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Influence on polymorphism of visfatin (rs 4730153) and nesfatin (rs 1330) genes on the development of the metabolic syndrome

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ABSTRACT

Introduction: Visfatin and nesfatin are active proteins that are involved in the regulation of physiological functions such as glucose metabolism and inflammatory response. This study aimed to assess the influence of visfatins and nystatins gene variants on the occurrence of the full metabolic syndrome or its components.

Material and methods: The research cohort comprised 285 diabetic patients, 132 men and 153 women. Specific DNA segments were amplified and labelled, and the rs 4730153 gene of visfatin and the rs 1330 gene of nesfatin were analysed. Real-Time PCR was conducted using fluorescence-labelled probes.

Results: The AA genotype of visfatin (rs 4730153) shows a positive association with glucose concentration or pharmacological treatment in patients with type 2 diabetes, compared to AG and GG genotypes. The GG genotype of nesfatin (rs 1330) shows a positive association with incidents of metabolic syndrome, compared to the AA genotype.

Conclusions: Visfatin polymorphism in rs 4730153 may be associated with inappropriate glucose concentration. Nesfatin polymorphism in rs 1330 may be associated with metabolic syndrome. Further studies in a larger group of patients are necessary to completely assess this association.

Keywords: visfatin, nesfatin, polymorphism, metabolic syndrome, glucose tolerance

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Introduction

The prevalence of obesity-related metabolic syndrome is rapidly increasing. This negatively affects the quality of affected patients' lives and raises healthcare system costs [1].

Visfatin, also known as PBEF1 — pre-B-cell colony-enhancing factor, is a protein belonging to the class of adipocytokines. The protein is encoded by the PBGEF gene located on chromosome 7. In addition to adipose tissue, visfatin is also present in bone marrow, liver, skeletal muscle, bone, macrophages, lymphocytes, and neutrophils [2]. Its uncontrolled production can be observed in obesity and other diseases. Plasma visfatin level can serve as a marker predicting the incidence of type 2 diabetes (T2DM), obesity, metabolic syndrome and cardiovascular disease [3, 4]. Additionally, this adipocytokine may be a valuable marker of inflammation associated with atherosclerosis complications [5, 6]. However, its greatest importance is its hypoglycaemic effect. It stimulates insulin secretion, improves pancreatic beta-cell function, and modulates the expression of genes involved in maintaining glucose homeostasis [7].

Based on studies demonstrating a positive correlation between serum visfatin levels and metabolic syndrome or its certain components, particularly T2DM, it was hypothesized that genetic polymorphisms within the visfatin gene could affect the frequency of above mentioned medical conditions.

The second molecule of interest in this study is nesfatin-1. It is formed from precursor protein nucleobindin-2

(NUCB2). It is a part of the melanocortin signalling pathway in the hypothalamus and plays a role as an appetite-regulating molecule. It is characterized by its anorexigenic and anti-hyperglycaemic effects. The potential role of NUCB2 in the maintenance of homeostasis was evaluated in a study conducted on rat models. Intracerebroventricular injection of nesfatin-1 resulted in decreased food intake, whereas injection of an antibody-neutralizing nesfatin-1 stimulated rat appetite [8].

Protein nucleobindin-2/Nesfatin-1 is expressed widely in various tissues and organs in the body, including the gastrointestinal tract. The presence of nesfatin has been proven in adipocytes, gastric endocrine cells, and islet cells. Nesfatin participates in many processes regulating energy homeostasis. It is involved in glucose metabolism [9]. Recent research on the function of NUCB2/Nesfatin-1 in peripheral tissues has found its presence in islet β-cells and has been shown to enhance glucose-induced insulin secretion [10].

Several single nucleotide polymorphisms had been associated with obesity phenotype for different adipokinins. Because of nesfatin's functions, it was hypothesized that genetic polymorphisms in the NUCB2 gene may influence the development of obesity and metabolic syndrome.

