

Identification of Relationships Between Interleukin 15 mRNA and Brain-Derived Neurotrophic Factor II mRNA Levels With Formal Components of Temperament in Asthmatic Patients

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Abstract Asthma is a chronic inflammatory and heterogeneous disease developing mostly through allergic inflammation, which modifies the expression of various cytokines and neurotrophins. Previous studies suggest the involvement of interleukin (IL)-15 in the regulation of immune response in asthma. Brain-derived neurotrophic factor (BDNF) II plays an important role as a regulator of development and survival of neurons as well as maintenance of their physiological activity. Chronic stress associated with asthma and elevated IL-15 mRNA and BDNFII mRNA levels may affect the mood and a subjective sensation of dyspnoea-inducing anxiety. Psychopathological variables and numerous cytokine/neurotrophin interactions influence the formation of temperament and strategies of coping with stress. The aim of the study was to identify the role of IL-15 mRNA and BDNFII mRNA

expressions and their effect on components of temperament and strategies of coping with stress in asthmatics. A total of 352 subjects (176 healthy volunteers and 176 asthmatic patients) participated in the study. The Formal Characteristic of Behaviour-Temperament Inventory (FCB-TI), Coping Inventory for Stressful Situations (CISS), Beck Depression Inventory, State-Trait Anxiety Inventory, and Borg Rating of Perceived Exertion (RPE) Scale were applied in all the subjects. The expression of IL-15 and BDNFII gene was measured using quantitative real-time polymerase chain reaction (qRT-PCR). Different levels of IL-15 and BDNFII expressions between healthy volunteers and patients were revealed in the study. IL-15 enhanced the BDNFII mRNA expression among patients with bronchial asthma. The depression level negatively correlated with the BDNFII mRNA expression.

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This neurotrophin modified the temperament variable. BDNFII significantly affected (proportional relationship) the level of briskness in asthmatic patients. BDNFII might influence the level and style of coping with stress (emotion-oriented style). This hypothesis requires further studies on protein functional models. The obtained data confirms the role of IL-15 and BDNFII in the pathomechanisms of depression and formation of selected traits defining the temperament in asthmatics.

Keywords Personality traits (temperament) · Strategies of coping with stress · Depression · Anxiety · Dyspnoea · IL-15 mRNA expression · BDNFII mRNA expression · Asthma · Inflammation

Background

Asthma is a chronic heterogeneous inflammatory disease of the bronchi, whose expression is determined by genetic and environmental factors. Mutual interactions of these variables significantly affect the clinical course of the disease [1, 2]. Asthma-related comorbidities and affective variables remarkably model the intensity of symptoms and the response to treatment [3–5]. Chronic inflammation, specific changes in microvascularisation, neuronal degeneration and plasticity depend on the expression of numerous cytokines and the activity of neurotrophic factors influencing formal components of temperament and strategies of coping with stress in asthmatic patients [3, 6–8]. These sets of hereditary personality traits are genetically determined and also rely on the impact of many external factors [3, 9–13]. Interleukins (IL) and neurotrophic growth factors can strongly influence formal components of temperament and strategies of coping with stress in asthmatic patients, being responsible for the clinical course of the disease. IL-15 has a significant effect on neural behaviour and serotonin transmission in the hippocampus [14, 15]. The expression level of interleukin alone as well as its receptor can be increased by proinflammatory and autoimmune challenges at the blood–brain barrier in different regions of the central nervous system (CNS) [16–19]. IL-15 binds to the heterotrimeric receptor complex causing activation of Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway in the target cells [20, 21]. This pathway is regulated by gamma-aminobutyric acid (GABA), transmission, circadian rhythms, locomotor activity, as well as anxiety level, and it affects CNS functions [15, 18, 19, 22, 23]. Some neurotrophic factors have been found to demonstrate elevated expression in allergic diseases, e.g. in asthma. Increased levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) have been observed in the airways [7, 24–27]. The airway epithelium is a main source of NGF and BDNF; however, they are also subjected to constitutive expression in

macrophages and monocytes [7]. BDNF belongs to the neurotrophin family and can occur in many isoforms (specific splice variant expression). The role of a neurotrophin and its isoforms in asthma remains unclear. Proinflammatory cytokines, chemokines, adhesive molecules and acute phase reactants influence, firstly, the changed metabolism of particular neurotransmitters, i.e. serotonin (5HT) and dopamine, secondly, activation of corticotrophin-releasing hormone in the paraventricular nucleus and the following production and/or secretion of adrenocorticotrophic hormone and glucocorticoids (cortisol) and, thirdly, disruption of synaptic plasticity through alterations in some growth factors (e.g. BDNF) [28–34]. All these factors lead to stress–immune interactions and the secondary development of affective disorders, e.g. depression [34]. Thus, BDNF regulates the level of patients' mood. Its expression is affected by psychoactive substances, nutritional disorders, pain, infections, stress and metabolic disorders. The BDNF impact on the development of Alzheimer's and Huntington's disease as well as on autism and depression has been shown [6, 35–40]. Attention should be paid to the fact that asthma is a chronic stress for the patient, and inflammatory mediators in this disease are factors that change cerebral metabolism. Both the disease and cytokines have common genetic factors. Additionally, medications (e.g. glucocorticosteroids) used in asthma can induce mood disorders. The asthmatic patient experiences chronic stress and is characterized by acquired helplessness, accompanied by the deficit of positive stimuli and the excess of negative ones. This contributes to activation of the autonomous nervous system and the hypothalamic-pituitary-adrenal axis, leading to hippocampus impairment and inducing mood and anxiety disorders [3–5, 8, 10, 13, 41–44]. The above factors influence and determine formal components of temperament and strategies of coping with stress in asthmatic patients [3, 5, 8–13, 45, 46].

