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The Expression Levels of SIRT1 Splicing Isoforms and Genes Regulating Mitochondrial Homeostasis in the Liver of Patients with Type 2 Diabetes Mellitus and Obesity

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ABSTRACT

Aim. To evaluate the association between the expression levels of sirtuin 1 (*SIRT1)* splicing isoforms and the genes associated with mitochondrial homeostasis (*PGC-1a, PPAR-γ, PPAR-α, TFAM, MFN2, OPA1,* and *DRP1*) in the liver of patients with type 2 diabetes mellitus (T2DM).

Material and methods. The study included 59 patients who were divided into two groups: 1) control group, body mass index (BMI) < 30 kg/m^2, without cardio-metabolic disorders; 2) patients with T2DM, BMI > 30 kg/m^2. A biochemical analysis of the patients' blood parameters was performed, and the expression level of the genes of interest in the liver tissue was studied using quantitative RT-PCR.

Results. It was found that the *SIRT1* splicing isoforms *V1, V2*, and *V3* were stably expressed in the liver of patients with T2DM. *SIRT1* isoforms occur not only individually, but also in various combinations. The expression of the *SIRT1 V3* isoform was significantly increased in the group of patients, while the remaining analytes did not significantly differ between the groups. The *SIRT1 V3* isoform positively correlated with glucose levels. It is worth noting that the total *SIRT1* did not show significant correlations with the genes of interest and biochemical parameters, which only confirms the need to study the expression of isoforms separately.

Conclusion. *SIRT1* isoforms were stably expressed in the liver, and the expression level of *SIRT1 V3* isoform was significantly higher in patients with T2DM. The results of this work can serve as a basis for further studies of interactions between *SIRT1* splicing isoforms and mitochondrial homeostasis proteins at the post-translational level.

Keywords: type 2 diabetes mellitus, alternate splicing, mitochondria, mitochondrial fission and fusion, liver

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association (2000) and the Protocol to the Convention on Human Rights and Biomedicine (1999). The study was approved by the local Ethics Committee of Immanuel Kant Baltic Federal University (Minutes No. 40 dated June 26, 2023). All participants signed a consent form to participate in the study.

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Исследование уровней экспрессии сплайсинговых изоформ SIRT1 и генов – регуляторов митохондриального гомеостаза в печени больных сахарным диабетом 2-го типа и ожирением

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РЕЗЮМЕ

Цель. Оценка ассоциации между уровнем экспрессии изоформ сиртуина 1 (*SIRT1*) и генами белков, связанных с митохондриальным гомеостазом (*PGC-1α*, *PPAR-γ*, *PPAR-α*, *TFAM, MFN2, OPA1, DRP1*) в печени больных сахарным диабетом второго типа (СД2).

Материалы и методы. В исследование включено 59 пациентов, которые были разделены на две группы: 1) контрольная группа, индекс массы тела (ИМТ) менее 30 кг/м^2, без кардиометаболических нарушений; 2) пациенты с СД2, ИМТ более 30 кг/м^2. Выполнялся биохимический анализ показателей крови пациентов, а уровень экспрессии генов интереса в печеночной ткани изучали с помощью количественной полимеразной цепной реакцией с обратной транскрипцией.

Результаты. Обнаружено, что сплайсинговые изоформы *SIRT1 V1, V2* и *V3* стабильно экспрессировались в печени у больных СД2. Выявлено, что изоформы *SIRT1* встречаются не только по отдельности, но и в различных сочетаниях. Экспрессия изоформы *SIRT1 V3* значимо повышалась в группе больных, в то время как остальные аналиты значимо не различались между группами. Изоформа *SIRT1 V3* положительно коррелировала с уровнем глюкозы. Стоит отметить, что общий *SIRT1* не показал значимых корреляций с генами интереса и биохимическими показателями, что только подтверждает необходимость изучения экспрессии изоформ отдельно.

Заключение. Изоформы *SIRT1* стабильно экспрессировались в печени, уровень экспрессии изоформы *SIRT1 V3* был значимо выше у больных СД2. Результаты работы могут послужить основой для дальнейших, более точечных исследований взаимодействий между сплайсинговыми изоформами *SIRT1* с белками митохондриального гомеостаза на посттрансляционном уровне.

Ключевые слова: сахарный диабет 2-го типа, альтернативный сплайсинг, изоформы сиртуина 1, митохондриальный гомеостаз, митохондриальная динамика

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Для цитирования: Воронова С.С., Бограя М.М., Горбачева А.М., Вульф М.А., Газатова Н.Д., Литвинова Л.С. Исследование уровней экспрессии сплайсинговых изоформ SIRT1 и генов – регуляторов митохондриального гомеостаза в печени больных сахарным диабетом 2-го типа и ожирением/ Дифференцировочный статус клеток рака молочной железы, его сопряженность с метастазированием и с продукцией опухолью цитокинов при различных молекуляных подтипах. *Бюллетень сибирской медицины*. 2026;26(1):32–42. https://doi.org/10.20538/1682-0363-2026-1-32-42.

