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Influence of visfatin’s gene variations on late diabetic complications

Running title: Late diabetic complications influenced by visfatin’s genes

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Abstract
Visfatin (nicotinamide phosphoribosyltransferase) is adipokine which performed many function in organism. It can be expressed in different tissues such as brain, kidneys and visceral adipose tissue. Visfatin takes part in many molecular processes including apoptosis, inflammation, cell proliferation. It affects the glucose metabolism and is involved in pathogenesis of diabetes, insulin resistance, arteriosclerosis and obesity. Moreover studies suggest that visfatin also may be associated with development of diabetic nephropathy and retinopathy.

The goal of the study is the assessment of influence of different visfatin’s gene variants on occurrence of late diabetic complications.

Study group consisted 272 patients with diabetes- 139 men and 133 women from Southern Poland. Selected DNA fragments were amplified and marked. Visfatin’s gene in rs4730153 was examined. The Real Time PCR was conducted with fluorescence-labelled probes.

The most common genotypes were heterozygote AG- 138 patients (51%) and homozygote GG- 89 patients (33%).

In the study group there are 92 diabetics with retinopathy, 26 with nephropathy, 88 with neuropathy and 103 with macroangiopathy.

It has been assessed using χ² test that there are no differences between variability of different variants of visfatin’s gene in distribution of genotypes. According to Hardy-Weinberg's test the variety of population is maintained.

Key words: visfatin, diabetes mellitus, SNP, polymorphism, gene, late complications

Introduction
Diabetes mellitus is a silent pandemic of the 21st century [1]. Its prevalence is estimated to rise from 425 million people in 2017 to 629 million by 2045 [2]. Many
patients with type 2 diabetes (T2DM) develop microvascular complications (approximately 40% of those develop diabetic kidney disease (DKD), which is a leading cause of end-stage renal disease (ESRD) globally [3] and approximately 40 - 80% develop diabetic retinopathy (DR) [4].

DKD as well as DR are often diagnosed at advanced stages [5]. The multifactorial pathogenesis of DKD and DR consists of a combination of metabolic, environmental, and genetic factors [1]. A prior genome-wide association study (GWAS) identified genes with polymorphisms associated with increased incidence of DKD and DR [6]. However, further studies to prove the role of these polymorphisms in DKD and DR occurrence and progression in different diabetic populations are needed, in order to guide strategies that prevent and treat DKD and DR.

Visfatin, a recently discovered adipocytokine is the extracellular isoform of the NAMPT enzyme. Its gene is located on chromosome 7 and the exact location is as follows: band 7q22.3, starting 106,248,298 bp, ending 106,286,326 bp. Visfatin is able to cause insulin-mimetic effect in cells. As it downregulates amount of glucose release from the liver, visfatin accelerates triglycerides synthesis and increases glucose metabolism in monocytes and adipocytes. It plays an important role in the pathogenesis of T2DM and its complications such as diabetic nephropathy and retinopathy. Nevertheless, there are limited studies about the association between visfatin and diabetic complications in these diseases.

The role of visfatin in diabetic retinopathy is poorly understood. In humans, only one study investigated the differences in visfatin concentrations between patients with and without diabetic retinopathy. Y. Wang et al. have found elevated vitreous and serum level of visfatin in diabetic patients with proliferative diabetic retinopathy compared to these with nonproliferative diabetic retinopathy, without diabetic retinopathy and nondiabetic controls.[7]

Patients with diabetic nephropathy show markedly increased serum levels of visfatin comparing to non-diabetic group [8,9,10]. Mageswari R et al. suggest that visfatin level could be an index of severity of diabetic kidney disease.

There are many studies investigating the role of Single Nucleotide Polymorphism (SNP) in visfatin gene. It has been researched that the variations are involved in pathogenesis of diabetes, obesity as well as may regulate plasma insulin levels and plasma glucose levels.

In light of the positive GWAS outcomes in relation to the visfatin gene and the contradictory outcomes of follow-up studies — as well as the lack of studies performed in a Polish population — this study assessed the association of rs4730153 visfatin gene variants with DKD and DR in a group of Polish T2DM patients (the industrial region of Silesia, Poland).

Our study is first one to investigate the influence of SNP in visfatine gene on diabetic retinopathy and nephropathy occurrence.

