

Analysis of the differences in whole-genome expression related to asthma and obesity

Marta Gruchała-Niedoszytko¹, Marek Niedoszytko², Bahram Sanjabi³,
Pieter van der Vlies³, Piotr Niedoszytko⁴, Ewa Jassem², Sylwia Małgorzewicz¹

¹ Department of Clinical Nutrition, Medical University of Gdansk, Gdańsk, Poland

² Department of Allergology, Medical University of Gdansk, Gdańsk, Poland

³ Department of Genetics, University Medical Center, Groningen, Netherlands

⁴ Department of Medical Rehabilitation Medical University of Gdansk, Gdańsk, Poland

KEY WORDS

asthma, gene
expression,
inflammation, obesity,
prediction model

ABSTRACT

INTRODUCTION Concomitant obesity significantly impairs asthma control. Obese asthmatics show more severe symptoms and an increased use of medications.

OBJECTIVES The primary aim of the study was to identify genes that are differentially expressed in the peripheral blood of asthmatic patients with obesity, asthmatic patients with normal body mass, and obese patients without asthma. Secondly, we investigated whether the analysis of gene expression in peripheral blood may be helpful in the differential diagnosis of obese patients who present with symptoms similar to asthma.

PATIENTS AND METHODS The study group included 15 patients with asthma (9 obese and 6 normal-weight patients), while the control group—13 obese patients in whom asthma was excluded. The analysis of whole-genome expression was performed on RNA samples isolated from peripheral blood.

RESULTS The comparison of gene expression profiles between asthmatic patients with obesity and those with normal body mass revealed a significant difference in 6 genes. The comparison of the expression between controls and normal-weight patients with asthma showed a significant difference in 23 genes. The analysis of genes with a different expression revealed a group of transcripts that may be related to an increased body mass (*PI3*, *LOC100008589*, *RPS6KA3*, *LOC441763*, *IFIT1*, and *LOC100133565*). Based on gene expression results, a prediction model was constructed, which allowed to correctly classify 92% of obese controls and 89% of obese asthmatic patients, resulting in the overall accuracy of the model of 90.9%.

CONCLUSIONS The results of our study showed significant differences in gene expression between obese asthmatic patients compared with asthmatic patients with normal body mass as well as in obese patients without asthma compared with asthmatic patients with normal body mass.

Correspondence to:

Marta Gruchała-Niedoszytko, MD,
PhD, Katedra Żywnienia Klinicznego,
Gdański Uniwersytet Medyczny,
ul. Dębinki 7, 80-210 Gdańsk, Poland,
phone/fax: +48 58 349 27 23,
e-mail: mg@gumed.edu.pl

Received: July 2, 2015.

Revision accepted: August 7, 2015.

Published online: August 7, 2015.

Conflict of interest: none declared.

Pol Arch Med Wewn. 2015;

125 (10): 722-730

Copyright by Medycyna Praktyczna,

Kraków 2015

INTRODUCTION Obesity has become a serious medical issue in most societies in the 21st century. The effect of increased body mass on asthmatic patients has become a rationale for the concept of the asthma phenotype related to obesity, which was listed among the 5 major phenotypes of the disease in the 2014 edition of the Global Initiative for Asthma (GINA).¹ Obesity affects the respiratory system in several ways, ranging from the impairment of chest mechanics to the development of inflammatory processes in bronchial tissue. Systemic inflammation related to obesity

is caused by fat tissue, which acts as an endocrine organ producing proinflammatory adipokines. A recent ex-vivo study on human alveolar macrophages proved the distinguishing role of leptin in obesity-related asthma. Leptin levels in bronchoalveolar lavage fluid were the highest in overweight and obese subjects with asthma compared with normal-weight asthmatics and with overweight and obese patients without asthma. Cultured primary alveolar macrophages obtained from overweight and obese subject with asthma were the most sensitive to *Escherichia*

TABLE 1 Demographic data of the study groups

Parameter	Study groups		
	asthma and normal body mass, n = 6	asthma and obesity, n = 9	obesity without asthma, n = 13
sex, female/male, n	4/2	4/5	11/2
age, y	50 (38–66)	46 (34–64)	47 (24–65)
body mass index, kg/m ²	23 (19–25)	33 (30–39)	39.7 (31–57)

Data are presented as number or median (interquartile range).

coli lipopolysaccharides and generated the highest levels of proinflammatory cytokines, such as interleukin (IL) 5, IL-10, interferon γ , and tumor necrosis factor α (TNF- α), compared with other groups, while the preexposure to leptin enhanced the proinflammatory response. This leptin-sensitive proinflammatory macrophage phenotype in the context of higher leptin concentrations in obesity may play a role in the pathogenesis of obesity-related asthma.² Obesity has a significant adverse effect on asthma control, as reflected by more severe symptoms and increased use of medications. There are also data suggesting that the high serum level of leptin is associated with greater severity of clinical asthma.

