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Research Article

Genetic Variability in Selected *ZnT8* SNPs in the Opolskie Voivodeship (Poland) - Relationship with Type 2 Diabetes and its Complications and Accompanying Diseases

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Abstract

Introduction: Type-2 Diabetes Mellitus (DM2) is of multigeneous origin, and its course may be modified by autoimmune mechanisms. It can be assumed that the clinically different course of diabetes depends, among others, on the genetically determined efficiency of the mechanisms of zinc homeostasis maintenance. The study of the relationship of mutations in the gene encoding *ZnT8* with the tendency to the occurrence of diabetes and its course may result in new therapeutic possibilities in the future. It is also interesting because in leukocytes only *ZnT8* shows significant individual variability in expression under physiological and pathological conditions, which indicates its genetic determinants.

Selection of SNP SLC30A8 GENE polymorphisms: In the study group, Single Nucleotide Polymorphism (SNP) was determined in the following *ZnT8* alleles: rs2466293, rs2466294, rs2466295, rs3802177, rs10282940, rs11558471, rs13266634.

Study group: A group of 467 people was examined, including 45 subjects constituting the control group. The group of 422 people were patients with type 2 diabetes. The group of people subjected to the study are randomly selected representatives of the population of the Opolskie Voivodeship, specific in terms of origin- indigenous and immigrant population from the today's Ukraine territory that prior to Second World War belonged to Poland.

The control group consisted of healthy people with no family history of type 2 diabetes, type 1 diabetes, obesity, hypertension and early symptoms of atherosclerosis-strokes and heart attacks before the age of 50. The group of patients with DM2 was additionally divided into subgroups depending on the presence of other health problems.

Results: As to rs13266634, the C/C mutation seems to predispose to DM2 development. The mutation does not contribute to occurrence of additional clinical problems in DMt2 patients besides a predisposition to heart failure. In rs11558471, the A/A mutation predisposes to the development of DM2. The mutation does not contribute to the development of additional clinical problems in patients with DM2 with the exception of dilated cardiomyopathy where it may be protective. In rs2466294, rs3802177, rs2466293 and rs10282940 SNP mutations in these alleles did not affect the development of DM2 in the study population.

Conclusion: The present study should be treated as the successive, little appendix to the needed widened research and observations explaining the complexity of the processes leading to the disclosure of DM2 and the progression of organ changes. Such studies will allow the development of new curation methods and more individualized treatment in the future.

Keywords: Opolskie voivodeship; Type-2 diabetes mellitus; Leukocytes; Obesity; Hypertension; Strokes

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Introduction

Zinc is an essential micronutrient in the body's homeostasis. It performs numerous structural and functional functions. Disturbances in zinc homeostasis leading to its accumulation in the intracellular space led to cell dysfunction and organ damage. The intracellular zinc homeostasis is caused by, inter alia, membrane transporters, *ZnT* and Zip, whose expression under physiological conditions is different in various organs. Expression of *ZnT8* physiologically takes place mainly in beta cells of the pancreas. This transporter is responsible for the transfer of Zn ions to the secretory vesicles, where zinc stabilizes insulin molecules [1-4]. Under pathological conditions, hyperglycemia stimulates the influx of Zn into the cell. In unregulated diabetes, *ZnT8* expression may appear in other tissues, which is most

likely related to the compensation mechanisms enhancing the outflow of zinc from the cell as a counteracting its excessive intracellular accumulation. Expression increases with increasing levels of HbA1c. In this context, the appearance of *ZnT8* expression in leukocytes may indicate the growing problems with the compensation of metabolic disorders in diabetes, as well as favour the appearance of antibodies against *ZnT8*.

Type 2 Diabetes Mellitus (DM2) is of multigeneous origin, and its course may be modified by autoimmune mechanisms [5]. It can be assumed that the clinically different course of diabetes depends, among others, on the genetically determined efficiency of the mechanisms of zinc homeostasis maintenance. The study of the relationship of mutations in the gene encoding *ZnT8* with the tendency to the occurrence of diabetes and its course may result in new therapeutic possibilities in the future. It is also interesting because in leukocytes only *ZnT8* shows significant individual variability in expression under physiological [6] and pathological [7-11] conditions, which indicates its genetic determinants.

Materials and Methods

Study group

A group of 467 people was examined, including 45 subjects constituting the control group. The group of 422 people were patients with type 2 diabetes. The group of people subjected to the study are randomly selected representatives of the population of the Opolskie Voivodeship, specific in terms of origin- indigenous and immigrant population from the today's Ukraine territory that prior to Second World War belonged to Poland.

