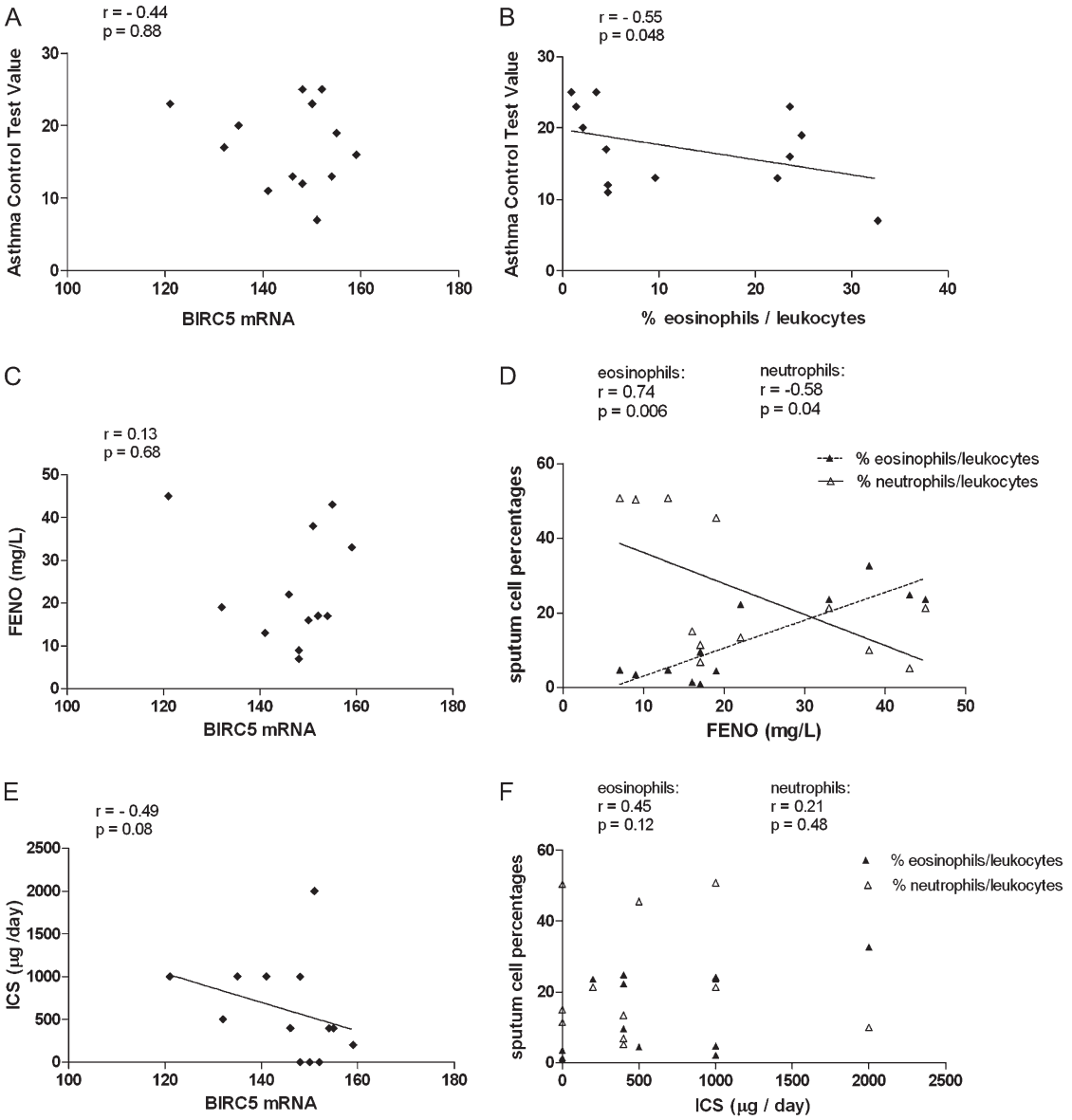

Fig. 2. Correlations between sputum BIRC5 mRNA levels and clinical parameters of asthma patients [ACT values (A), FENO (C) or ICS dosages (E)] are represented in Column 1 and correlations between sputum eosinophil/neutrophil cell percentages and clinical parameters [ACT values (B), FENO (D) or ICS dosages (F)] are represented in Column 2. Correlations were calculated using Spearman non-parametric test. Correlation curves are presented only when the results are significant. ACT, Asthma Control Test; FENO, exhaled nitric oxide level.

these associations were also more prominent when studying only women (OR = 3.096, 95% CI = 1.569–6.109, $P = 0.001$ for rs8073069 and OR = 2.808, 95% CI = 1.372–5.746, $P = 0.005$ for rs8073903).