Tsaroucha et al³ analyzed the concentrations of 3 adipose tissue-derived hormones—leptin, adiponectin, and ghrelin—in relation to severity of asthma in women. In their study, both the leptin level and leptin-to-adiponectin ratio discriminated between severe and mild or moderate asthma in women. Furthermore, these markers were significantly higher during asthma exacerbation, while serum adiponectin and ghrelin concentrations were significantly lower. These findings suggest that fat tissue-derived hormones may be involved in the pathogenesis of severe asthma in women.³ Secondly, obesity is linked to chronic, low-grade systemic inflammation because adipose tissue releases proinflammatory cytokines including TNF- α , IL-6, plasminogen activator inhibitor 1, eotaxin, and vascular endothelial growth factor (VEGF), which activate the inflammatory state at sites distant to fat tissue. This systemic inflammation may also exacerbate asthma.^{4,5}

Obesity is also associated with an increased incidence of comorbidities such as cardiovascular diseases, type 2 diabetes, and neoplasms related to estrogens. Patients with a coexistence of obesity and asthma differ from other subjects in their response to pharmacotherapy (impaired effect of inhaled steroids) and show different immunological patterns, which results in an increased exacerbation rate. Recently, an animal study showed that hyperinsulinemia accompanying obesity enhances vagally induced bronchospasm, owing to the inhibition of neuronal M2 muscarinic receptors and increased acetylcholine release from airway parasympathetic nerves.⁶ Additionally, obese patients show increased 17 β -estradiol concentrations. 17 β -estradiol may enhance IL-3 and IL-4 levels in monocytes and promote a

Th2 phenotype, which explains (or possibly explains) the relationship between obesity and atopy among women. In a study by Vieira et al,⁷ the occurrence of specific immunoglobulin E (IgE) in obese women was seen almost 3 times more often than in nonobese women. The plasma concentrations of 17 β -estradiol, the ratio of 17 β -estradiol to sex hormone-binding globulin, the fasting insulin resistance index, and C-peptide and leptin concentrations were higher in the obese group than in the nonobese group, after adjustment for the use of oral contraceptives. The authors suggested that the relationship between atopy and obesity may be due to an increased production of 17 β -estradiol by adipose tissue.⁷

These findings may lead to novel methods of treatment, such as the anti-inflammatory therapy using metformin and statins as an alternative step for future treatment of obese adult asthmatics who do not respond to standard therapy.⁵ On the other hand, patients with obesity may present with symptoms that can mimic asthma, such as impaired exercise tolerance, dyspnea, and sleep apnea. Thus, a differential diagnosis in such cases is crucial to the initiation of proper treatment.

The aim of this study was to identify genes that are differentially expressed in the peripheral blood of obese and normal-weight asthmatic patients. Additionally, we aimed to evaluate whether the analysis of gene expression in peripheral blood may be helpful in the differential diagnosis of obese patients who present with symptoms similar to asthma.

PATIENTS AND METHODS **Patients** The study group included 15 patients with asthma (9 obese and 6 normal-weight patients), treated at the Department of Allergology and Pneumology, University Hospital in Gdańsk, Poland. The control group included 13 obese patients in whom asthma was excluded, treated at the Department of Clinical Nutrition, University Hospital in Gdańsk, Poland (TABLE 1). The inclusion criteria for the asthma group were partly or well-controlled asthma and step 4 treatment according to the GINA guidelines, including inhaled steroids and long-acting β -agonists in all cases.¹ All studied patients had a history of bronchial obstruction with positive reversibility test results. Additionally, all asthma cases were atopic with inhalant allergy confirmed with a skin prick test with pollens or house dust mites (or both). The exclusion criteria were as follows: lack of consent, pregnancy, severe chronic or malignant disease, diabetes, hypertension, and coronary artery disease.

In addition to regular procedures performed in asthmatic patients on an outpatient basis, blood samples were obtained from each individual. In control subjects, blood samples were obtained before starting the dietary and exercise treatment of obesity.

Collection of blood samples RNA samples were collected using the PAXgene Blood RNA Tubes

(Qiagen, Venlo, Netherlands). All tubes were immediately frozen and stored at -20°C until RNA isolation (a maximum period of 2 months). RNA was isolated using the PAXgene Blood RNA Kit CE (Qiagen). All RNA samples were stored at -80°C until labeling and hybridization.

The quality and concentration of RNA was determined using the Agilent 2100 Bioanalyzer and the Agilent RNA 6000 Nano Kit (Agilent, Amstelveen, Netherlands). Samples with an RNA integrity number of more than 7.5 were used for further analysis on expression arrays.

Gene expression analysis For amplification and labeling of RNA with the Illumina TotalPrep 96 RNA Amplification Kit (Applied Biosystems, Nieuwerkerk aan den IJssel, Netherlands), 200 ng of RNA from each sample was used. The Human HT-12_V3 expression arrays (Illumina, San Diego, California, United States) were processed according to the manufacturer's protocol. Slides were scanned immediately using the Illumina BeadStation iScan (Illumina).

Image and data analysis The first-line check, background correction, and Quantile normalization of the data were done with the Genomestudio Gene Expression Analysis module v 1.0.6 Statistics (Illumina, San Diego, United States). Entities for which at least 75% of the samples had a signal intensity value above the 20th percentile in 100% of the samples of at least 2 groups were included in further analysis.

Data analysis was performed using the GeneSpring package version 8.0.0 (Agilent Technologies, Santa Clara, California, United States) according to the manufacturer's instructions. Genes

for which expression was significantly different between the compared groups were chosen based on a $\log_2$ -fold change exceeding 2 in gene expression, a t test P value of less than 0.05, and corrected for multiple testing using the Benjamin-Hochberg method ($P < 0.05$). The naive Bayes prediction model was used to build a prediction model differentiating between obese patients with and without asthma.

The functional annotation of genes was described using the Genecodis functional annotation web-based tool⁹ and the PANTHER database.^{9,10}

Clinical data for this study were analyzed with GeneSpring (Agilent Technologies).

All patients gave their written informed consent to participate in the study. The study was approved by the Medical Ethics Committee of the Medical University of Gdansk, Gdańsk, Poland.

RESULTS The whole-genome expression analysis was performed in RNA samples isolated from all blood cells in whole blood. From the 47 323 probes present in the array, 32 379 transcripts had sufficient data for further analysis.