Aim of the Study

The aim of the study was to identify the role of IL-15 mRNA and BDNFII mRNA (specific splice variant II expression), as well as to evaluate the effect/correlation of their expressions on formal components of temperament (briskness, perseverance, sensory sensitivity, emotional reactivity, endurance, activity) and strategies of coping with stress (task-oriented coping, emotion-oriented coping, avoidance-oriented coping, distraction seeking, social diversion) in asthmatic patients.

Material and Methods

Ethical Approval

The study was approved by the local ethics committee (consent of the Research Review Board of the Medical University

of Lodz, Łódź, Poland; no. RNN/133/09/KE). At the beginning of the study, the participants were invited to attend the investigations voluntarily, and written informed consent was obtained from every patient prior to enrolment.

The study was performed by two investigators who were unaware of the participants' phenotypes.

A total of 176 patients with asthma (112 women and 64 men) and 176 healthy volunteers (117 women and 59 men) participated in the study. Mean age in the healthy group was 46.77 years (from 20 to 80 years), and standard deviation (SD) was 16.10 years. Mean age of asthmatic patients was 49.94 years (from 19 to 81 years), SD being 15.42 years. Table 1 presents a detailed characteristic of the study groups.

Patients with asthma were selected from the patients of the Department of Internal Medicine, Asthma and Allergy of the Norbert Barlicki University Clinical Hospital No. 1 in Łódź, Poland, the Department of Pneumology and Allergology of the Norbert Barlicki University Clinical Hospital No. 1 in Łódź, Poland, and the Specialist Outpatient Clinic of Pulmonary Diseases and Allergology at the same hospital [3, 4, 8, 13]. The patients who had been diagnosed with chronic obstructive pulmonary disease, cardiac failure of III and IV class according to the NYHA Functional Classification, renal failure, liver insufficiency, thyroid diseases, diabetes, autoimmune diseases, neoplastic diseases, unstable angina pectoris and other severe systemic diseases were excluded from the study [3, 4, 8, 13, 47–50].

Healthy subjects included in the study were randomly selected from a general population. The control group consisted only of healthy subjects who met all the required criteria: negative results of skin prick tests for common allergens, lack of first-degree relatives with asthma, allergy or atopy, lack of

therapy with rifampicin, phenobarbital, phenytoin or ephedrine, lack of a physical examination and anamnesis confirming the occurrence of asthma, other pulmonary diseases, allergy, atopy and hypersensitivity to non-steroidal anti-inflammatory medications and lack of a spirometric test confirming obstruction [3, 4, 8, 13, 47–50].

Functional tests of the respiratory system were conducted in compliance with ERS/ATS guidelines [51]. Skin prick tests were carried out in compliance with EAACI guidelines [52]. Temperament was assessed with the application of the Formal Characteristic of Behaviour (FCZ; briskness, perseverance, sensory sensitivity, emotional reactivity, endurance, activity)-Temperament Inventory (KT; STRELAU) [53]. Coping Inventory for Stressful Situations (CISS: SSZ—task-oriented coping, SSE—emotion-oriented coping, SSU—avoidance-oriented coping, ACZ—distraction seeking, PKT—social diversion) was used to evaluate the degree of coping with stress [54]. In all subjects, the authors applied the Beck Depression Inventory in a version recommended by Puzyński and Wciórka [55, 56]. Trait and state anxiety was measured by the Spielberger State-Trait Anxiety Inventory, a version modified by Wrześniewski et al. and issued by the Psychological Test Laboratory of the Polish Psychological Association [57].

Venous blood samples were collected from the participants onto tripotassium ethylenediaminetetraacetic acid (EDTA-K3; SARSTEDT AG & Co., Nümbrecht, Germany). DNA was obtained from the peripheral blood leukocyte fraction. The genetic material was isolated using QIAamp DNA Blood Mini Kit (QIAGEN Inc.) according to the guidelines provided by the manufacturer [48–50].

Total RNA (10 µg) was extracted from the peripheral blood lymphocytes using a RNA extraction reagent (TRI Reagent®

Table 1 Description of spirometric characteristics of the studied groups

Parameters	Patients with asthma		Healthy		<i>p</i>
	<i>N</i> , %		<i>N</i>		
Sex: f vs m	112, 63.64 % vs 64, 36.36 %		117, 66.48 % vs 59, 33.52 %		0.5762
Division of asthma according to GINA:			—		—
-Mild	30, 17.05 %				
-Moderate	84, 47.73 %				
-Severe	62, 35.22 %				
	<i>N</i>	Me (IQR)	<i>N</i>	Me (IQR)	
Age [years]	176	52.0 (37.0–62.0)	175	48.0 (31.0–58.0)	0.0731
FEV1% predicted value	175	74.0 (60.0–88.0)	176	96.0 (90.0–102.0)	<0.0001
FVC% predicted value	175	93.0 (80.0–104.0)	176	101.0 (93.0–111.0)	<0.0001
FEV1% FVC%	175	67.69 (57.14–77.0)	176	78.6 (74.4–83.3)	<0.0001

FEV1 forced expiratory volume in 1 s (expressed in litres), *FEV1% A/N%*—percentage ratio of the measured to predicted value (expressed as percent of the predicted value), *FVC* forced vital capacity (expressed in litres), *FVC% A/N%*—percentage ratio of the measured to predicted value (expressed as percent of the predicted value), *FEV1% FVC index* FEV1 to FVC ratio-forced vital capacity (expressed as absolute numbers)