**Materials and Methods**
Materials used for this genetic study were samples of venous blood taken from willing subjects. All patients partaking in this study has signed written consent. Study group consisted of 272 individuals. Among them 139 were men and 133 women. All of these patients had been previously diagnosed with T2DM.

For time needed to gather representative group for research, blood samples collected from subjects were stored in proper temperature at minus 70 Celsius degrees.

In laboratory of Clinical Hospital 1 in Zabrze the DNA material was isolated from obtained blood. Next step was to prepare proper concentration of the DNA which was 15 ng/μl. Then with spectrophotometer we checked the purity of samples.

Using fluorescent-labeled TaqMan Pre-designed SNP Genotyping Assay probes allelic discrimination was performed in Roche Lightcycler 96 thermocycler. Alleles were marked as A in VIC and G in FAM.

Finally statistical analysis was made to present result of the study. The significance between distributions of genotypes and alleles, presence of diabetic retinopathy, nephropathy, neuropathy and macroangiopathy were tested using Pearson’s χ² test. We used non-parametric ANOVA analysis to examine the association of visfatin polymorphism in rs4730153 with occurrence of late complications of diabetes mellitus. P values <0.05 were considered as statistically significant. The statistical software STATISTICA 13 for Windows (TIBCO Software Inc., Palo Alto, CA, USA) was used to perform all analyses.

Results

In order to analyze the dependence between visfatin’s gene in rs4720153 and late complications of T2DM the number of each patients’ alleles and genotypes were profiled with clinical data and occurrence of complications.

Among studied patients 29 were in the healthy weight range (10,5%), 115 overweight (42,5%) and 128 obese (47%) due to the WHO Body Mass Index (BMI) (Table 1). Waist to hip ratio (WHR) of patients was also assessed with similar results: 17 patients with normal ratio (6,25%), 64 over-weight (23,5%) and 191 obese (70,25%).

Table 1

| Genotype Distribution | Number of Patients |
|-----------------------|--------------------|
| AA        | 17 (33%) |
| AG        | 139 (51%) |
| GG        | 33 (17%) |

Regarding treatment schedule, at the moment of testing 101 individuals were treated with insulin injections, 171 were taking oral medicaments and 72 were prescribed both of them. Hypertension was present in 74,3% patients (202 patients). Patients with dyslipidemia stated 54,8 % (149) of all group.
We took into consideration 4 late complications of T2DM in anamnesis: retinopathy, nephropathy, peripheral neuropathy and macroangiopathy. Conducted analysis provided results as presented below.

Retinopathy occurred in 92 patients (33.8%). Regarding all of the patients, there were more ones without the retinopathy among AA and AG genotype. The above indicates more people with genotype GG developing this T2DM complication. (Table 3)

Table 3

Nephropathy was present in 26 cases (9.6%). Out of the patients who did not suffer with nephropathy 51% stated AG genotype. The greatest difference can be seen in GG genotype: 46 % among group of occurred by retinopathy to only 31% among patients without this complication. (Table 4)

Table 4

Considering the next studied complication, 88 patients of T2DM group had neuropathy, which is 32.3% of all. With slight difference of occurrence in AA genotype, the other two present as follows: more non-neuropathy patients in comparison to ones with neuropathy in AG genotype and consequently, less patient without this complication than these with neuropathy in GG genotype. (Table 5)

Table 5

Macroangiopathy showed most often occurrence. 37.9 % of the studied patients (103) had already been diagnosed with the disease while genotype determining. In this case, group with AA and GG genotype have not significantly more patients with the complication. (Table 6)

Table 6

24 (8,8%) of patients suffered with macroangiopathy and neuropathy altogether. Most of them presented GG genotype (45,8%), second AG (37,5%) and 16,7% AA genotype.

Only 6 (2,2%) individuals presented all of the mentioned complications.

We may note among all of the studied complications is that in AG genotype group there are always more patients without the disease than with one. Exactly opposite we may note in GG genotype in all of the studied cases.

We have not observed any statistically significant differences in genotype distribution between groups in all researched areas.