Additionally, we found that minor allele of rs1508147 was also significantly associated with the increased risk of non-allergic asthma in the female cohort (OR = 3.057, 95% CI = 1.445–6.466, $P = 0.003$).

To determine the effects of the studied SNPs on serum eosinophil level, linear regression analysis was performed. Rs9904341 was found to be significantly correlated with both absolute and relative serum eosinophil counts (OR = 0.917, 95% CI = −0.145 to −0.026, $P = 0.004$ and OR = 0.262, 95% CI = −2.132 to −0.468, $P = 0.002$, respectively). No such correlation could be observed for serum IgE levels.

To obtain more statistical evidence for the associations, we performed haplotype analyses. In the majority of the cases, the most significant results were observed when performing two-marker analyses with SNPs rs8073903 and rs8073069. The corresponding results are presented in Table 5. Between asthma cases and controls, a strong significant difference was found for the haplotype consisting of the wild-type alleles (TG) of rs8073903 and rs8073069, as the haplotype was less frequent among the asthma patients compared with controls (57% versus 65%, $P = 0.004$), whereas the haplotype carrying the minor alleles (CC) was found to be more prevalent in the asthma group (31% versus 25%, $P = 0.019$). Further analyzing the asthma subgroups, we found that CC haplotype showed a very strong association with non-allergic asthma, as it was significantly more

Table 3. Description of selected SNPs

SNP rs#	Position according to NCBI Genome Build 37.1	Position relative to the start codon	Alleles on the forward strand	Gene location	MAF in controls	MAF in cases	*P*-value[a]	HWE in controls
rs3764384	chr17:76207728	−2670	C/T	5′ near gene	0.39	0.36	0.77	0.00005[b]
rs8073903	chr17:76209754	−644	T/C	5′ near gene	0.34	0.42	0.30	0.16
rs8073069	chr17:76209773	−625	G/C	5′ near gene	0.25	0.32	0.34	0.05
rs17878467	chr17:76210157	−241	C/T	5′ near gene	0.09	0.10	1	0.88
rs9904341	chr17:76210367	−31	G/C	5′ UTR	0.35	0.34	1	0.55
rs1508147	chr17:76222588	12190	G/A	3′ near gene	0.42	0.44	0.88	0.06

[a]*P*-values between allele frequencies were calculated by Fisher's -exact test. MAF: minor allele frequency.
[b]SNP was excluded because of the significant deviation from HWE.

prevalent in those subjects than in other patients (46% versus 61%, $P = 0.00005$).

Regarding the eosinophilia, we found that any haplotypes carrying the wild-type allele of rs9904341 were more frequent in patients with high relative eosinophil level, while haplotypes carrying its minor allele were observed less frequently in this group compared with that with normal eosinophil ratio (for TGCCG haplotype consisting of all five studied SNPs: 20% versus 33%, $P = 0.015$). However, these haplotype associations had less statistical significance than the single-marker analysis revealed for rs9904341 alone.

None of the identified haplotypes showed significant correlation with atopic, infection-induced or exercise-induced asthma.

Discussion

Up to the present, this is the first human case–control study investigating the potential role of BIRC5 in asthma development.

In this study, we measured the BIRC5 mRNA level in sputum samples of asthma patients and compared it with those of healthy subjects. Conforming to our expectations based on previous research, we found that the mean BIRC5 expression level was significantly higher in the airways of patients with asthma than of healthy controls.

Furthermore, we demonstrated that the eosinophil ratio in induced sputum of asthma patients was in correlation with the BIRC5 mRNA expression level. This observation supports previous data that describe the anti-apoptotic effect of survivin on eosinophil cells (14).