The control group consisted of healthy people with no family history of type 2 diabetes, type 1 diabetes, obesity, hypertension and early symptoms of atherosclerosis - strokes and heart attacks before the age of 50. The group of patients with DM2 was additionally divided into subgroups depending on the presence of other health problems. Persons with type 2 diabetes were patients of the Department of Internal Diseases and Diabetes Clinic of the Provincial Hospital in Opole (2016-2018), and the control group were students of the Faculty of Physical Education and Physiotherapy at the Opole University of Technology.

In the study group, Single Nucleotide Polymorphism (SNP) was determined in the following *ZnT8* alleles:

rs2466293

rs2466294

rs2466295

rs3802177

rs10282940

rs11558471

rs13266634

In the group with diabetes, the occurrence of the above SNPs was analysed in relation to age, the onset of obesity, atrial fibrillation, nephropathy, arterial hypertension, circulatory failure, left ventricular hypertrophy, left ventricular dilatation, advanced atherosclerosis (stroke, myocardial infarction, limb arteriosclerosis. lower). Then, the prevalence of the above-mentioned (except age) in the group with diabetes was compared with the group of healthy subjects, without family history of type 2 and type 1 diabetes, collected on the basis of the questionnaire.

The research protocol was approved by the Bioethics Committee operating at the Regional Medical Chamber in Opole. People taking part in the study gave their consent to have blood taken for the tests. In the case of people with diabetes, there was no need for additional blood sampling because blood samples taken for routine morphology or glycated hemoglobin (HbA1c) tests were used for genetic tests.

Genetic methods

Selection of SNP SLC30A8 GENE polymorphisms: The study of the variability of the *SLC30A8* gene focused on polymorphisms in the non-coding region of the 3'UTR that may be functional in the post-transcriptional regulation of gene expression. Moreover, one SNP polymorphism (rs13266634) with functional significance documented in the literature, located in the coding region of the gene, was selected. The mini-sequencing technique was used to analyse selected single nucleotide polymorphisms of the SNP of the *SLC30A8* gene.

Isolation of Deoxyribonucleic Acid (DNA): Genotype analysis was preceded by the extraction of genomic DNA from peripheral blood leukocytes of test patients using the QIAamp DNA Blood Mini Kit (QIAGEN). Multiplex PCR amplification: amplification of the appropriate gene fragments was performed using the multiplex PCR technique. Seven pairs of primers were used in the reaction mixture to obtain the appropriate DNA fragments. The multiplex PCR primer sequences are summarized in Table 1.

Table 1: Multiplex PCR primer sequences.

Reference number (Ref SNP)	Starter sequence
rs13266634	F: 5' TGTGCAATCAGTGCTAATCTC 3;
	R: 5' AATGGTGAGTGAGTGCATCG 3'
rs2466295	F: 5' gatggatctcagtgcctcttc 3'
	R: (GACT) tcatgtcatggtgcaatgca 3'
rs2466294	F: 5' TGAACACAGCTACTGATCAG 3'
	R: 5' TGCTGCTATGCAGTTTCTGC 3'
rs3802177	F: 5' ATGGATCTCAGTGCCTCTTC 3'
	R: 5' CTGTGACTAGCTCAGTCAC 3'
rs11558471	F: 5' tggttgtagagacccagaa 3'
	R: 5' GCACTTGCTGCGTCTGATTC 3'
rs2466293	F: 5' gaactcctgtggttgacaatc 3'
	R: 5' tccacgagcatgatgtgagc 3'
rs10282940	F: 5' GCCTTCCATATGTTTGGTTAC 3'
	R: 5' GTGCCCACTCATGTTTGTAC 3'

Oligonucleotides (primers) were synthesized in the Laboratory of DNA Sequencing and Oligonucleotide Synthesis of Institute of Biochemistry and Biophysics, Polish Academy of Science AN. The QIAGEN Multiplex PCR Kit (QIAGEN) was used to perform the multiplex PCR. The amplification reaction was performed in a mixture that contained: 2.0 µl of DNA, 5.0 µl QIAGEN Multiplex PCR Master Mix, (containing HotStartTaq DNA polymerase, buffer and dNTP deoxynucleotides), 1.0 µl of primer mix (2 µM concentration of each primer), and 2.0 µl of water. The temperature profile of the amplification reaction was, as follows: initial denaturation (polymerase activation) 95°C for 15 min; denaturation 94°C for 30 sec; primer annealing 57°C for 90 sec; synthesis 72°C for 90 sec; additional elongation 72°C for 10 min.