Solution, Ambion, NY, USA), according to the standard acid-guanidinium-phenol-chloroform method [58]. The extracted RNA was analysed by agarose gel electrophoresis, and only cases with preserved 28S, 18S and 5S ribosomal RNA bands indicating good RNA quality were used in the study. Total RNA was digested with DNase (GIBCO) at room temperature for 15 min. The amount of purified RNA was determined using spectrophotometry at 260 nm in a Nanodrop analyser (ND-100; Nanodrop Technologies, Wilmington, DE, USA). The purity was verified according to the ratio of 260/280 nm measurements, such that values between 1.8 and 2.1 indicated that the quality of the RNA obtained was optimal and suitable for the quantitative real-time polymerase chain reaction (qRT-PCR) [58–60]. An Agilent Technologies Stratagene Mx3000P was used for IL-15 and BDNFII quantitative RT-PCR reaction. For each sample, the C_T (threshold cycle) values were calculated with the help of Mx-Pro software. The qRT-PCR amplification of the IL-15 and BDNFII genes was compared to that of glyceraldehydes 3-phosphate dehydrogenase (GAPDH), a housekeeping reference gene, and ΔC_T values were determined ($\Delta C_T = C_{T, \text{GENE}} - C_{T, \text{GAPDH}}$). The qRT-PCR data was automatically calculated with the data analysis module. The results were analysed according to the $2^{-\Delta\Delta C_T}$ method. Validation of PCR efficiency was performed with a standard curve [58–62]. The complementary DNA (cDNA) was subjected to real-time quantitative PCR using gene-specific primers for IL-15, BDNFII and GAPDH with the use of the EvaGreen® and Master Mixes for qRT-PCR (Biotium, Inc., CA, USA) and Phusion® High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Inc., MA, USA). A detailed description of conditions of the qRT-PCR reaction is presented in Table 2.

Statistical Analysis

Nominal variables were given as numbers with appropriate percentage, whereas continuous variables as means with standard deviations or medians with interquartile ranges. Chi-squared test with Yate's correction, Fisher's exact test and Freeman-Halton extension of it were used to test the associations. Continuous variables were analysed using the Mann-Whitney's U test or the analysis of variance (ANOVA) depending on the number of compared groups. Correlation

coefficients were calculated with Spearman's rank test. P levels lower than 0.05 were considered as statistically significant. Statistica 10.0 package (Statsoft, Tulsa, OK, USA) was used for statistical analysis. Due to the repeated testing of research hypotheses, a modified Benjamini–Hochberg procedure (p corrected) was used.

Results

The expression of BDNFII was evaluated in the study population. The expression was found to be heterogenous. The results were observed to be separated into two subpopulations with a low and high level of the neurotrophin. The variable had features of a normal distribution. Analyses showed that the high expression was <-2.095 (35th percentile) and a low one was >-2.095 . The 35th percentile became a cutoff point. Phenotypes of the BDNFII mRNA expression are presented in Fig. 1.

The observed phenomenon of a low/high expression of BDNFII mRNA was considered in further analyses. There was a statistically significant difference in the levels of the BDNFII and IL-15 expression between healthy volunteers and asthmatic patients. The effects of the BDNFII mRNA expression on the level of IL-15 were evaluated. A positive correlation ($R=0.02$), however statistically non-significant, was stated between the neurotrophin and the cytokine ($p=0.0655$) (Fig. 2).

The effect of IL-15 on the level of the BDNFII expression was investigated only in the group of asthmatic patients. A positive strongly significant correlation ($R=0.30$, $p=0.0154$) between the studied cytokine and the neurotrophin was revealed (Fig. 3).

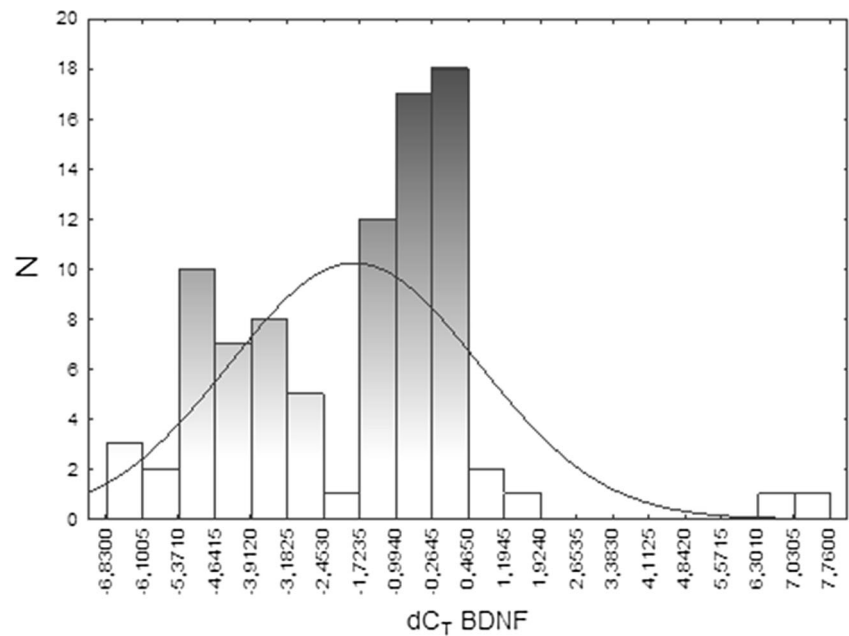
Correlations between formal components of temperament and strategies of coping with stress and the BDNFII mRNA expression were assessed in the entire study population as well as in the group of patients with asthma (Table 3). Attention was drawn in the entire population to an inversely proportional correlation between the emotion-oriented style (both for the absolute result–source data, $R=-0.29$, $p=0.017$, as well as for the result of the normalized test scale–sten scores, $R=-0.27$, $p=0.021$) out of strategies of coping with stress and the BDNFII expression. This correlation appeared to be non-significant after the statistical correction (p corrected).