Asthma and obesity The comparison of the gene-expression profiles in patients with asthma and obesity versus patients with asthma and normal body mass revealed a $\log_2$ -fold change difference exceeding 2 in gene expression in 19 transcripts. A significant difference corrected for multiple testing was found in 6 of the analyzed transcripts (FIGURE 1).

The gene-expression analysis comparing obese patients without asthma and asthmatic patients with normal body mass revealed a $\log_2$ -fold change difference exceeding 2 in gene

FIGURE 1 Hierarchical clustering dendrogram of genes that were differentially expressed ($\log_2$ -fold change > 2 , $P < 0.05$ corrected for multiple testing by the Benjamini-Hochberg method $P < 0.05$) between asthmatic patients with obesity and those with normal body mass. Each column represents a patient sample, and each row, an individual gene. For each gene, green color represents underexpression; red color, overexpression; and black signal, missing data.

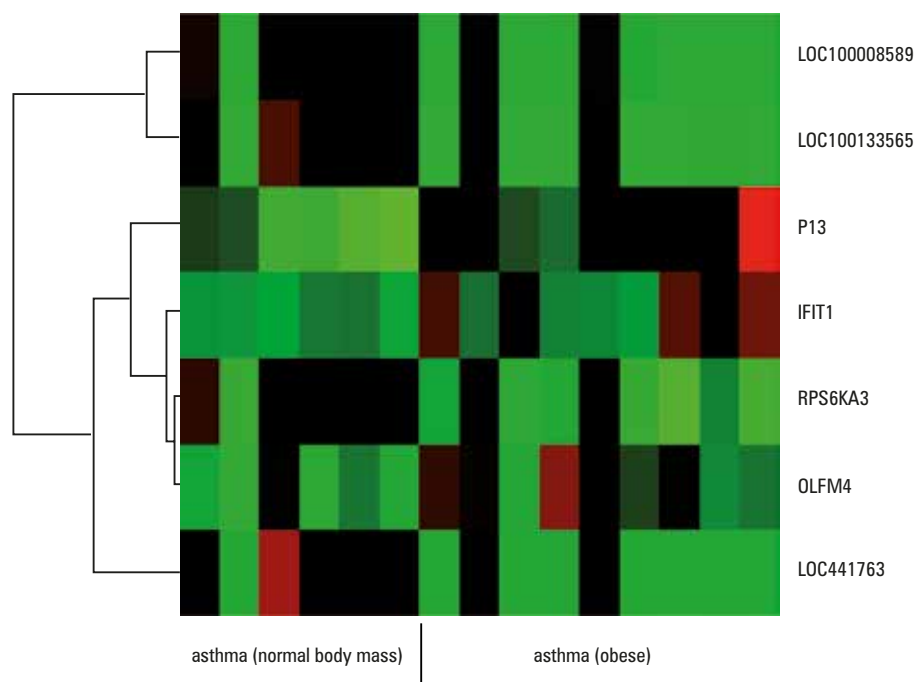
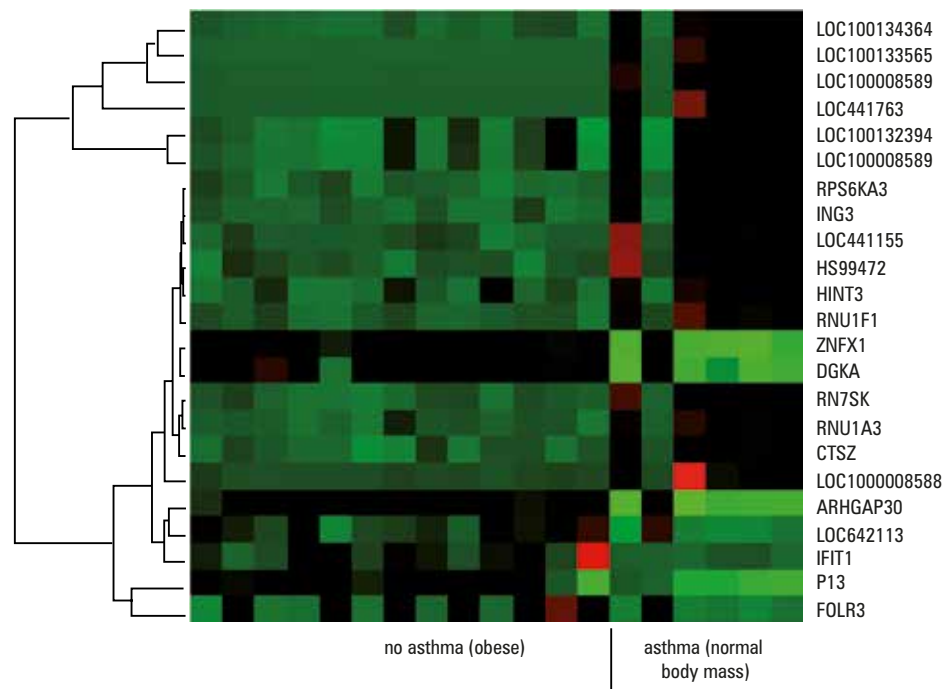


FIGURE 2 Hierarchical clustering dendrogram of genes that were differentially expressed ($\log_2$ -fold change > 2 , $P < 0.05$ corrected for multiple testing by the Benjamini–Hochberg method $P < 0.05$) between asthmatic patients with normal body mass and obese patients without asthma. Each column represents a patient sample, and each row, an individual gene. For each gene, green color represents underexpression; red color, overexpression; and black signal, missing data.



expression in 23 transcripts. A significant difference corrected for multiple testing was present in all 23 entities (FIGURE 2).