The study aimed to investigate the association between different variants of the visfatin gene in rs 4730153 and nesfatin gene rs 1330 with metabolic syndrome. To determine this serum concentration of glucose, triglyceride and HDL cholesterol were measured and also blood pressure and waist size were monitored in the study cohort.

Material and methods

The blood samples were collected from patients who have resided in southern Poland and who presented to the primary care clinic. The total number of patients included in the research was 285 (153 women and 132 men) and everybody gave written consent. The average age of those patients was 53 years.

Metabolic syndrome was diagnosed in patients who have met at least three of the following criteria:

— waist size equal or bigger than 80 cm in women or equal or bigger than 94 cm in men,
— concentration of triglyceride greater than 1.7 mmol/L or current treatment of hypertriglyceridemia,
— concentration of HDL-C less than 1.0 mmol/L in men and less than 1.3 mmol/L in women or current treatment of this lipid disorder,
— blood pressure Systolic equal to or greater than 130 mmHg or diastolic equal to or greater than 85 mm or successfully controlled previously diagnosed hypertension,
— concentration of glucose equal to or more than 100 mg/dL or normal level in patients pharmacologically treating type 2 diabetes.

As the next step, isolation of the DNA from collected blood samples, which up to this point were stored at –70°C was performed using the column method. Determined was the concentration of genetic material and adjusted it to 15 ng/μL by mixing DNA with water according to the established dilution protocol. The final level of dilution was verified using the spectrophotometer denoviX.

Subsequently, allelic discrimination was performed using fluorescently labelled probes, according to the producer protocol. The PCR reaction was accomplished with the use of Roche Lightcycler 96. Alleles were labelled A in VIC and G in FAM. The assay was performed separately for (rs 4730153) and nesfatin (rs 1330) genes.

Having completed the laboratory part, a statistical analysis was performed. The first step was to assess the data distribution by the Shapiro-Wilk test. The authors determined statistically significant differences in the distribution of genotypes (AA, AG, GG), gender, and prevalence of criteria for metabolic syndrome recognition by using the Anova Kruskala-Wallisa rank test with multiple comparisons. The Mann-Whitney U test was performed to compare valuable data between the two groups.

Results

There were no significant differences between visfatin genotypes (rs 4730153) and all but one criterion of metabolic syndrome (Tab. 1).

Specifically, the AA genotype of visfatin (rs 4730153), as opposed to AG and GG genotypes, does have a positive association with serum glucose levels or pharmacological treatment in type 2 diabetes patients. (Tab. 2).

Associations between nesfatin genotypes (rs 1330) and individual diagnostic criteria of metabolic syndrome did not reach statistical significance (Tab. 3). However there was a statistical significance between the GG genotype of nesfatin (rs 1330) with metabolic syndrome in general, in comparison association to AA genotype (Tab. 4).

Table 1. Comparison of visfatin genotypes (rs 4730153) distributions in patients with and without criteria of metabolic syndrome

Genotype visfatin rs 4730153							P-value
	AA		AG		GG		
Gender	**n**	**%**	**n**	**%**	**n**	**%**	
Women	25	16.34%	67	43.79%	61	39.87%	
Men	23	17.42%	60	45.45%	49	37.12%	
Waist size (waist circumference > 80 cm for women, > 94 cm for men)	**n**	**%**	**n**	**%**	**n**	**%**	0.4741
Yes	38	79.17%	89	70.08%	81	73.64%	
No	10	20.83%	38	29.92%	29	26.37%	
Triglyceride (concentration > 1.7 mmol/L or treatment of hypertriglyceridemia)	**n**	**%**	**n**	**%**	**n**	**%**	0.9572
Yes	23	52.08%	64	50.39%	55	50%	
No	25	47.91%	63	49.61%	55	50%	
HDL-C (concentration < 1.0 mmol/L in men and < 1.3 mmol/L in women or treatment of this lipid disorder)	**n**	**%**	**n**	**%**	**n**	**%**	0.7583
Yes	28	58.33%	80	62.99%	71	64.54%	
No	20	41.67%	47	37.01%	39	35.45%	
Blood pressure (systolic ≥ 130 mmHg or diastolic ≥ 85 mm or treatment of previously diagnosed hypertension)	**n**	**%**	**n**	**%**	**n**	**%**	0.3262
Yes	30	62.5%	84	66.14%	63	57.27%	
No	18	37.5%	43	33.86%	47	42.73%	
Glucose (concentration ≥ 100 mg/dL or pharmacological treatment of type 2 diabetes)	**n**	**%**	**n**	**%**	**n**	**%**	0.0176
Yes	13	27.08%	17	13.39%	11	10%	
No	35	72.92%	110	86.61%	99	90%	
Metabolic syndrome	**n**	**%**	**n**	**%**	**n**	**%**	0.8042
Yes	28	58.33%	70	55.12%	58	52.72%	
No	20	41.67%	57	44.88%	52	47.27%	