Table 2 Description of conditions of the qRT-PCR reaction to evaluate the expression of mRNA of studied genes

Studied gene	Technique	Primers	Primer annealing temperature
IL-15	qRT-PCR	Forward primer (5'-GTGTTGATGAACATTTGCAC-3') and reverse primer (5'-AAGTTATTTCACTTGACTCCG-3')	61 °C
BDNFII	qRT-PCR	Forward primer (5'-CAAAAAGTGGAGAACATTTGC-3') and reverse primer (5'-AACTCCAGTCAATAGGTCAG-3')	61 °C

IL interleukin, qRT-PCR quantitative real-time PCR

Fig. 1 Distribution of the BDNFII mRNA expression in the study population (all participants). A detailed description can be found in the text. The authors' own drawing-up. d delta, C_T threshold cycle, N number of observations



Moreover, no relationship between temperamental variables, the level of depression, anxiety (as a trait), dyspnoea, the degree of asthma control and the BDNFII expression was found in the entire population (Table 3). However, the correlation between anxiety-state and BDNF levels was found (Table 3).

A changeable level of a high/low BDNFII mRNA expression was considered in the analyses. The authors noted a relationship between the emotion-oriented coping style (source

data, $p = 0.046$, as well as sten scores, $p = 0.039$) and the level of a high/low BDNFII mRNA expression in the entire population. The statistical correction (p corrected) did not confirm the statistical significance (Table 4).

In the entire population, no relationship between temperamental variables, the level of depression, anxiety (as a state not a trait), dyspnoea, the degree of asthma control and the high/low BDNFII mRNA expression was recorded either (Table 4).

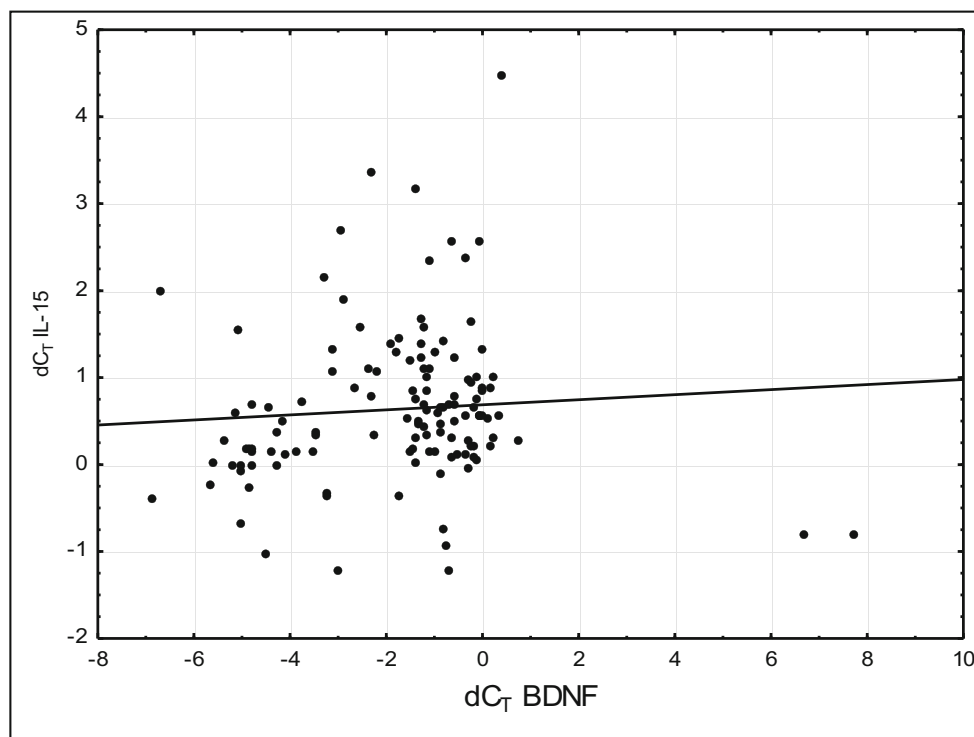


Fig. 2 Correlation between the BDNFII expression and the IL-15 expression (entire population). A detailed description can be found in the text. The authors' own drawing-up. d delta, C_T threshold cycle