PI3, *LOC100008589*, *RPS6KA3*, *LOC441763*, *IFIT1*, and *LOC100133565* transcripts were defined as possibly related to the increased body mass (TABLE 2).

Asthma and control groups The following analysis focused on the identification of genes that were differentially expressed in subjects with asthma (both obese and with normal body mass) in comparison with controls. The results revealed that a significant difference corrected for multiple testing was present in 95 entities. A $\log_2$ -fold change difference exceeding 2 in gene expression was found in 5 transcripts including *HS.99472*, *ARID4B*, *LOC643313*, *C14ORF4*, and *HS.193767*.

Prediction model differentiating obese patients with and without asthma In order to identify genes that could be used to differentiate obese controls and obese asthmatics, we analyzed genes showing a significant difference in expression corrected for multiple testing ($P < 0.05$) in 2 groups: 1) the set differentially expressed in obese controls and normal-weight asthmatics (1486 transcripts) and 2) genes differentially expressed in the whole asthma group in comparison with obese controls (95 transcripts). The difference in the transcription of 1425 entities was found only in the comparison of obese controls and normal-weight asthmatics; 61 genes presented a common pattern of expression in both analyses; another 34 genes were differentially expressed only in the comparison of the entire asthma group versus controls (*LOC100132528*, *SAMD3*, *MEI1*, *C5ORF41*, *TERF2IP*, *BOLA2B*, *HS.563552*, *UFC1*, *PATL2*, *C3ORF75*, *GLO1*,

ITPRIP, *LOC197135*, *PHLPP1*, *C6ORF170*, *RICS*, *FKTN*, *PPM1F*, *SNAPC4*, *RAD51*, *LOC646808*, *LOC643313*, *LILRB3*, *ANKRD13A*, *RECQL*, *HS.193767*, *LOC728732*, *MIR21*, *RHOQ*, *ZNF93*, *C9ORF103*, *RC3H2*, *ZNF626*, and *SAMD3*) corrected for multiple testing ($P < 0.05$). The last group of transcripts was used to construct a prediction model. Of this patient group, according to the proposed model, 92% of obese controls and 89% of obese asthmatic patients were classified correctly, resulting in the overall accuracy of the model of 90.9%.

Functional annotation of differentially expressed genes Functional annotation was assigned by the Genecodis tool⁸ and PANTHER database,^{9,10} based on the genes with the $\log_2$ -fold change difference exceeding 2 in gene expression. The main pathways of the differentially expressed genes were platelet-derived growth factor (PDGF), integrin, transcription, and inflammation (TABLE 3).

DISCUSSION Our study showed differences in gene expression in peripheral blood related to obesity both in obese asthmatic patients and in obese patients without asthma. Secondly, the prediction model based on the results of gene expression allowed to differentiate obese patients with asthma from obese controls with high accuracy.

Asthma and obesity are one of the most serious health problems in modern society. The overlap between these conditions causes clinical difficulties in differential diagnosis. A better understanding of the pathology of asthma, especially its phenotype related to obesity, may lead to the development of effective and tailored treatment strategies. As the gene expression analysis has become a daily routine method in

TABLE 2 Genes that were differentially expressed; log₂-fold change >2; *P* <0.05 corrected for multiple testing *t* test Benjamini–Hochberg method *P* <0.05

Obese patients with asthma vs patients with asthma and normal body mass				Obese controls vs patients with asthma and normal body mass			
symbol (cytogenic localization)	regulation	FC	<i>P</i> value (corrected)	symbol (cytogenic localization)	regulation	FC	<i>P</i> value (corrected)
genes with similar differences in both comparisons							
PI3 (20q13.12)	up	2.59019	0.0054217135	PI3 (20q13.12)	up	2.800159	9.669405E-4
LOC100008589 (1st) (22p12)	down	−15.45324	0.043978054	LOC100008589 (1st) (22p12)	down	−3.6162987	0.0013241239
RPS6KA3 (Xp22.12)	down	−2.101352	0.043978054	RPS6KA3 (Xp22.12)	down	−2.6177142	3.0760257E-6
LOC441763 (16p11.2)	down	−11.751951	0.043978054	LOC441763 (16p11.2)	down	−27.957869	1.64401E-6
IFIT1 (10q23.31)	up	2.064402	0.043978054	IFIT1 (10q23.31)	up	2.1131089	0.007211569
LOC100133565 (chr. 12)	down	−17.774307	0.043978054	LOC100133565 (chr. 12)	down	−47.53617	1.64401E-6
genes with different expression in 1 comparison							
OLFM4 (13q14.3)	up	2.0234244	0.043978054	LOC100008589 (2nd) (22p12)	down	−41.281216	1.64401E-6
				ZNFX1 (20q13.13)	up	2.000805	1.64401E-6
				DGKA (12q13.2)	up	2.151851	5.8393896E-5
				ARHGAP30 (1q23.3)	up	2.3613424	3.5922574E-5
				LOC642113 (chr. 2)	up	2.0307086	0.03201332
				ING3 (7q31.31)	down	−2.0347931	5.828479E-6
				RNU1F1 (1p36.1)	down	−2.1985717	5.828479E-6
				Hs.99472 (10p12.31)	down	−2.1621523	1.5372529E-5
				RNU1A3 (1p36.1)	down	−2.4608004	1.73829E-5
				LOC100132394 (Xq22.3)	down	−3.2237225	0.0021874802
				LOC441155 (6q12)	down	−2.0805464	1.73829E-5
				LOC100008588 (22p12)	down	−5.064386	1.8518655E-5
				LOC100134364 (chr. X)	down	−7.300454	8.25479E-5
				RN7SK (6p12.2)	down	−2.2607615	5.815962E-6
				HINT3 (6q22.32)	down	−2.0507286	3.535359E-4
				CTSZ (20q13.32)	down	−2.4224112	1.64401E-6

oncology and hematology, it is likely that this method will also be used in allergology, where a correct diagnosis is crucial for long-term patient treatment.¹¹⁻¹⁴

For this study, we analyzed RNA samples isolated from whole blood. As the mechanism of obesity in asthma and subsequent inflammation is largely unknown and probably related to more than 1 or 2 specific molecular pathways and processes, the advantage of this method is to include all possible

processes into the analysis. Another advantage of this standardized method is that it also reduces the effect of sample handling on the result.