Several aspects of our results are in line with previous publications. First, we confirmed that better asthma control was significantly correlated with lower sputum eosinophil count (19, 20). Also, consistent with other studies, we found significant correlation between sputum eosinophil levels and clinical severity, as mild asthmatic subjects had significantly lower percentage of sputum eosinophils than moderate-to-severe asthmatics (5). The phenomenon that BIRC5 mRNA level is correlated with eosinophilia but not with ACT may simply arise from the attenuated statistical interaction between two variables in indirect relationship. Furthermore, conforming to previous reports, we observed strong correlation between exhaled nitric oxide level and sputum eosinophil count (21). At some points, however, our data differ from prior studies. In this study, we were not able to show significant correlation between the amount of ICSs and sputum eosinophil levels, however, it can be assumed that it is because of the relatively small sample size used for sputum analysis. Also, as we have no available data on when ICS were administered we cannot exclude the possibility that various time intervals between ICS administration and sputum induction influence our results.

To see if common polymorphisms in the 5′ and 3′ regulatory regions of BIRC5 gene have any impact on the asthma phenotypes, we conducted SNP association analysis. Two of our studied SNPs, rs8073903 and 8073069, both are located in the 5′ region, showed significant associations with asthma. When examining the male and female subjects separately, we found that the observed associations were even more prominent among women. Additionally, in this subgroup, rs1508147 also proved to be associated with asthma. When studying the relationships between the SNPs and different asthma phenotypes, we observed more apparent predisposing effects of these polymorphisms in the non-allergic asthma subgroup. Furthermore, consistent with the results of genotyping analyses, haplotypes carrying the minor alleles of these SNPs were found to be associated with a significantly increased risk of asthma and non-allergic asthma. Because eosinophilia is more prominent in non-allergic asthma than in allergic asthma (22), these results were highly consistent with our expectations. Altogether, these association data indicate a strong gender-dependent effect of *BIRC5* regulatory polymorphisms on apoptotic processes and thus support the results of our expression analysis.

In a gene expression measurement carried out on esophageal cancer patients, Yang *et al.* (23) found that the −625 G/C (rs8073069) polymorphism in the BIRC5 promoter was significantly associated with increased survivin expression. This suggests that the potential predisposing effects of the minor allele of this SNP in asthma patients may be resulted from its association with higher BIRC5 mRNA expression. The importance and functional role of this SNP are further strengthened by the findings that in patients who received only chemotherapy, only rs8073069 had a significantly increased risk on the prognosis of non-small cell lung cancer survival, and in another study, only this SNP showed a difference in the median progression-free survival (23, 24). Besides its effect in other diseases, the present study supports the role of rs8073069 in the development of asthma. Contrary to this

Table 4. Results of association analyses of BIRC5 SNPs with different asthma phenotypes