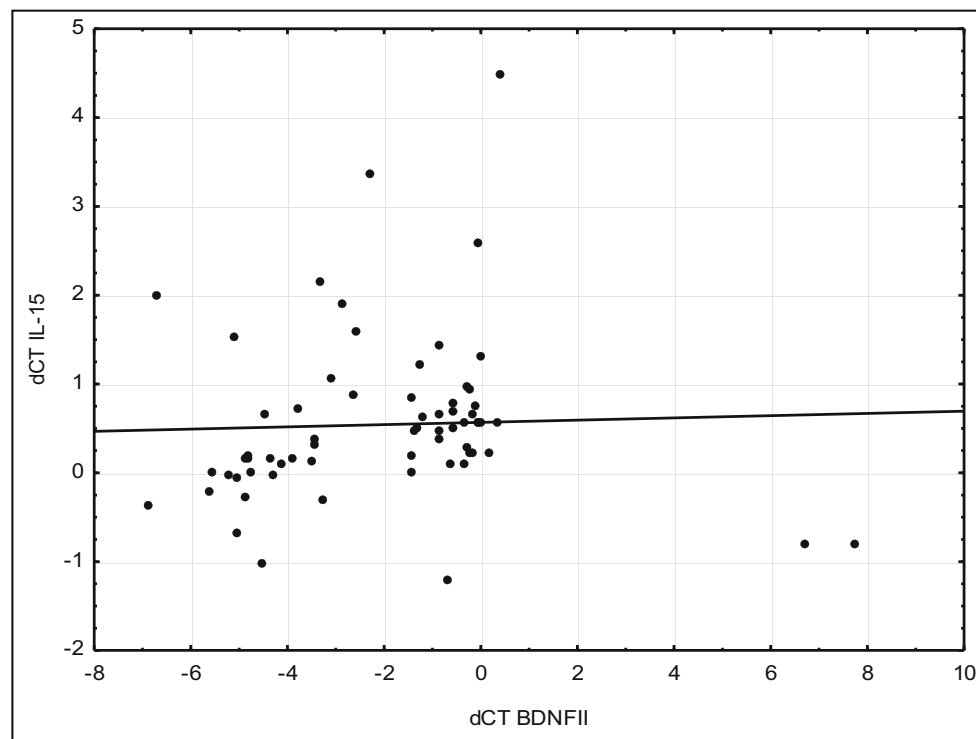


Fig. 3 Correlation between the BDNFII expression and that of IL-15 (only asthmatic patients). A detailed description can be found in the text. The authors' own drawing-up. *d* delta, C_T threshold cycle

The study participants were also analysed separately in subgroups. For asthmatic patients, the relationships with the emotion-oriented style both for absolute and source data were noted; however, after the statistical correction, they appeared to be at $p > 0.05$ (Table 3). In the group of asthmatics, the authors observed a strong correlation between briskness of formal components of temperament for source data and the BDNFII mRNA expression. This correlation was positive and positively verified by the statistical correction ($p = 0.001$, p corrected = 0.022). It is worth mentioning that sten scores were statistically non-significant after using the analytical correction. The details are shown in Table 3. Additionally, the inversely proportional correlation ($R = -0.21$) between the level of depression and the BDNFII expression at the significance level of $p = 0.037$ (Table 3) was noted. There were no relationships between the level of anxiety as a state and a trait, the level of dyspnoea, the degree of asthma control and the BDNFII mRNA expression (Table 3).

Analysing the subgroup of patients with asthma in the context of its impact on the high/low BDNFII mRNA expression (Table 4), the correlation was found between the emotion-oriented coping style for the absolute result (source data, $p = 0.021$) and the result of normalized test scale (sten scores, $p = 0.019$), whose significance level after the statistical correction was $p > 0.05$. Briskness was also correlated with the low/high expression of the neurotrophin; however, after the statistical correction, p corrected was 0.146. No statistical

relationships were noticed for other temperamental components, as well as for the level of depression, anxiety, dyspnoea and the degree of asthma control (Table 4).

Levels and the effect of IL-15 on formal components of temperament and strategies of coping with stress as well as on psychopathological variables were evaluated in the entire study population and in the subgroup of asthmatic patients. Additionally, the effect of this cytokine expression on the level of dyspnoea and the degree of asthma control was considered in the analyses (Table 3).

The authors recorded a correlation between activity (source data, $R = -0.35$; sten scores, $R = -0.33$) of formal temperamental components and the IL-15 expression at the level of $p = 0.028$ and 0.044, respectively, in the entire study population. These results were $p = 0.246$ and $p = 0.264$, respectively, after the statistical correction. No statistical relationships were registered for strategies of coping with stress, psychopathological variables (level of depression and anxiety), level of dyspnoea and asthma control.

In the subgroup of asthmatic patients, among all analysed variables of formal components of temperament, strategies of coping with stress, as well as psychopathological variables, only internal inversely proportional ($R = -0.34$) relationship of activity expressed by the source data ($p = 0.038$) was observed, which after the correction showed an insignificant level of p (p corrected = 0.258). Details are shown in Table 3.

Table 3 Analysis of the relationships between parameters of CISS tests and FCZ-KT questionnaire trait scores in groups determined by the BDNFII mRNA and IL-15 mRNA expressions in the studied groups