Discussion

The aim of the study was to investigate the correlation between different variants of visfatin gene in rs 4730153 and occurrence of late complications of T2DM in population of Southern Poland. We wanted to assess whether the studied SNP of visfatin gene is associated with increased risk of developing late complications of diabetes.
The T2DM is a growing problem nowadays; in 2013 there were 3 million patients diagnosed with diabetes in Poland [11]. What is more 30% patients diagnosed with myocardial infarction have also diabetes; one in seven patients with new diagnosed diabetes will develop acute coronary syndrome in next 10 years, 60% patients with duration of diabetes more than 15 years have retinopathy and 15%- nephropathy [12].

As has been mentioned, the level of proinflammatory visfatin is elevated in patients with diabetes mellitus. Many studies indicate that visfatin may lead to vascular disorders in different mechanisms: through visfatin ability to induce MMP-9 and nuclear factor-κB which are involved in instability of atherosclerotic plaque.

Moreover, visfatin may be engaged in endothelial disfunction [13]. It has been shown that in diabetic macroangiopathy the level of serum visfatin is significantly lower in comparison to patients with non-complicated diabetes. Additionally, serum level of visfatin can be negatively correlated with lipid profile [14].

It was described that visfatin as an adipokine with proangiogenic features may take some role in pathogenesis of diabetic retinopathy. In patients with diabetic retinopathy the concentration of visfatin in serum and vitreous is elevated and correlated with severity of disease [15].

It has been reported that visfatin may stimulate expression of endothelial nitric oxide in renal cells in patients with diabetic nephropathy. This observation seems to be confirmed by the fact that patients with diabetic nephropathy have increased level of visfatin in serum [16] especially in IV stage of disease in comparison to stage III [17]. Level of visfatin in serum can predict severity of diabetic nephropathy.

There are only several researches which investigated the role of visfatin polymorphism in rs4730153. Ones of them indicates that there is no association of T2DM and studied SNP, however the ratio visceral/subcutaneus visfatin expression is corelated with visfatin polymorphism in rs4730153 [18].

Other studies note the role of rs4730153 in pathogenesis of obesity; it was also described that plasma visfatin level is increased in patients with obesity. One study indicates that genotype AA in rs4730153 of visfatin gene may decrease the risk of cardiovascular disorders both in patients with normal body weight and with obesity. Variant AA of the rs4730153 is related with fasting blood glucose, fasting blood insulin and HOMA-IR (homeostasis model assessment-insulin resistance) [19].

Other research was carried out on Chinese obese children taking part in aerobic excercise training program. It has been observed statistically significant differences in level of triacylglycerols (TG) and HOMA-β value before and after exercise programme according to genotype in rs4730153. Genotype GG seems to reduce TG level and increase sensitive to insulin induced by excercises [20].

There are contradictory data on association between rs4730153 and BMI; some studies suggest that there is no association of rs4730153 and BMI [18], but on the other hand another one shows borderline significant correlation between rs4730153 and decreased BMI [21].
The present study was carried out since there are no studies about the role of visfatin polymorphism in rs4730153 in long term complications of diabetes mellitus. Our study is the first one which investigates the association between visfatin polymorphism and late complications of diabetes. We had not observed any statistically significant association between different variants of visfatin gene in rs 4720153 and occurrence of late complications of diabetes. However, duration of diseases and intensity of glycemic control influence strongly the development of diabetes complications [22]. Also the disproportion in number of patients with/ without specific complications could have affected the results.

Our study had some limitations: we did not measure the concentration of visfatin in serum; we also did not examine the expression of visfatin by measuring visfatin mRNA, so we could not asses how studied SNP influences on visfatin expression. Further studies with complete assessment of visfatin expression not only in serum, but also in organs involved in diabetic complications are necessary to assess the role of visfatin polymorphism in rs4730153 in development of long term complications of diabetes mellitus.

In comparison to other visfatin’s SNPs rs4720153 is yet to be widely researched.

**Conclusions**

Collected data showed that SNP in rs4730153 of visfatin using χ² test does not show statistically significant correlation in any of late complication of T2DM. All of the complications were present more often in heterozygote patients (AG) which may result from the largest group of patient with the genotype. Most tested patients were obese and suffered already with hypertension and dyslipidemia.

Because of a quite small group and not much of any other research on this polymorfism, further study is needed to know the role of SNP of visfatine in T2DM late complications.