Gene expression profile Although we focused on the differences in gene expression between obese and normal-weight patients with asthma and between normal-weight patients with asthma and obese patients without asthma (TABLE 2), we also found interesting similarities between these

TABLE 3 Gene co-occurrence annotations found by Genecodis^{12,13} and (Panther Pathways),^{14,15} which were differentially expressed ($P < 0.05$ corrected for multiple testing by the Benjamini–Hochberg method $P < 0.05$) in normal-weight asthmatic patients and obese controls

Genes	NGR	NG	Hyp	Hyp*	Annotations
<i>ELF2, ITPR3, PLCG1, NCK2, FLI1, PIK3R1, ELF1, PIK3CD, RPS6KA5, BRAF, STAT5A, PDPK1, VAV3, MAP3K2, ELF4, RAF1, ARHGAP1, RPS6KA3, NIN, PKN2, JAK1</i>	128	21	2.83858E-10	3.63338E-08	(PANTHER Pathways) P00047: PDGF signaling pathway
<i>ARL1, ELMO1, PIK3R1, RRAS, PIK3CD, ACTN4, BRAF, ELMO2, ITGAL, ACTG1, ITGAX, CDC42, CRKL, MAP3K2, RAF1, ARF1, ARF3, RAPGEF1, CRK, RAP1A</i>	157	20	6.41022e-08	4.10254E-06	(PANTHER Pathways) P00034: integrin signaling pathway
<i>CREB1, GTF2F1, GTF2E2, GTF2A1, CREB3L2, PRKAR2A, GTF2H1, POLR2C, PRKAR1A, EP300, CREBBP</i>	47	11	1.16215E-07	4.9585E-06	(PANTHER Pathways) P00055: transcription regulation by bZIP transcription factor
<i>ITPR3, PLCG1, CXCR3, RRAS, CIDEA, MYH9, PIK3CD, PLCB3, RGS17, NFKB2, ITGAL, ACTG1, PDPK1, CDC42, CAMK2G, PRKCB, MAP3K2, RAF1, ROCK1, NFATC1, PLCB2</i>	198	21	6.9568E-07	2.22618E-05	(PANTHER Pathways) P00031: inflammation mediated by chemokine and cytokine signaling pathway
<i>ITPR3, PIK3R1, PIK3CD, PLCB3, PRKAR2A, PRKAR1A, PRKCB, ECE1, RAF1, ADCY7, PLCB2</i>	72	11	1.01347E-05	0.000162155	(PANTHER Pathways) P00019: endothelin signaling pathway
<i>ITPR3, SYK, PIK3CD, NFKB2, VAV3, PRKCB, MAP3K2, RAF1, LYN, NFATC1</i>	59	10	9.90636E-06	0.000181145	(PANTHER Pathways) P00010: B-cell activation

Abbreviations: Hyp, P values were obtained by the Hyper geometric analysis; Hyp*, P values were obtained by the hypergeometric analysis, corrected by the false discovery rate method; NGR, number of annotated genes in the reference list; NG, number of annotated genes in the input list

groups. The *PI3* gene is involved in the PI3K kinase pathway, which impacts asthma in several ways. The *PI3K* activity is increased after ovalbumin allergen provocation. It increases the expression of VEGF, which enhances vascular permeability and Th2 sensitization, including IL-13 and IL-6 levels.^{15,16} Furthermore, *PI3K* signaling affects the deeper bronchus layer, increasing airway smooth muscle proliferation.¹⁷ It is also involved in eosinophil cationic protein, eosinophil peroxidase, and myeloperoxidase release in allergic rhinitis and asthma, irrespective of the allergen-challenge model.¹⁸ Additionally, *PI3K* signaling is increased in obesity induced by a high-fat diet.¹⁹ The *IFIT1* gene is involved in antiviral response; additionally, its expression is increased in Th2-cytokine pretreated epithelial cells.²⁰ The *ARID4B* gene is involved in the development of neoplasms, spermatogenesis, and alcohol abuse.²¹ The protein belongs to the retinol-binding protein family, which additionally regulates adipogenesis.²²

A previous study on the gene expression analysis of peripheral blood mononuclear cells (PBMCs) of 34 560 oligonucleotide probes identified 8 candidate genes affecting the development of asthma. By using more strict criteria, the authors found that the best model that may be a useful biomarker for asthma consisted of only 3 genes: *MEPE*, *MLSTD1*, and *TRIM37*. Further analyses identified the best model for classification of asthma severity (*CCT5*, *NOX5*, *LMAN1*, *KNS2*, *MLSTD1*, *TRIM37*).¹¹ Unfortunately, the function of the genes in the pathomechanism of asthma has not been determined yet. A recent microarray analysis of PBMCs in childhood asthma revealed that the combination of *ADAM33*, *SMAD7*, and *LIGHT* genes was involved in immune response, stress

response, and apoptosis, which may provide a useful model for childhood asthma.¹²