Parameters			dC _T BDNFII								dC _T IL-15							
			Entire population				Asthmatic patients				Entire population				Asthmatic patients			
Studied variable			<i>N</i>	<i>R</i>	<i>p1</i>	<i>p2</i>	<i>N</i>	<i>R</i>	<i>p1</i>	<i>p2</i>	<i>N</i>	<i>R</i>	<i>p1</i>	<i>p2</i>	<i>N</i>	<i>R</i>	<i>p1</i>	<i>p2</i>
CISS	SSZ	Source data	68	−0.08	0.50	0.11	24	−0.18	0.40	0.63	37	0.05	0.77	0.77	36	0.06	0.71	0.71
		Sten scores	69	−0.13	0.30	0.11	25	−0.15	0.47	0.63	38	0.07	0.69	0.76	37	0.08	0.62	0.76
	SSE	Source data	69	−0.29	0.02	0.17	24	−0.42	0.04	0.25	37	−0.17	0.31	0.44	36	−0.22	0.20	0.44
		Sten scores	70	−0.27	0.02	0.17	25	−0.40	0.05	0.25	38	−0.09	0.58	0.73	37	−0.14	0.42	0.73
	SSU	Source data	69	−0.08	0.49	0.17	24	−0.08	0.72	0.72	37	−0.26	0.12	0.37	36	−0.24	0.16	0.37
		Sten scores	70	−0.12	0.31	0.17	25	−0.10	0.64	0.71	38	−0.29	0.08	0.37	37	−0.26	0.12	0.37
	ACZ	Source data	69	−0.21	0.08	0.38	24	−0.26	0.23	0.63	37	−0.26	0.13	0.37	36	−0.24	0.16	0.37
		Sten scores	70	−0.20	0.10	0.38	25	−0.18	0.40	0.63	38	−0.23	0.16	0.37	37	−0.22	0.19	0.37
	PKT	Source data	69	0.21	0.08	0.50	24	0.18	0.40	0.63	37	−0.18	0.29	0.44	36	−0.17	0.33	0.44
		Sten scores	70	0.20	0.10	0.50	25	0.14	0.51	0.63	38	−0.22	0.19	0.37	37	−0.20	0.22	0.37
FCZ-KT	Briskness	Source data	69	0.15	0.21	0.73	24	0.60	<0.01	0.02	38	0.07	0.69	0.91	37	0.08	0.65	0.91
		Stanine scores	69	0.12	0.33	0.73	24	0.49	0.02	0.09	38	0.11	0.51	0.91	37	0.12	0.50	0.91
	Perseverance	Source data	69	−0.01	0.91	0.95	24	−0.04	0.87	0.95	38	−0.22	0.18	0.54	37	−0.26	0.12	0.54
		Stanine scores	69	−0.01	0.95	0.95	24	−0.01	0.95	0.95	38	−0.28	0.09	0.37	37	−0.31	0.06	0.37
	Emotional reactivity	Source data	69	0.03	0.83	0.95	24	−0.20	0.36	0.73	38	0.00	0.99	0.99	37	0.03	0.87	0.99
		Stanine scores	69	−0.05	0.69	0.92	24	−0.27	0.20	0.59	38	−0.05	0.74	0.91	37	−0.03	0.88	0.91
	Sensory sensitivity	Source data	69	−0.12	0.32	0.73	24	−0.28	0.19	0.59	38	−0.09	0.59	0.91	37	−0.14	0.42	0.91
		Stanine scores	69	−0.12	0.33	0.73	24	−0.13	0.55	0.73	38	−0.15	0.36	0.87	37	−0.20	0.23	0.87
	Endurance	Source data	69	0.10	0.42	0.73	24	0.15	0.48	0.73	38	0.03	0.84	0.91	37	0.07	0.67	0.91
		Stanine scores	69	0.08	0.49	0.74	24	0.15	0.50	0.73	38	0.05	0.78	0.91	37	0.09	0.60	0.91
	Activity	Source data	69	0.14	0.27	0.73	24	0.15	0.48	0.73	38	−0.35	0.03	0.26	37	−0.34	0.04	0.26
		Stanine scores	69	0.10	0.42	0.73	24	−0.08	0.69	0.83	38	−0.33	0.04	0.26	37	−0.32	0.06	0.26
STAI	Form X-1		211	−0.01	0.84		99	−0.21	0.04		176	−0.06	0.42	0.42	117	−0.03	0.42	
	Form X-2		211	−0.01	0.85		99	−0.05	0.63		176	−0.05	0.54	0.54	117	−0.10	0.54	
Beck Depression Inventory			211	−0.06	0.40		99	−0.10	0.34		176	−0.07	0.34	0.34	117	−0.10	0.34	
Borg Scale			211	0.08	0.25		100	−0.02	0.86		175	0.02	0.79	0.79	118	0.05	0.79	
Asthma Control Test (ACT)			109	0.08	0.42		109	0.08	0.42		128	−0.04	0.62	0.62	128	−0.04	0.62	

Detailed data presented in the text. The author's own analysis

Bold Statistically significant values

CISS strategies of coping with stress (SSZ—task-oriented coping, SSE—emotion-oriented coping, SSU—avoidance-oriented coping, ACZ—distraction seeking, PKT—social diversion). FCZ-KT temperamental components (briskness, perseverance, emotional reactivity, sensory sensitivity, endurance, activity). ACT™ Asthma Control Test, Beck Depression Inventory depression level, STAI Form X-1 The State-Trait Anxiety Inventory-State, STAI Form X-2 The State-Trait Anxiety Inventory-Trait, Borg Scale dyspnoea level, dC_T delta cycle threshold values, *p1* coefficient of statistical significance, *p2* corrected coefficient of statistical significance, *N* number, *R* rank correlation coefficient

Discussion

Asthma is a heterogeneous disease accompanied by pathophysiological and biochemical changes. They are an outcome of the activity of external, i.e. environmental, and internal factors, which are regulated by various gens. Asthma is a chronic disease leading to dysfunction and impairment of organs, where clinical symptoms of the disease can be observed. Stress constantly accompanying asthmatic patients induces

exhaustion of the organism and its immune and hormonal reserves, suppressing the action of neurotransmitters. Stress reaction results in internalization of negative emotions through maintaining prolonged tension and state of readiness to act [63]. The patient experiences strong negative emotions. Thus, asthma becomes a psychosomatic disease [9–11, 63]. Strong dysregulating feelings accompanying asthma, such as anxiety, fear, panic, phobia, hostility, lowered mood or aggression, cause the disproportion between demands (put forward

Table 4 Analysis of the relationships (median values with interquartile ranges) between parameters of CISS tests and FCZ-KT questionnaire trait scores in groups determined by the low/high BDNFII mRNA expression in the studied groups