Table 2. The result of the Mann-Whitney U-test demonstrating the difference between genotypes of visfatin (rs 4730153) and glucose (concentration ≥ 100 mg/dL or pharmacological treatment of type 2 diabetes), showing statistical difference $p < 0.05$

Compared group	P-value	Z
AA-GG	0.006150	2.739679
AA-AG	0.026498	2.218845

Discussion

Previous studies have shown an association of visfatin with metabolic syndrome, including metabolic factors that contribute to its development, such as obesity, impaired glucose or lipid metabolism, and elevated blood pressure [3, 5, 11–16]. A study conducted by Masood et al. [17] showed that the frequency of mutant alleles of the visfatin gene (rs 2302559 and rs 1215113036) was significantly higher in individuals with metabolic syndrome compared to those without the condition. The findings suggest that individuals with any genetic variations in visfatin may be at an increased risk of developing metabolic syndrome.

In the study of the visfatin gene rs 4730153, the authors observed a correlation between the AA genotype and the occurrence of T2DM or elevated blood glucose levels. The findings align with those of a study conducted by Larrad et al. [12], which revealed a significant association between the AA genotype of the rs 4730153 SNP and fasting glucose, fasting insulin,

Table 3. Comparison of nesfatin genotypes (rs 1330) distributions in patients with and without criteria of metabolic syndrome

Genotype nesfatin rs 1330							**P-value**
	AA		**AG**		**GG**		
Gender	**n**	**%**	**n**	**%**	**n**	**%**	
Women	66	51.97%	77	55.80%	10	50%	
Men	61	48%	61	44.20%	10	50%	
Waist size (waist circumference > 80 cm for women, > 94 cm for men)	**n**	**%**	**n**	**%**	**n**	**%**	0.9152
Yes	94	74.02%	100	72.46%			
No	33	25.98%	38	27.54%			
Triglyceride (concentration > 1.7 mmol/L or treatment of hypertriglyceridemia)	**n**	**%**	**n**	**%**	**n**	**%**	0.2750
Yes	57	44.88%	73	52.90%	12	60%	
No	70	55.12%	65	47.10%	8	40%	
HDL-C (concentration < 1.0 mmol/L in men and < 1.3 mmol/L in women or treatment of this lipid disorder)	**n**	**%**	**n**	**%**	**n**	**%**	0.3332
Yes	75	59.06%	89	64.49%	15	75%	
No	52	40.94%	49	35.51%	5	25%	
Blood pressure (systolic ≥ 130 mmHg or diastolic ≥ 85 mm or treatment of previously diagnosed hypertension)	**n**	**%**	**n**	**%**	**n**	**%**	0.1696
Yes	72	56.69%	90	65.22%	15	75%	
No	55	43.31%	48	34.78%	5	5%	
Glucose (concentration ≥ 100 mg/dL or pharmacological treatment of type 2 diabetes)	**n**	**%**	**n**	**%**	**n**	**%**	0.2365
Yes	20	15.75%	16	11.59%	5	25%	
No	107	84.25%	122	88.41%	15	75%	
Metabolic syndrome	**n**	**%**	**n**	**%**	**n**	**%**	0.0296
Yes	60	47.24%	81	58.70%	15	75%	
No	67	52.76%	57	41.30%	5	25%	