The multiplex PCR efficiency and specificity of the products obtained were checked by electrophoresing a 5 µl reaction mixture on a 2% agarose gel. The amplification products were purified by removing free nucleotides and oligonucleotide fragments with enzymes: alkaline phosphatase (Shrimp Alkaline Phosphatase, SAP,

Applied Biosystems Thermo Fisher Scientific) and exonuclease I (Exonuclease I, Exo I, Thermo Scientific).

Mini-sequencing, also known as the SNaPshot technique, is one of the genotyping methods that allows the identification of genetic variants of the SNP type. The mini-sequencing technique involves performing the PCR in the presence of primers, probes and dideoxynucleotides, which allows the unlabelled primers to be extended by one nucleotide. Primers used in the mini-sequencing process are designed so that their 3' end after hybridization is immediately in front of the polymorphic site tested. Each dideoxynucleotide is labelled with a fluorescent dye, which enables its subsequent identification during capillary electrophoresis. The sequence of the mini-sequencing primers is provided in Table 2.

The SNaPshotTM Multiplex Kit-Applied Biosystems® was used to perform the mini-sequencing. The kit includes AmpliTag®DNA Polymerase-DNA polymerase, Reaction Buffer, fluorescently labelled dideoxynucleotides Fluorescently labelled ddNTPs. The SNaPshot reaction was performed separately for the F and R primers. Composition of the reaction mixture: 1.5 µl SNaPshot Mix, 0.25 µl SNaPshot F or R primer mix, 2.75 µl deionized water, 0. 5 µl Multiplex PCR products. Conditions for the SNaPshot reaction: denaturation 96°C for 10 sec, primer annealing 57°C for 5 sec, synthesis 60°C for 30 sec.

Mini-sequencing products prior to capillary electrophoresis were purified from unused dideoxynucleotides by alkaline phosphatase-SAP digestion. 0.5 µl of SAP was added to 5 µl of the SNaPshot mixture. The mixture was incubated at 37°C for 1 hour and then the enzyme was deactivated at 85°C for 15 min.

Capillary electrophoresis procedure: samples for capillary electrophoresis were prepared according to the protocol of the mini-sequencing kit manufacturer (ABI Prism® SNaPshot TM Multiplex Kit Protocol). 1 µl of the purified mini-sequencing products were combined with 10 µl of the solution mixture containing Hi-Di Formamide and the GeneScan™ 120 LIZ® Size Standard. The mixture of Hi-Di Formamide with the size standard was prepared in the proportion of 30 µl GeneScan™ 120 LIZ® Size Standard to 1 ml of Hi-Di Formamide. After mixing, the prepared samples were denatured at 95°C for 5 min, and then cooled to 4°C.

The analysis of mini-sequencing products was performed using the ABI PRISM 3130 Genetic Analyzer (Life Technologies). The presence of the GeneScan size standard- 120 LIZ during electrophoretic separation allowed, additionally, the identification of the analyzed DNA fragments in the range from 15 bp to 120 bp.

The results were analyzed using GeneMapper ID v 3.2 software (Life Technologies).

Statistical methods

The Pearson's chi-square test, NW chi-square test and Spearman's rank coefficient were used to assess the dependence of variables and their significance.

Results

An example of an evaluation for rs13266634 in DM2 with and without heart failure is provided in Table 3.

Patients with DM2

This analysis compared the DM2 group with the comorbid disease and the DM2 group without this condition. Comparing patients with DM2 only in the case of rs13266634, there were a statistically significant difference between the group with and without heart failure. In the case of rs13266634, there was statistically more C/C in the diabetics with heart failure than in the diabetics without heart failure. That finding can indicate that the C/C mutation may be associated with a predisposition to develop heart failure in people with DM2 (Table 3).

Concerning the remaining examined SNPs: rs2466293, rs2466294, rs2466295, rs3802177, rs10282940, rs11558471, there were no statistically significant differences between the patient groups.