Studied variable			Studied populations							
			Entire population				Asthmatic patients			
			dC _T high BDNFII	dC _T low BDNFII	p1	p2	dC _T high BDNFII	dC _T low BDNFII	p1	p2
CISS	SSZ	Source data	58 (53–62)	57 (52–60)	0.40	0.44	58 (54–62)	53 (50–60)	0.38	0.51
		Sten scores	6 (5–7)	5 (5–6)	0.33	0.41	6 (5–7)	5 (4–6)	0.30	0.50
	SSE	Source data	48 (38–56)	41 (34–47)	0.05	0.16	52 (45–57)	42 (37–45)	0.02	0.11
		Sten scores	6 (4–7)	5 (3–6)	0.04	0.16	7 (6–8)	5 (4–6)	0.02	0.11
	SSU	Source data	48 (39–54.5)	46 (41–51)	0.60	0.60	49 (44–56)	45 (42–51)	0.41	0.51
		Sten scores	6 (5–8)	6 (5–7)	0.33	0.41	6 (5.5–8)	5 (5–7)	0.21	0.41
	ACZ	Source data	21.5 (18–27.5)	21 (17–23)	0.10	0.17	23 (19–29)	19 (15–24)	0.07	0.24
		Sten scores	6 (5–8)	6 (5–7)	0.07	0.16	7 (5–8.5)	5 (4–7)	0.10	0.24
FCZ-KT	PKT	Source data	16 (14–18.5)	18 (16–20)	0.06	0.16	17 (15–20)	17 (15–20)	0.82	0.82
		Sten scores	5 (4–6)	6 (5–7)	0.08	0.16	6 (5–6.5)	6 (5–7)	0.76	0.82
	Briskness	Source data	14.5 (11.5–17)	16 (12–18)	0.34	0.88	11 (8–13)	16 (14–17)	0.01	0.15
		Stanine scores	5 (4–6)	5 (4–7)	0.44	0.88	4 (3–4)	5 (4–6)	0.14	0.55
	Perseverance	Source data	13 (9–15)	13 (11–16)	0.93	0.95	14 (8–16)	12 (11–13)	0.14	0.55
		Stanine scores	5 (3–6)	5 (4–6)	0.86	0.95	6 (3–7)	5 (4–6)	0.19	0.58
	Emotional reactivity	Source data	14.5 (12–16.5)	14 (12–17)	0.72	0.95	13 (11–16)	14 (12–14)	0.45	0.92
		Stanine scores	5 (3–6)	4 (3–6)	0.95	0.95	5 (2–7)	4 (3–4)	0.52	0.92
	Sensory sensitivity	Source data	12 (7.5–14.5)	11 (6–14)	0.41	0.88	13 (10–15)	10 (8–12)	0.73	0.92
		Stanine scores	5 (4–6)	4 (3–6)	0.24	0.88	5 (4–6)	4 (4–5)	0.73	0.92
	Endurance	Source data	8.5 (4–11.5)	8 (4–14)	0.72	0.95	4 (3–9)	7 (3–11)	0.73	0.92
		Stanine scores	4.5 (3–6)	5 (3–7)	0.78	0.95	4 (3–5)	4 (2–7)	0.77	0.92
	Activity	Source data	8 (5–11)	8 (6–12)	0.37	0.88	6 (4–11)	9 (8–12)	0.91	0.95
		Stanine scores	5 (4–7)	5 (4–7)	0.44	0.88	5 (4–7)	5 (5–6)	0.95	0.95
STAI	Form X-1		37 (30–43)	37 (31–44)	0.40	–	37 (29–42.5)	39 (32–44)	0.05	–
	Form X-2		41 (35–48)	42 (35–48)	0.59	–	42.5 (37–49)	42 (35–47)	0.67	–
Beck Depression Inventory			9 (3–15)	7.5 (4–13)	0.58	–	11.5 (5.5–19)	9 (5–14)	0.63	–
Borg Scale			2 (0–4)	3 (0–4)	0.89	–	4 (2–5)	4 (2–5)	0.41	–
Asthma Control Test (ACT)			–	–	–	–	17.5 (14–21)	19 (13–22)	0.43	–

Detailed data presented in the text. The author's own analysis

CISS strategies of coping with stress (SSZ—task-oriented coping, SSE—emotion-oriented coping, SSU—avoidance-oriented coping, ACZ—distraction seeking, PKT—social diversion), FCZ-KT temperamental components (briskness, perseverance, emotional reactivity, sensory sensitivity, endurance, activity), ACT™ Asthma Control Test, Beck Depression Inventory depression level, STAI Form X-1 The State-Trait Anxiety Inventory-State, STAI Form X-2 The State-Trait Anxiety Inventory-Trait, Borg Scale dyspnoea level, dC_T delta cycle threshold values, p1 coefficient of statistical significance, p2 corrected coefficient of statistical significance

by patients themselves, their families, physicians or environment), expectations and a real inability to cope with them [63]. When we consider asthma as a psychosomatic disease, it seems particularly interesting to understand the role of the BDNFII neurotrophin (specific splice variant II expression) and the IL-15 cytokine in the regulation of inflammation ongoing in the course of this nosologic entity and in modelling the expression of personality components, temperament and the style of coping with stress. The study attempts to indicate and elucidate a crucial role of BDNF, especially its isoform II in understanding the pathogenesis of asthma as a psychosomatic disease.

Neuronal mechanisms are activated by allergic stimuli and play a significant role in immunoregulation of allergic inflammation through numerous cytokines and mediators via the tyrosine kinase receptor A (TrkA, NTRK1) located on mastocytes [7]. Moreover, neurotrophins, e.g. BDNFII, undergo constitutive expression on macrophages and monocytes. Neurotrophic factors have been found to undergo a higher expression in allergic diseases [7]. Experiments on animal models have shown that neurotrophins are involved in the complex bronchial hyperresponsiveness and airway obstruction. They are secreted during the inflammatory response with

the participation of eosinophils, which enhances the spreading inflammation [7, 24, 25]. Additionally, the airway epithelium is a source of BDNFII. The present study has revealed that the level of BDNFII increases with the increase in the IL-15 expression (Figs. 2 and 3). In the light of our analyses, IL-15 alone does not seem to exert a significant effect on the level of formal components of temperament and strategies of coping with stress, as well as on the level of depression or anxiety (as a state and a trait). However, internal analyses in subgroups indicate its potential involvement only in the regulation of patients' activity, which in the authors' opinion requires further research.