The adipose tissue of obese patients consists of adipocytes, macrophages, T cells, B cells, and eosinophils. All these immune cells play a crucial role not only in the development and acceleration of low-grade systemic inflammation and metabolic disturbances but also in allergic inflammation. Recent data have confirmed the relationship between gene expression in PBMCs (lymphocytes and monocytes) and visceral fat accumulation. Animal studies suggested that changes in gene expression in PBMCs can be used as early predictors of obesity; in particular, the expression of energy homeostatic genes such as the genes for neuropeptide Y and fatty acid synthesis (*FASN*, *SREBP1*) and adipogenesis (*PPARG*).¹³ In another study, the progression of overexpression of *scl27α2* was strongly associated with weight gain in rats owing to hyperlipidemic diet; that is why, it may be also a molecular predictor of early development of overweight.¹⁴ The gene expression analysis of PBMCs from obese patients showed that the expression of several genes related to circadian rhythm, inflammation, oxidative stress, and immune response was significantly positively correlated with visceral fat adiposity (eg, *SCL46A3*) or was negatively correlated with visceral fat adiposity (eg, *PER1*, *ZNF174*). It suggests that low-grade systemic inflammation in adipose tissue may reflect the expression of PBMC genes similarly to asthma, as described above. Interestingly, the activation and count of lymphocytes, monocytes, and neutrophils are similar in obesity and asthma.²³

The pathway analysis of the genes differentially expressed in the group of normal-weight asthmatic patients and obese controls showed several annotations that may help elucidate the

underlying mechanism. The most significant was the PDGF signaling pathway, which may be related to obesity. A genome-wide association study showed that PDGF signaling was the most significant mechanism associated with metabolic syndrome.²⁴ Integrin signaling is involved in the adiponectin pathway.²⁵ The imbalance between normal adipogenesis and osteogenesis mediated by integrin $\alpha v/\beta 1$ has been shown to be related to the development of obesity.²⁶ Interestingly, both PDGF and integrin pathways also play an important role in the pathogenesis of asthma.²⁷⁻³⁰ PDGF may be involved in airway remodeling, and IL-13-mediated inflammation—in chronic asthma.^{29,30} Integrin is involved in airway inflammation both in the epithelial layer (by recruiting dendritic cells)²⁸ and in the muscular layer (by affecting smooth muscle cells)²⁷. The endothelin pathway may be responsible for the development of obesity because the *EDNRB* gene (coding endothelin receptor type B) may influence the susceptibility to obesity. On the other hand, endothelin is a cofactor of IL-25 in the murine model of inflammation in asthma.³¹

The regulation of transcription, inflammation, and B-cell activation may also be related to both diseases because they are both chronic inflammatory conditions.⁵

Our results confirm the observations that asthma and obesity have common genetic mechanisms in 8%.^{32,33} Recent reports have emphasized the link between allergic inflammation and atherosclerosis, lipid metabolism, and venous thromboembolism.³³⁻³⁵ A study by Inouye et al³⁵ described a tissue-specific gene network associated both with blood lipid levels and with key components of inflammation and allergy. On the other hand, airway inflammation in some obese asthmatics is hypocellular, with a higher blood eosinophil count and higher eotaxin and IL-5 concentrations, while some studies showed lower concentrations of exhaled nitric oxide and increased neutrophil count.^{4,33} Genetic studies indicated several mechanisms involved in the pathogenesis of asthma, including innate immunity as well as airway hyperresponsiveness and remodeling.¹ The regulation of serum IgE is associated with single nucleotide polymorphisms in 1q23, 5q31, and 12q13.³⁶ Our study underlines the nonatopic mechanisms involved in the pathogenesis of inflammation present in obese asthmatics, which does not exclude a possible atopic inflammation in atopic individuals.

Clinical relevance of the results We managed to identify a group of genes related to the development of asthma, which could be used to construct a prediction model differentiating obese asthmatics from obese patients without asthma with similar symptoms caused by obesity. The proposed model allowed to correctly classify 92% of obese patients without asthma and 89% of obese asthmatic patients, resulting in the overall accuracy of 90.9%. These findings may be used

in further studies on the development of a clinical diagnostic tool.

Some limitations of the current phase and future phases of our study need to be discussed. The necessary next step of the gene expression study is to validate the gene profile in independent groups of obese and normal-weight asthmatic patients, obese nonasthmatic controls, and, additionally, normal-weight subjects without asthma as “supernormals”.

The number of biomarkers that can be measured in asthmatic patients is limited. In peripheral blood, we can measure only specific IgE and total IgE levels, eosinophil count, serum periostin levels, and prostaglandin E_2 levels.^{37,38} Sputum quantitative assay, exhaled breath analysis, and urinary metabolomic analysis may also be performed.³⁷ However, none of these parameters is specific for obesity-related asthma.

Conclusions The results of our study show significant differences in gene expression between obese and normal-weight asthmatic patients. However, changes in gene expression caused by asthma and obesity have common pathways. The analysis of gene expression might be a useful tool in difficult cases of obese patients suspected of asthma.

Contribution statement MGN conceived the idea of the study, participated in data collection, and drafted the manuscript. MN conceived the idea of the study, performed the statistical analysis, and drafted the manuscript. PN conceived the idea of the study, participated in data collection, and drafted the manuscript. BS and PV performed the genetic analysis. EJ and SM participated in the concept of the paper and revised the draft.