Patients with DM2 and the control group (PO)

As part of the analysis, the group with DM2 with a given comorbid condition and without this condition was compared (as one group together) with the control group of normal subjects without a history of family history of DM2. The results obtained are depicted in Table 4.

rs13266634

In the DM2 group with and without nephropathy considered together (no differences between these groups), there were more C/C, and in the PO group - more C/T. In the DM2 group with and without obesity in total (no differences between these groups), there were more C/C, and in the PO- group C/T. In the DM2 group with and without hypertension in total (no differences between these groups), there were more C/C, and in the PO group- C/T. In the DM2 group with and without dilated cardiomyopathy in total (no differences between these groups), there were more C/C, and in the PO group - more C/T. In the DM2 group with and without hypertrophic cardiomyopathy in total (no differences between these groups), there were more C/C, and in the PO group- more C/T. In the group with DM2 with and without heart failure in total (no differences between these groups), there were more C/C, and in the PO group- C/T. In the DM2 group with and

Table 2: Mini-sequencing Primer Sequence (SNaPshot).

Reference number (Ref SNP)	Starter sequence SNaPshot
rs13266634	F: 5'CTTTATCAACAGCAGCCAGC 3'
	R: 5' CCGAACCACTTGGCTGTCCC 3'
rs2466295	F: 5' (GACT)ACTAAATAAATAGATAAAAT 3'
	R: 5' (GACT) tcatgtcatggtgcaatgca 3'
rs2466294	F: 5' (GACTGACTGACT) CTCTTCCTTCATGGTGAATG 3'
	R: 5' (GACTGACTGACT) TTATCTATTTATTTAGTT 3'
rs3802177	F: 5' (GACTGACTGACTGACT) ATAAAATGTGCATTGCACCATGA 3'
	R: 5' (GACTGACTGACTGACT) GGAACCAAAGGAAGAAATTCAT 3'
rs11558471	F: 5' (GACTGACTGACTGACTGACTGACT) AATTTAGATATTTACCTGCA 3'
	R: 5' (GACTGACTGACTGACTGACTGACT) TGCATCTGCTTTATTCCTTC 3'
rs2466293	F: 5' (GACTGACTGACTGACTGACTGACTGACT) ggttccaaaccaaaccca 3'
	R: 5' (GACTGACTGACTGACTGACTGACTGACT) TCTGGAAATCCTATGTCA 3'
rs10282940	F: 5' (GACTGACTGACTGACTGACTGACTGACT) CCTCTATTTCCTGATCAGT 3'
	R: 5' (GACTGACTGACTGACTGACTGACTGACT) ACATTTGAATAGGGAGGTTT 3'

Table 3: rs13266634 in diabetes type 2 with and without heart failure.

rs13266634	DM2 + heart failure - yes	DM2 + heart failure - no	Total
C/T	17	80	97
% of the column	23.61%	38.83%	
% of the line	17.53%	82.47%	
% of the total	6.12%	28.78%	34.89%
C/C	50	107	157
% of the column	69.44%	51.94%	
% of the line	31.85%	68.15%	
% of the total	17.99%	38.49%	56.47%
T/T	5	19	24
% of the column	6.94%	9.22%	
% of the line	20.83%	79.17%	
% of the total	1.80%	6.83%	8.63%
Total	72	206	278
% of the total	25.90%	74.10%	100.00%
Statistics	Chi^2	df	p
Pearson's Chi^2	6.758874	df=2	p=0.03407
NW Chi^2	6.954987	df=2	p=0.03088
Spearman's rank	-0.106979	t=-1.788	p=0.07495

without advanced atherosclerosis in total (no differences between these groups), there were more C/C, and in the PO group- more C/T. Finally, in the DM2 group with and without atrial fibrillation in total (no differences between these groups), there were more C/C, and in the PO group- more C/T.

Interpretation: As to rs13266634, the C/C mutation seems to predispose to DM2 development. The mutation does not contribute to occurrence of additional clinical problems in DMt2 patients besides a predisposition to heart failure.

rs2466295

Here, C/T occurred in the DM2 group with nephropathy statistically more often than in the group DM2 without nephropathy and the PO group. There were no statistically significant differences in the other groups.

Interpretation: in rs2466295, the C/T mutation may be related with a predisposition to diabetic nephropathy.

rs2466294

There were no statistically significant differences between the studied groups.

Interpretation: SNP mutations in this allele did not affect the development of DM2 in the study population.

rs3802177

There were no statistically significant differences between the studied groups.

Interpretation: SNP mutations in this allele did not affect the development of DM2.

rs11558471

In the DM2 group with and without nephropathy in total (no differences between these groups), there were more A/A, and in the PO group- more A/G. In the DM2 group with and without obesity in total (no differences between these groups), there was more A/A, and in the PO- group more A/G. In the DM2 group with and without hypertension in total (no differences between these groups), there were more A/A, and in the PO group- more A G. There were more A/A in the DM2 group without dilated cardiomyopathy, and more A/G in the group DM2 with dilated cardiomyopathy and in the group PO. In the DM2 group with and without hypertrophic cardiomyopathy in total (no differences between these groups), there were more A/A, and in the PO group- more A/G. In the DM2 group with and without heart failure in total (no differences between these groups), there were more A/A, and in the PO group- more A/G.