The BDNFII expression, observed in this study, is heterogeneous because the study groups are not homogeneous (Fig. 1). Numerous external factors affect both healthy and ill subjects influencing complex adaptive processes in various stressful situations for different age groups, e.g. comorbidities, airway infections, diabetes and metabolic syndromes and disorders [6]. Levels of BDNFII alone may change in stressogenic situations and bind to some personality traits even in healthy subjects [6]. Additionally, asthma alone is not a homogeneous disease and manifests itself in many coexisting and overlapping phenotypes. Involvement of comorbidities, which can superimpose processes affecting maintenance of health and well-/ill-being, plays also a significant role. The most important outcome resulting from our study is the confirmation that BDNFII exerts an impact on the level of depression (inversely proportional correlation), the anxiety as a state and temperamental traits, i.e. on briskness intensity (directly proportional correlation). BDNFII is thus a significant mediator involved in behavioural interactions between the environment and the human organism [6]. The present study correlates the expression of peripherally circulating BDNFII with intensification of depression in asthmatic patients. The relationship between the severity of mood lowering and the BDNFII mRNA expression in serum of patients suffering from asthma has been revealed. The present study confirms previous literature reports on the existence of a negative correlation between the severity of depression symptoms and the BDNFII mRNA expression [6, 64–67]. However, no effects of this neurotrophin either on the level of anxiety as a trait or the intensity of perceived dyspnoea have been noted (Table 3).

It is worth noticing that the BDNFII mRNA expression positively correlates with the level of briskness of formal temperamental components in the group of patients (Table 3). Briskness in the asthmatic patients, due to variation in intensity of symptoms and their paroxysms accompanied by a strong sensation of dyspnoea, anxiety and threat, influences the patients' tendency to change the pace of responding to changing symptoms and their perception. Asthma as a chronic disease that lasts for years can reduce the rate at which patients perform their daily life activities and cause behavioural changes in response to the change in external conditions (e.g. exposure to an antigen which induces instantaneous allergic

reaction) [9–11, 53]. This situation may result from the body's response to chronic pathological state and impaired locomotor activity.

Attention should be drawn in the present study to some tendencies in asthmatic patients associated with strategies of coping with stress, which from the "formal" point of view are statistically non-significant but clinically (research aspect) seem to be very interesting and require further multicentre investigations [3]. BDNFII both in the entire population [healthy vs patients] and in the group of asthmatic patients appears to affect the emotion-oriented style of strategies for coping with stress. This style reflects the characteristic features of patients treated in outpatient allergy and pulmonary clinics. The tendencies among asthmatics to concentrate on themselves, their own problems, experiences and emotions, frequently negative ones, are often observed by physicians, clinical psychologists and psychiatrists [3, 6]. Patients with asthma frequently present unstable emotions and anger, feeling of guilt, anxiety or failure in life. They usually blame themselves for the situation, i.e. for developing lung disease, and demonstrate the tendency towards wishful thinking to reduce the emotional tension associated with the disease and its consequences: dyspnoea, anxiety and depressed mood [3, 4, 6, 8, 13, 47]. Such activities do not always bring benefits. Sometimes they enhance the feeling of nervousness and tension and, in consequence, worsen depression [6]. While discussing this problem, the authors of the present study have found that the observed correlations between the level of the BDNFII mRNA expression and scoring associated with the emotion-oriented style of the CISS questionnaire have not been confirmed in the post hoc analysis. This can be interpreted in two ways. The first possibility is that such a relationship does not exist. The statistical model, which was used, indicates this possibility and it could be accepted to avoid type I error (rejecting the null hypothesis despite its correctness). The authors are however inclined to the second possibility which suggests that in the light of current evidence and theories, this relationship may exist. The Folkman and Lazarus model, the most currently relevant and useful theory about stress and coping strategies, says about two stages of the evaluation of stressogenic situation, namely cognitive and emotional. The cognitive stage consists of non-emotional assessment of the phenomenon, and the emotional one includes the process of providing a certain emotional background to the phenomenon (positive or negative).

Both processes take place *de facto* at the same time, so the way of coping with the stressful situation depends on the proportion between them. Since the CISS questionnaire is consistent with the theory of Folkman and Lazarus, and BDNFII is involved in changes in neural connections associated with cognitive processes of the central nervous system, the reasonable hypothesis implies that the higher the emotional reaction to the stress situation, the lower the involvement of

the cognitive element in coping with the problem/stress. So it seems to the authors that the correlation between the result obtained in the emotion-oriented coping strategy and the level of the BDNFII expression may have clinical relevance [68–73]. However, this hypothesis still requires verification.

Limitations

The study has an associational character. Therefore, the observed associations represent a cause-effect relationship confirmed only by means of statistical methods. The noticed correlations may be connected with many other systems of genetic and environmental factors. Hence, the outcomes of this study should be confirmed by functional research on identified protein factors, providing reliable evidence for the described cause-effect relationships.

The subsequent limitation of this study is a small size of the examined population. It would be advisable to verify our hypotheses on subpopulations consisting of at least 1000 people. Our findings should be regarded as preliminary, taking into account the fact that psychological and neurobiological interpretation of the observations requires more in-depth studies on psychopathological aspects of asthma, with consideration of pathways of statistical analyses.

Summary

It should be emphasized that IL-15 stimulates the proliferation and migration of T lymphocytes, being involved in regulation of the inflammatory response, and increases the BDNFII mRNA expression (specific splice variant II expression) in the group of patients with asthma (Fig. 3). IL-15 in the light of this study does not seem to exert a direct impact on formal components of temperament and strategies of coping with stress. BDNFII is however only one of the members of the neurotrophin family, which occurs in various isoforms and has changeable effects on the differentiation and survival of cells of the neural and immune system [72]. This neurotrophin unquestionably affects the intensity of depression and anxiety state and determines temperamental traits (briskness). In compliance with the findings of this study, BDNFII may also have an impact on the tendency of asthmatic patients to cope with stress, i.e. with the disease; however, this outcome requires further and in-depth neuroimmune and neurobiological research [73, 74]. This study contributes to a better understanding of the role of BDNF and its isoform in asthma.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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