REFERENCES

- 1 From the Global Strategy for Asthma Management and Prevention. Global Initiative for Asthma (GINA) 2015. <http://www.ginasthma.org/>. Accessed January 8, 2015.
- 2 Lugogo NL, Hollingsworth JW, Howell DL, et al. Alveolar macrophages from overweight/obese subjects with asthma demonstrate a proinflammatory phenotype. *Am J Respir Crit Care Med*. 2012; 186: 404-411.
- 3 Tsaroucha A, Daniil Z, Malli F, et al. Leptin, adiponectin and ghrelin levels in female patients with asthma during exacerbation periods. *J Asthma*. 2013; 50: 188-197.
- 4 Boulet LP. Obesity and atopy. *Clin Exp Allergy*. 2015; 45: 75-86.
- 5 Linderholm AL, Bratt JM, Schuster GU, et al. Novel therapeutic strategies for adult obese asthmatics. *Immunol Allergy Clin North Am* 2014; 34: 809-823.
- 6 Nie Z, Jacoby DB, Fryer AD. Hyperinsulinemia potentiates airway responsiveness to parasympathetic nerve stimulation in obese rats. *Am J Respir Cell Mol Biol*. 51: 251-261.
- 7 Vieira VJ, Ronan AM, Windt MR, Tagliaferro AR. Elevated atopy in healthy obese women. *Am J Clin Nutr*. 2005; 82: 504-509.
- 8 Carmona-Saez P, Chagoyen M, Tirado F, et al. GENECODIS: a web-based tool for finding significant concurrent annotations in gene lists. *Genome Biol*. 2007; 8: R3.
- 9 Thomas PD, Kejariwal A, Campbell MJ, et al. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. *Nucleic Acids Res*. 2003; 31: 334-341.
- 10 Mi H, Lazareva-Ulitsky B, Loo R, et al. The PANTHER database of protein families, subfamilies, functions and pathways. *Nucleic Acids Res*. 2005; 33: 284-288.
- 11 Shin SW, Oh TJ, Park SM, et al. Asthma-predictive genetic markers in gene expression profiling of peripheral blood mononuclear cells. *Allergy Asthma Immunol Res*. 2011; 3: 265-272.

- 12 Kong Q, Li WJ, Huang HR, et al. Differential gene expression profiles of peripheral blood mononuclear cells in childhood asthma. *J Asthma*. 2015; 52: 343-352.
- 13 Oliver P, Reynés B, Caimari A, et al. Peripheral blood mononuclear cells: a potential source of homeostatic imbalance markers associated with obesity development. *Pflügers Arch*. 2013; 465: 459-468.
- 14 Caimari A, Oliver P, Rodenburg W, et al. Slc27a2 expression in peripheral blood mononuclear cells as a molecular marker for overweight development. *Int J Obes (Lond)*. 2010; 34: 831-839.
- 15 Lee KS, Kim SR, Park SJ, et al. Mast cells can mediate vascular permeability through regulation of the PI3K-HIF-1 α -VEGF axis. *Am J Respir Crit Care Med*. 2008; 178: 787-797.
- 16 Krishnamoorthy N, Oriss TB, Paglia M, et al. Activation of c-Kit in dendritic cells regulates T helper cell differentiation and allergic asthma. *Nat Med*. 2008; 14: 565-573.
- 17 Hu R, Pan W, Fedulov AV, et al. MicroRNA-10a controls airway smooth muscle cell proliferation via direct targeting of the PI3 kinase pathway. *FASEB J*. 2014; 28: 2347-2357.
- 18 Kämpe M, Lampinen M, Stolt I, et al. PI3-kinase regulates eosinophil and neutrophil degranulation in patients with allergic rhinitis and allergic asthma irrespective of allergen challenge model. *Inflammation*. 2012; 35: 230-239.
- 19 Nteeba J, Ross JW, Perfield JW, Keating AF. High fat diet induced obesity alters ovarian phosphatidylinositol-3 kinase signaling gene expression. *Reprod Toxicol*. 2013; 42: 68-77.
- 20 Herbert C, Zeng QX, Shanmugasundaram R, et al. Response of airway epithelial cells to double-stranded RNA in an allergic environment. *Transl Respir Med*. 2014; 2: 11.
- 21 Liangpunsakul S, Lai X, Ross RA, et al. Novel serum biomarkers for detection of excessive alcohol use. *Alcohol Clin Exp Res*. 2015; 39: 556-565.
- 22 Zizola CF, Frey SK, Jitngarmkusol S, et al. Cellular retinol-binding protein type I (CRBP-I) regulates adipogenesis. *Mol Cell Biol*. 2010; 30: 3412-3420.
- 23 Yamaoka M, Maeda N, Nakamura S, et al. A pilot investigation of visceral fat adiposity and gene expression profile in peripheral blood cells. *PLoS One*. 2012; 7: e47377.
- 24 Shim U, Kim HN, Sung YA, Kim HL. Pathway Analysis of Metabolic Syndrome Using a Genome-Wide Association Study of Korea Associated Resource (KARE) Cohorts. *Genomics Inform*. 2014; 12: 195-202.
- 25 Ramezani-Moghadam M, Wang J, Ho V, et al. Adiponectin reduces hepatic stellate cell migration by promoting tissue inhibitor of metalloproteinase-1 (TIMP-1) secretion. *J Biol Chem*. 2015; 290: 5533-5542.
- 26 Chen Q, Shou P, Zhang L, et al. An osteopontin-integrin interaction plays a critical role in directing adipogenesis and osteogenesis by mesenchymal stem cells. *Stem Cells*. 2014; 32: 327-337.
- 27 Shkumatov A, Thompson MA, Choi KM, et al. Matrix Stiffness-Modulated Proliferation and Secretory Function of the Airway Smooth Muscle Cells. *Am J Physiol Lung Cell Mol Physiol*. 2015; 308: L1125-L1135.
- 28 Zhao L, Yang W, Yang X, et al. Chemerin suppresses murine allergic asthma by inhibiting CCL2 production and subsequent airway recruitment of inflammatory dendritic cells. *Allergy*. 2014; 69: 763-774.
- 29 Johnson JR, Folestad E, Rowley JE, et al. Pericytes contribute to airway remodeling in a mouse model of chronic allergic asthma. *Am J Physiol Lung Cell Mol Physiol*. 2015; 308: L658-L671.
- 30 Lu J, Zhu Y, Feng W, et al. Platelet-derived growth factor mediates interleukin-13-induced collagen I production in mouse airway fibroblasts. *J Biosci*. 2014; 39: 693-700.
- 31 Martínez-Barquero V, de Marco G, Martínez-Hervas S, et al. Polymorphisms in endothelin system genes, arsenic levels and obesity risk. *PLoS One*. 2015; 10: e0118471.
- 32 González JR, Cáceres A, Esko T, et al. A common 16p11.2 inversion underlies the joint susceptibility to asthma and obesity. *Am J Hum Genet*. 2014; 94: 361-372.
- 33 Dixon AE, Holguin F, Sood A, et al. American Thoracic Society Ad Hoc Subcommittee on Obesity and Lung Disease. An official American Thoracic Society Workshop report: obesity and asthma. *Proc Am Thorac Soc*. 2010; 7: 325-335.
- 34 Potaczek D. Links between allergy and cardiovascular or hemostatic system. *Int J Cardiol*. 2014; 170: 278-285.
- 35 Inouye M, Silander K, Hamalainen E. An immune response network associated with blood lipid levels. *PLoS Genet*. 2010; 6: e1001113.
- 36 Sharma V, Michel S, Gaertner V, et al. Fine-mapping of IgE-associated loci 1q23, 5q31, and 12q13 using 1000 Genomes Project data. *Allergy*. 2014; 69: 1077-1084.
- 37 Dasgupta A, Nair P. When are biomarkers useful in the management of airway diseases? *Pol Arch Med Wewn*. 2013; 123: 183-188.
- 38 Mastalerz L, Kumik J, Kasperkiewicz H, et al. Altered metabolism of prostaglandin E2 in asthma patients with aspirin hypersensitivity. *Pol Arch Med Wewn*. 2013; 123: 423-424.