In the DM2 group with and without advanced atherosclerosis in total (no differences between these groups), there were more A/A, and in the PO group- more A/G. There were more A/A in the DM2 group with and without atrial fibrillation in total (no difference between these groups), and in the PO group A/G.

Interpretation: In rs11558471, the A/A mutation predisposes to the development of DM2. The mutation does not contribute to the development of additional clinical problems in patients with DM2 with the exception of dilated cardiomyopathy where it may be protective.

s2466293

There were no statistically significant differences between the studied groups.

Interpretation: SNP mutations in this allele did not affect the development of DM2.

Table 4: DM2 with a given comorbid condition and without it (as one group together) with a control group without a family history of DM2.

	DM-A/PO	DM-B/PO	DM-C/PO	DM-D/PO	DM-E/PO	DM-F/PO	DM-G/PO	DM-H/PO
rs13266634	CC/CT	CC/CT	CC/CT	CC/CT	CC/CT	CC/CT	CC/CT	CC/CT
rs2466295	CT*	ns	ns	ns	ns	ns	ns	ns
rs2466294	ns	ns	ns	ns	ns	ns	ns	ns
rs3802177	ns	ns	ns	ns	ns	ns	ns	ns
rs11558471	AA/AG	AA/AG	AA/AG	AA/AG	AA/AG**	AA/AG	AA/AG	AA/AG
rs2466293	ns	ns	ns	ns	ns	ns	ns	ns
rs10282940	ns	ns	ns	ns	ns	ns	ns	ns

A - DM2 with and without nephropathy
B - DM2 with and without obesity
C - DM2 with and without arterial hypertension
D - DM2 with left ventricular hypertrophy and with no left ventricular hypertrophy
E - DM2 with left ventricular dilatation and with no left ventricular dilatation
F - DM2 with and without heart failure
G - DM2 with and without advanced atherosclerosis
H - DM2 with and without atrial fibrillation
*In the DMt2 group with nephropathy, C/T occurred more frequently than in the non-nephropathic diabetic group and the PO group (taken together).
**In the DM2 group without dilated cardiomyopathy, there were more A/A, and in the group with dilated cardiomyopathy and PO (taken together)- A/G were more frequent.

rs10282940

There were no statistically significant differences between the studied groups.

Interpretation: SNP mutations in this allele did not affect the development of DM2.

Discussion

Research on gene variability, incl. *ZnT8* is important for future research in the field of pharmacogenetics- as sites of potential action of medicinal substances. As part of the studied *ZnT8* SNPs regarding the rs13266634 allele, it was found that in the studied population the C/C mutation predisposes to the development of DM2. This mutation, however, did not contribute to the development of additional, studied clinical problems in patients with DM2, with the exception of the presence of circulatory failure, to which it may predispose.

There are data confirming this relationship in the available literature. In the Chinese population, the C/C mutation in the rs13266634 allele was found to be more frequent in DM2 subjects and in subjects with impaired glucose tolerance [12]. A meta-analysis of European and Asian studies also showed such a relationship [13]. Contrary to this, no such relationship was found in the Iranian population [14].

It was also shown that the C/C mutation was accompanied by lower serum Zinc concentrations (Zn-s), while the highest serum zinc levels were found in subjects with the T/T mutation [15]. DM2 occurrence was found more frequently with the C/C mutation and, independently, with lower Zn-s [15]. Also, metabolic syndrome was more common in people with the C/C mutation [16,17]. Patients with the C/C mutation had more frequent postoperative hyperglycemia after major abdominal surgery [18]. In the rs13266634 allele, the C/C mutation predisposes to autoimmunity, i.e., it must be associated with greater expression of *ZnT8*, greater possibility of p/*ZnT8* antibody formation, and, consequently, faster progression of diabetes and its complications [18].

The presented by us observations indicate that in the rs13266634 allele, the C/C mutation may be related to the predisposition to the development of heart failure in people with DM2. This could be the result of association with atherogenic lipid profile [19] and a faster development of coronary atherosclerosis. Thus, this allele could be a potential target for drugs slowing DM2 complications development [20]. In patients with simultaneous HIV and HCV infection with the C/C mutation, lower blood HDL (atherogenic lipid profile) was found, and the highest HDL levels were found at C/T and T/T [19]. This fact is consistent with the observed by us tendency to more frequent occurrence of heart failure with this mutation.