INTRODUCTION

The incidence of type 2 diabetes mellitus (T2DM) has increased alarmingly over the last 40 years [1]. The liver is particularly affected by T2DM, recent meta-analyses confirm that patients with T2DM develop steatosis, steatohepatitis, and fibrosis of the liver tissue in around 60% of cases [2]. Insulin resistance combined with adipose tissue dysfunction is considered to be the main cause of the development of comorbid liver pathology in T2DM [3]. Active lipolysis, which occurs as a result of adipose tissue dysfunction [3], leads to an increase in the amount of free fatty acids (FFA) in the bloodstream [4].

The liver actively accumulates FFA, but their excess is associated not only with steatosis but also with the development of oxidative stress. Thus, the increased β-oxidation of FFA leads to the accumulation of reactive oxygen species (ROS) in hepatocytes [5]. Oxidative stress is an integral part of T2DM [6] and has a detrimental effect on the cell. Oxidative stress leads to a violation of mitochondrial homeostasis [7] and the development of mitochondrial dysfunction in the form of dysregulation of mitophagy [8] and mitochondrial dynamics (MD) [9], a decrease in the copy number of mitochondrial DNA (mtDNA) [10], and mitochondrial biogenesis [11].

It is well known that mitochondrial dysfunction plays a key role in the pathogenesis of T2DM, as insulin resistance and hyperglycemia eventually lead to dysregulation of energy substrate consumption in all body tissues [12]. This not only destabilizes energy homeostasis, but subsequently also impairs the formation of ROS, MD, and apoptosis: an imbalance of these processes in T2DM subsequently leads to a lack of vital functions, including disruption of hepatocytes and cardiac muscle cells, hindered production of insulin by beta cells, impaired homeostasis of neurons, etc. [13].

Mitochondrial homeostasis is a complex process controlled at many molecular levels: by MD (mitochondrial fusion/division), mitophagy, mitochondrial dissociation, and antioxidant systems [12]. Certain other processes also have recently become the focus of research: mtDNA heteroplasmy, the non-coding RNAs, and their epigenetic regulation of the mitochondrial genome and transcriptome [13]. From this perspective, the phenomenon of mitochondrial hormesis (a slight increase in ROS concentration leading to an improvement in mitochondrial homeostasis) is also interesting. It has been shown that mitochondrial hormesis can activate *AMPK* and *PGC-1a* through *SIRT1/3*, thus restoring normal mitochondrial homeostasis [14]. Therefore, investigating the molecular mechanisms of mitochondrial homeostasis maintenance/disruption is an important task in the context of investigating the pathogenesis of T2DM comorbidities.

Mitochondrial homeostasis in the cell is regulated by both the nuclear and mitochondrial genomes [7]. Thus, the main players are: 1) transcription factors – *TFAM, NRF-1*, and *NRF-2*, which control the expression of mtDNA genes [7]; 2) receptors activated by peroxisome proliferators (*PPARs*), which belong to the nuclear hormone receptor superfamily [7, 15]. As a transcription factor, PPARα regulates the expression of genes encoding enzymes that metabolize fatty acids and the activity of mitochondrial β-oxidation in the liver [16]. PPARγ, in turn, stimulates *de novo* lipogenesis [17]; 3) *PGC-1* family transcriptional coactivators (*PGC-1a, PGC-1b,* and *PRC*) that contribute to the maintenance of mitochondrial biogenesis [18], particularly *PGC-1a* [19]; 4) proteins associated with mitochondrial division (DRP1) and fusion [20] (*MFN1, MFN2*, and *OPA1*).

Many researchers are now focusing on the obvious aspects of impaired mitochondrial homeostasis in the liver, such as the role of transcription factors associated with mitochondrial biogenesis [21] or the expression of genes that regulate oxidative phosphorylation [22]. Of course, these are important links in the pathogenesis of mitochondrial homeostasis disorders, but it is equally important to investigate regulators at a higher level, in particular the histone deacetylase sirtuin 1 (*SIRT1*).

SIRT1 has been extensively studied in the context of T2DM and its comorbidities [23, 24]. *SIRT1* is known to regulate the work of many proteins associated with mitochondrial homeostasis at the post-translational level [25]. Deacetylation increases the activity of the transcriptional coactivator *PGC-1a* [26, 27] and the transcription factor *PPARα* [28] and represses the activity of *PPARγ* [29, 30]. Deacetylation, also by *SIRT* family proteins (*SIRT1* and *SIRT3*), is associated with the regulation of balance in the MD. It is known that deacetylation of *DRP1* leads to a reduction in the activity of this protein, suppressing mitochondrial division [31], while deacetylation of *MFN2* [32] and *OPA1* [33], on the contrary, stimulates their active fusion. Furthermore, it has been shown that *SIRT1* splicing isoforms may be involved in the regulation of oxidative phosphorylation and ATP formation by deacetylation of mitochondrial respiratory chain complex 1 [34].