Analiza różnic w ekspresji genomu związanych ze współistnieniem astmy i otyłości

Marta Gruchała-Niedoszytko¹, Marek Niedoszytko², Bahram Sanjabi³,
Pieter van der Vlies³, Piotr Niedoszytko⁴, Ewa Jassem², Sylwia Małgorzewicz¹

¹ Katedra Żywnienia Klinicznego, Gdański Uniwersytet Medyczny, Gdańsk

² Klinika Alergologii, Uniwersytet Medyczny w Gdańsku, Gdańsk

³ Katedra Genetyki, Uniwersyteckie Centrum Kliniczne, Groningen, Holandia

⁴ Klinika Rehabilitacji, Gdański Uniwersytet Medyczny, Gdańsk

SŁOWA KLUCZOWE

astma, ekspresja
genów, model
predykcyjny, otyłość,
zapalenie

STRESZCZENIE

WPROWADZENIE Współistniejąca otyłość znacznie pogarsza kontrolę astmy. U otyłych chorych na astmę zaobserwowano bardziej nasilone objawy choroby oraz zwiększone zużycie leków.

CELE Głównym celem badania była identyfikacja genów o różnej ekspresji we krwi obwodowej otyłych chorych na astmę, chorych na astmę o prawidłowej masie ciała oraz osób otyłych bez astmy. Drugim celem było określenie, czy analiza ekspresji genów we krwi obwodowej może być pomocna w diagnostyce różnicowej u chorych otyłych z objawami przypominającymi astmę.

PACJENCI I METODY Grupa badana obejmowała 15 chorych na astmę (9 otyłych i 6 o prawidłowej masie ciała), natomiast grupa kontrolna – 13 osób otyłych, u których wykluczono astmę. Analiza ekspresji całego genomu wykonana została na próbkach RNA krwi obwodowej.

WYNIKI Porównanie profilu ekspresji u chorych na astmę z otyłością i chorych o prawidłowej masie ciała wykazało statystycznie istotną różnicę w przypadku 6 genów. Porównanie ekspresji między grupą kontrolną a chorymi na astmę o prawidłowej masie ciała wykazało istotne różnice w przypadku 23 genów. Porównanie wyników genów o odmiennej ekspresji wykazało grupę transkryptów, które mogą być związane ze zwiększoną masą ciała (*PI3*, *LOC100008589*, *RPS6KA3*, *LOC441763*, *IFIT1*, *LOC100133565*). W oparciu o wyniki ekspresji genów skonstruowano model predykcyjny, który pozwolił na prawidłową klasyfikację 92% chorych otyłych i 89% otyłych chorych na astmę, wykazując 90,9% dokładność.

WNIOSKI Wyniki naszego badania wskazują na istotne różnice w ekspresji genów u otyłych chorych na astmę w porównaniu z chorymi o prawidłowej masie ciała oraz u osób otyłych bez astmy w porównaniu z chorymi na astmę o prawidłowej masie ciała.

Adres do korespondencji:
dr med. Marta Gruchała-Niedoszytko,
ul. Dębinki 7, 80-210 Gdańsk,
tel./fax: 58 349 27 23, e-mail:
mg@gumed.edu.pl

Praca wpłynęła: 02.07.2015.

Przyjęta do druku: 07.08.2015.

Publikacja online: 07.08.2015.

Nie zgłoszono sprzeczności
interesów.

Pol Arch Med Wewn. 2015;

125 (10): 722-730

Copyright by Medycyna Praktyczna,

Kraków 2015