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**List of abbreviations**

T2DM- Type 2 Diabetes Mellitus  
WHO- World Health Organization  
SNP- single nucleotide polymorphism  
BMI- Body Mass Index  
WHR- Waist to hip ratio  
TG- triacylglycerols  
HOMA-IR- homeostasis model assessment-insulin resistance
Statement of competing interests

The authors report no competing interests.

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**Table legend**

Table 1
Distribution of genotypes of visfatin’s SNP in rs4730153 in reference to BMI

Table 2
Distribution of genotypes of visfatin’s SNP in rs4730153 in research group

Table 3
Distribution of genotypes of visfatin’s SNP in rs4730153 in reference to retinopathy occurrence

Table 4
Distribution of genotypes of visfatin’s SNP in rs4730153 in reference to nephropathy occurrence

Table 5
Distribution of genotypes of visfatin’s SNP in rs4730153 in reference to neuropathy occurrence

Table 6
Distribution of genotypes of visfatin’s SNP in rs4730153 in reference to macroangiopathy occurrence

Tables

Table 1

| Genotype | normal body weight | overweight | obese |
|----------|-------------------|------------|-------|
| AA       | n                 | 4          | 26    | 15    |
|          | %                 | 9          | 58    | 33    |
| AG       | n                 | 15         | 57    | 66    |
|          | %                 | 11         | 41    | 48    |
| GG       | n                 | 10         | 32    | 47    |
|          | %                 | 11         | 36    | 53    |

p=0,1962

Table 2

| Genotype | Amount of carriers | Percentage of group |
|----------|--------------------|---------------------|
| AA       | 45                 | 17%                 |
| AG       | 138                | 51%                 |
| GG       | 89                 | 33%                 |

Table 3

| Genotypes | with retinopathy | without retinopathy | p value | OR (95% CI) |
|-----------|------------------|---------------------|---------|------------|
|           | N    | %     | N     | %     |   |       |
| AA        | 13   | 28,89 | 32    | 71,11 | -  | 1,00 (Reference) |
| AG        | 43   | 31,16 | 95    | 68,84 | 0,7742 | 1,114 (0,532-2,332) |
| GG        | 36   | 40,45 | 53    | 59,55 | 0,1914 | 1,672 (0,773-3,615) |
| AG+GG     | 79   | 34,80 | 148   | 65,20 | 0,4447 | 0,761 (0,378-1,533) |

Table 4

| Genotypes | with nephropathy | without nephropathy | p value | OR (95% CI) |
|-----------|-----------------|---------------------|---------|------------|
|           | N    | %     | N     | %     |   |       |
| AA        | 2    | 4,44  | 45    | 95,56 | -  | 1,00 (Reference) |
| AG        | 12   | 8,70  | 126   | 91,30 | 0,3606 | 2,048 (0,441-9,518) |
| GG        | 12   | 13,48 | 77    | 86,52 | 0,1245 | 3,351 (0,716-15,673) |
| AG+GG     | 24   | 10,57 | 203   | 89,43 | 0,2165 | 2,542 (0,579-11,162) |
Table 5

| Genotypes | with neuropathy | without neuropathy | p value | OR (95% CI) |
|-----------|----------------|-------------------|---------|-------------|
|           | N  | %   | N   | %     |         |              |
| AA        | 16 | 35,56 | 29  | 64,44 | -        | 1,00 (Reference) |
| AG        | 37 | 26,81 | 101 | 73,19 | 0,2631   | 0,664 (0,324-1,360) |
| GG        | 35 | 39,33 | 54  | 60,67 | 0,6713   | 1,175 (0,558-2,472) |
| AG+GG     | 72 | 31,72 | 155 | 68,28 | 0,6155   | 0,842 (0,430-1,648) |

Table 6

| Genotypes | with macroangiopathy | without macroangiopathy | p value | OR (95% CI) |
|-----------|----------------------|-------------------------|---------|-------------|
|           | N  | %   | N   | %     |         |              |
| AA        | 19 | 42,22 | 26  | 57,78 | -        | 1,00 (Reference) |
| AG        | 49 | 35,51 | 89  | 64,49 | 0,4190   | 0,753 (0,379-1,497) |
| GG        | 35 | 39,33 | 54  | 60,67 | 0,7469   | 0,887 (0,428-1,838) |
| AG+GG     | 84 | 37,00 | 143 | 63,00 | 0,5103   | 0,804 (0,420-1,540) |