	rs8073903			rs8073069			rs17878467			rs9904341			rs1508147		
	P-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI
Asthma	**0.004**	1.458	[1.126–1.889]	0.017*	1.393	[1.061–1.830]	0.263	1.276	[0.832–1.959]	0.373	0.887	[0.683–1.154]	0.08	1.266	[0.972–1.648]
Asthma in females	**0.003**	1.872	[1.232–2.843]	**0.003**	1.887	[1.238–2.878]	0.918	1.033	[0.547–1.954]	0.585	0.893	[0.596–1.340]	0.017*	1.683	[1.096–2.585]
Asthma in males	0.195	1.245	[0.896–1.734]	0.553	1.115	[0.778–1.596]	0.163	1.516	[0.845–2.724]	0.491	0.885	[0.627–1.251]	0.756	1.055	[0.752–1.480]
Allergic asthma	0.927	1.016	[0.720–1.434]	0.813	0.956	[0.660–1.386]	0.576	1.174	[0.669–2.062]	0.818	0.959	[0.675–1.364]	0.618	1.095	[0.765–1.569]
Allergic asthma in females	0.089	1.739	[0.917–3.299]	0.339	1.383	[0.711–2.691]	0.387	1.502	[0.597–3.783]	0.11	0.622	[0.347–1.114]	0.210	1.512	[0.791–2.893]
Allergic asthma in males	0.449	0.845	[0.548–1.305]	0.421	0.825	[0.518–1.317]	0.783	1.108	[0.531–2.313]	0.488	1.178	[0.740–1.876]	0.853	1.043	[0.662–1.647]
Infection-induced asthma	0.792	0.952	[0.661–1.371]	0.500	0.871	[0.584–1.301]	0.365	1.300	[0.736–2.297]	0.220	0.788	[0.539–1.153]	0.870	0.968	[0.663–1.417]
Infection-induced asthma in females	0.518	0.806	[0.420–1.549]	0.528	0.822	[0.448–1.511]	0.920	0.953	[0.377–2.416]	0.528	0.822	[0.448–1.511]	0.670	0.867	[0.448–1.680]
Infection-induced asthma in males	0.852	1.042	[0.672–1.617]	0.634	0.887	[0.544–1.449]	0.190	1.619	[0.787–3.333]	0.260	0.753	[0.460–1.234]	0.906	1.028	[0.644–1.642]
Non-allergic asthma	0.022*	1.622	[1.072–2.452]	**0.001**	2.010	[1.336–3.024]	0.347	0.700	[0.333–1.473]	0.099	1.428	[0.935–2.183]	0.101	1.433	[0.933–2.202]
Non-allergic asthma in females	**0.005**	2.808	[1.372–5.746]	**0.001**	3.096	[1.569–6.109]	0.440	0.648	[0.216–1.950]	0.141	1.606	[0.855–3.019]	**0.003**	3.057	[1.445–6.466]
Non-allergic asthma in males	0.708	1.111	[0.641–1.923]	0.172	1.464	[0.847–2.533]	0.461	0.681	[0.245–1.895]	0.242	1.420	[0.788–2.559]	0.081	1.701	[0.937–3.086]
Exercise-induced asthma	0.588	0.893	[0.594–1.344]	0.169	0.720	[0.450–1.151]	0.181	1.514	[0.823–2.786]	0.381	1.200	[0.797–1.808]	0.892	1.029	[0.672–1.579]
Exercise-induced asthma in females	0.921	1.036	[0.510–2.108]	0.696	0.859	[0.401–1.84]	0.538	1.342	[0.525–3.432]	0.729	0.889	[0.459–1.726]	0.500	1.298	[0.606–2.782]
Exercise-induced asthma in males	0.445	0.821	[0.495–1.362]	0.159	0.646	[0.352–1.187]	0.241	1.618	[0.723–3.625]	0.154	1.462	[0.867–2.466]	0.663	0.888	[0.522–1.512]
Absolute eosinophil count	0.232	1.036	[−0.023 to 0.094]	0.506	1.022	[−0.043 to 0.086]	0.366	1.043	[−0.051 to 0.138]	**0.004**	0.917	[−0.145 to −0.026]	0.384	1.028	[−0.036 to 0.092]
Relative eosinophil level	0.140	1.836	[−0.203 to 1.422]	0.436	1.413	[−0.530 to 1.222]	0.193	2.44	[−0.456 to 2.239]	**0.002**	0.272	[−2.132 to −0.468]	0.247	1.698	[−0.371 to 1.431]
IgE level	0.917	0.978	[0.649–1.476]	0.471	1.172	[0.760–1.810]	0.175	0.624	[0.316–1.234]	0.135	1.380	[0.904–2.106]	0.853	1.043	[0.664–1.639]

Linear regression models adjusted for age and gender were used to study correlations between absolute/relative eosinophil levels and BIRC5 SNPs, in other cases, we used logistic regression models adjusted for age and gender. Bold characters represent *P*-values reaching the Bonferroni-corrected significance threshold (0.01). In case of linear regression analyses, ORs were calculated from the B coefficients.
*indicate nominally significant *P*-values.