Table 4. Result of Mann-Whitney U test between genotypes of nesfatin (rs 1330) and occurrence of metabolic syndrome, showing statistical difference $p < 0.05$

Compared group	**P-value**	**Z**
GG-AA	0.0216271	2.296864

and HOMA-IR. Additionally, the GG genotype of the visfatin gene rs 4730153 was found to positively regulate glucose and lipid metabolism by enhancing tissue insulin sensitivity and reducing triglyceride levels [15]. Furthermore, research conducted on individuals with non-alcoholic fatty liver disease (NAFLD) demonstrated an association between the rs 4730153 visfatin polymorphism and lipid metabolism as well as insulin resistance [14].

On the contrary, a study conducted by Soltanzadeh et al. [18] found no association between the polymorphism of the visfatin gene rs 61330082 and T2DM or insulin resistance. Similar findings were noted in a cohort of 112 individuals, where no statistically significant association was found between glycaemia, triglycerides, HDL cholesterol levels, and body mass index in relation to the various alleles of the rs 4730153 visfatin gene [19]. The impact of a single nucleotide polymorphism may vary depending on the specific locus of change in its gene. Further research on the different polymorphisms of the visfatin gene is necessary to comprehensively evaluate their influence on glucose metabolism.

In the case of nesfatin genes, a previous study conducted by Zegers et al. [20] shows the association between 3 SNPs (rs 1330, rs 214101, and rs 757081) and obesity in the male population only. Moreover, the

effects of those SNPs were equally affected by BMI, weight, and fat-free mass in linear regression analysis. Additionally, a study of 578 T2DM patients and 1,609 healthy controls conducted by Li et al. [21] showed an association between five *NUCB2* SNPs (rs 10832756, rs 1330, rs 10766383, rs 10832757, rs 11024251) and increased risk for type 2 diabetes. Among those single nucleotide polymorphisms, rs 11024251 statistically was most strongly affecting T2DM (lowest P-value). The risk of developing T2DM was different among genders.

Also, a study conducted by Wang et al. [22] population showed that carriers of G allele in rs 757081 (C > G mutation) were associated with a decreased risk of developing T2DM. GG genotype was significantly associated with decreased levels of BMI and fasting plasma glucose in patients with T2DM. Another study's results showed that the NUCB2 variant rs 757081 C > G is associated with childhood adiposity. Children ages 5 and 8 being GG carriers were characterized by lower weight for height and body mass index scores. The non-obese subjects were more likely to have the homozygous GG genotype [23].

The authors have not observed previously suggested associations between nesfatin genotypes and particular diagnostic criteria for metabolic syndrome. The current research, however, does show the association between the GG nesfatin genotype (rs 1330) and the prevalence of metabolic syndrome as a general diagnosis. Further studies in a larger group of patients are necessary to completely assess this association.

The main limitations of this study are:

1) serum levels of nesfatin and visfatin molecules were not measured;
2) a small number of patients who were carriers of GG genotype of visfatin have been found in the study. The association that was observed in patients with the GG genotype may differ in larger groups.

Conclusion

The obtained results suggest that SNP in the rs 1330 nesfatin gene may be associated with the occurrence of metabolic syndrome. Results from the current study showed a significant association of the GG genotype with the incidence of metabolic syndrome, as compared to the AA genotype ($p = 0.0216271$).

The authors have not found a significant difference between SNP in rs 4730153 visfatin gene and metabolic syndrome. On the other hand, the results show that the AA genotype of visfatin (rs 4730153) shows an association with higher glucose concentration or pharmacological treatment of type 2 diabetes compared to AG ($p = 0.026498$) and GG ($p = 0.006150$) genotypes.

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