As for the rs2466295 allele, the C/T mutation may be associated with a predisposition to develop diabetic nephropathy. In the Chinese population, no association of mutations in this allele with the presence of DM2 was demonstrated [21]. However, other studies showed that this mutation is associated with DM1 by increasing a predisposition to autoimmunity [22,23]. So, the overlapping genetic predisposition to DM2 with autoimmunity may predispose to a more rapid development of diabetic nephropathy.

In the case of the rs3802177 allele, our study did not show statistically significant differences between the studied groups, which means that SNP mutations in rs3802177 did not affect the development of DM2 in the examined population. It should be noted, however, that other studies revealed an association of C/C mutations in rs3802177 with both gestational diabetes [24] and DM2 in Asian and European populations [25-27].

In relations to the rs11558471 allele in the studied population, the A/A mutation seems to predisposes to the development of DM2. This mutation does not contribute to the higher probability of developing additional clinical problems in DM2 patients, apart from dilated cardiomyopathy, as to which it may be of protective effect.

Literature data indicate an association of mutations in this allele with DM2, but they are not conclusive. Associations with mutations in rs11558471 with gestational diabetes in Asian and Caucasian populations have not been demonstrated [28]. In the Chinese population, the rs11558471 A/A mutation was associated with DM2 [21,25,26], as well as with abnormal postprandial glycemia [29-31]. In the Malay population, the AG genotype in this allele turned out to be more common in DM2 patients.

As for the rs2466294 allele, there were no statistically significant differences between the examined groups. SNP mutations in this allele did not affect the development of DM2 in our population. In the Iranian population, there was found a relationship between the C/T mutation in the considered allele and DM2 occurence [32]. There were no statistically significant differences between the studied groups with regard to the rs10282940 allele. Also, no data on the SNP of this allele have been found in the available literature.

It should be emphasized that DM2 is a multi-gene phenomenon that is revealed sooner or later through a person's environmental and behavioural factors. Considering the human body through the prism of homeostasis, it can be stated that proper glucose metabolism is a condition for efficient Zn homeostasis. Protein glycation in hyperglycemia disrupts proteins function through disturbing Zn homeostasis, including changes in gene expression. This also applies to the zinc carrying proteins- *ZnT* and Zip. The altered expression of *ZnT* genes may also be a compensatory mechanism secondary to disorders of glucose metabolism. The polygenic efficiency of Zn metabolism may have an influence on the course of also polygenically determined DM2.

The question arises whether SNPs may appear with age? The overwhelming majority of SNPs for *ZnT8* are innate genetic beauty. Theoretically, it can be assumed that in rapidly dividing cell lines, e.g., leukocytes or intestinal epithelial cells, such a mutation may be of acquired nature, modifying the course of metabolic processes in a given cell line (similarly to mutations causing neoplastic growth). As a side note, it should be noted that in normal leukocytes, only *ZnT8* expression shows high variability and is not present in all tested samples, unlike other zinc transporters from the *ZnT* group [6,7]. This phenomenon can also concern pathophysiological conditions. This variability of *ZnT8* expression in leukocytes is probably of genetic background.

In DM2 diabetes in postmenopausal women, higher HbA1c values were shown in persons with *ZnT8* expression in leukocytes compared to the group not showing this expression [8]. Higher expression of the *ZnT8* gene is associated with a greater tendency to autoimmunity and the formation of anti-*ZnT8* antibodies, which increases the risk of diabetes complications - polyneuropathy and nephropathy. In turn, obesity promotes autoimmunity [9].

It has been hypothesized that silencing the *ZnT8* gene could

attenuate the autoimmune-induced inflammatory process in the pancreas and its damage [11]. It was found that the SNP Arg325Trp (rs13266634) SNP polymorphism influences *ZnT8* expression and cytokine production in Peripheral Blood Mononuclear Cells (PBMCs) in diabetic patients. In the case of the Arg/Arg polymorphism, a higher concentration of free intracellular Zn, higher *ZnT8* expression and higher cytokine production were found [10].

The present study should be treated as the successive, little appendix to the needed widened research and observations explaining the complexity of the processes leading to the disclosure of DM2 and the progression of organ changes. Such studies will allow the development of new curation methods and more individualized treatment in the future.

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