Table 5. Summary of BIRC5 haplotypes that influence risk of asthma

	Haplotype	Frequency in group1	Frequency in group2	OR (95% CI)	*P*-value
		Control	Asthma		
Asthma, rs8073903-rs8073069	TG	0.651	0.574	0.721 (0.576–0.902)	**0.004**
	CC	0.253	0.311	1.336 (1.048–1.703)	**0.019**
	CG	0.096	0.111	1.182 (0.862–1.690)	0.379
		Other asthma	Non-allergic asthma		
Non-allergic asthma, rs8073903-rs8073069	TG	0.606	0.440	0.512 (0.341–0.769)	**0.001**
	CC	0.274	0.466	2.308 (1.528–3.486)	**0.00005**
	CG	0.120	0.077	0.606 (0.291–1.260)	0.182
		Low	High		
Serum eosinophil ratio, rs8073903-rs8073069-rs17878467-rs9904341-rs1508147	TGCCG	0.335	0.207	0.508 (0.296–0.871)	**0.015**
	TGCGG	0.235	0.318	1.491 (0.901–2.466)	0.1017
	CCCGA	0.252	0.228	0.893 (0.524–1.523)	0.6219
	CGTGA	0.087	0.148	1.830 (0.914–3.665)	0.0846

Differences of haplotype frequencies between study groups were calculated by MedCalc 10.0.2 software. Bold characters represent P-values reaching the 0.05 significance threshold.

SNP, other studies have not shown any association of rs8073903 (−644 C/T) with pathological outcomes, so far.

In a recent genome wide association study of global gene expression, Dixon *et al.* (25) found that rs1508147 had the highest impact on BIRC5 expression (LOD score = 19.89).

It is worth mentioning that according to HapMap data (release #27, NCBI Build 36) (26), rs1508147 is in strong LD ($D' = 1$, $r^2 = 1$) with 11 other SNPs (9 in introns, 2 in 3′-UTR region), which may carry the causative alleles for the observed effects. Additionally, the 3′ localization of rs1508147 and the two other SNPs in strong linkage disequilibrium raises the possibility of disrupting or creating a miRNA binding site, but with the available miRNA predicting tools, we were not able to confirm this hypothesis.

Since there is increasing evidence that BIRC5 plays an important role in the regulation of eosinophil survival, we investigated if there was any association between the studied SNPs and serum eosinophil or IgE levels. We found that individuals homozygous for the wild-type allele of rs9904341 had elevated absolute and relative eosinophil levels in their serum compared with those carrying either one or two copies of the rare allele. This result was confirmed by haplotype analysis, however, none of the haplotypes involving rs9904341 was found to carry higher risk for eosinophilia than the SNP alone. Although it is still plausible that there are other interacting genetic variants that modify the effect of rs9904341 but were not studied here.

Rs9904341 (−31 G/C) is the most studied genetic variation in the BIRC5 promoter region. It is located within the cell cycle-dependent element/cell cycle homology region of BIRC5 promoter and therefore regulates the cell-cycle dependent expression of the BIRC5 gene. There are many association studies reporting that −31C allele confers an increased risk of some cancer types, however, the results are quite contradictory (27–29). Moreover, in a recent meta-analysis of the genetic association studies of −31 G/C polymorphism, the increased cancer risk was proved to be significant in Asian population only (30).

It is also plausible that BIRC5 expression is regulated by not only these commonly studied polymorphisms but other genetic or epigenetic interactions, as it has already been discussed in connection with breast carcinomas (31). In addition, in case of some of our studied polymorphisms, the greater ORs resulting from association tests of haplotypes compared with those of individual SNPs also reflect a synergistic influence of the promoter variants on BIRC5 transcription. Therefore, we suppose that studies examining BIRC5 haplotypes rather than single SNPs may solve the problem of why contradictory results appeared in the topic.

In brief, our results provide evidences for the first time that sputum samples of patients with asthma contain elevated BIRC5 mRNA levels compared with healthy subjects. The observed correlation between sputum eosinophil levels and BIRC5 mRNA expression levels suggest that survivin has an important role in eosinophil accumulation detected in the airways of asthma patients. Supporting these findings, our SNP association analyses indicate that common polymorphisms located in the regulatory regions of BIRC5 have high impact on the development of asthma phenotypes.

Here, we have to mention that the main limitation of our study is probably the insufficient number of induced sputum samples for testing the direct relationship between the studied genetic variations and BIRC5 mRNA levels.

Concluding, we propose that in eosinophils, the apoptotic process may be regulated by the anti-apoptotic protein BIRC5 that can be regarded as a new therapeutic target in eosinophilic asthma.

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