Indeed, particular attention should be paid to the fact that the *SIRT1* gene is a multi-exon gene that undergoes alternative splicing [35]. Three isoforms of *SIRT1* are distinguished (Fig. 1). The first isoform (*V1*, transcript identifier ENST00000212015.11) is canonical and consists of nine exons (ex1–9) and has a length of 747 amino acid (AA) residues. This isoform contains two nuclear localization signal sites (NLS), encoded in ex1 and ex3, and two nuclear export signal sites (NES)in ex1 and ex7, respectively. Neither the second (*V2*) nor the third isoform (*V3*) has the first or third exon. These isoforms are therefore exclusively cytoplasmic, which has also been confirmed [35].

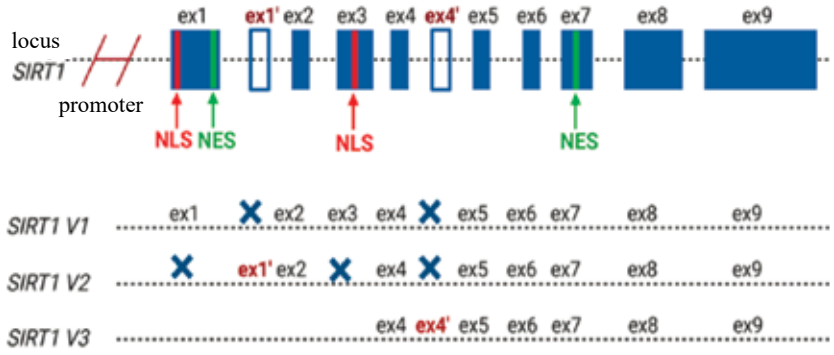

Fig. 1. Structure of SIRT1 isoforms [35]. Abbreviations: NLS – nuclear localization signal, NES – nuclear export signal

The second isoform (ENST00000432464.5) has a shortened N-terminus and consists of 452 AA residues. The transcript encoding *V2* has 8 exons: 7 exons similar to those of *V1* (ex2 and ex4–9) and one exon unique to this isoform (ex1'). However, since the reading frame covers the range from ex4 to ex9, *V2* differs from *V1* only by the shortened N-terminus and has no unique segments.

The third isoform (ENST00000406900.5) is the shortest with a length of 444 AA. *V3* has a unique sequence of 11 AA residues at the N-terminus. This is due to the fact that *V3*, like *V2*, has a variable exon, but unlike the second isoform, this exon (ex4') is included in the reading frame.

It is assumed [34] that these differences influence not only the localization of the isoforms but also their functional activity. However, this question has not yet been considered in the context of human liver tissue. Therefore, **the aim** of our study was to investigate the relationship between the expression level of *SIRT1* isoforms and the genes of proteins associated with mitochondrial homeostasis (*PGC-1a, PPARγ, PPARα, TFAM, MFN2, OPA1,* and *DRP1*) in the liver of patients with T2DM.

MATERIALS AND METHODS

The study participants were diagnosed by the assigned healthcare providers and admitted to Kaliningrad Regional Clinical Hospital. Before the elective abdominal surgery, which was performed under general anesthesia, the participants underwent a standard dietary adjustment. All obese patients stopped taking medication that affects carbohydrate and lipid metabolism 36 hours before the surgery. On the day of the surgery, venous blood samples were taken in the morning following overnight fasting. During surgery, liver samples up to 0.5 cm^3 in volume were collected for RNA isolation (biopsies were preserved in 600 µl RNAlater solution (Ambion, Waltham, Massachusetts, USA)). The study was conducted in accordance with the Declaration of Helsinki of the World Medical Association (2000) and the Protocol to the Convention on Human Rights and Biomedicine (1999). The study was approved by the local Ethics Committee of Immanuel Kant Baltic Federal University (Decision of the Ethics Committee of IKBFU No. 40 dated June 26, 2023). All participants signed a consent form to participate in the study.

Inclusion criteria were as follows: individuals over 21 years of age referred for elective abdominal surgery for various indications: hernioplasty, gastric resection, and pathology of the gallbladder and biliary tract (cholelithiasis, cholecystitis, polyposis, cyst, etc.). Participants had to confirm their willingness to participate in the study by providing a written informed consent.

Exclusion criteria were the following: infectious liver diseases, concurrent somatic-symptom and infectious diseases in the acute inflammatory stage, a known infection with the human immunodeficiency virus or any malignant or benign neoplasms. Individuals under the age of 21 or those who refused to undergo medical and laboratory examinations during the study or to sign a consent form were also excluded.

The study enrolled 59 patients with a mean age of 49.15 ± 10.96 years; 23 men and 36 women), who were divided into two groups: 1) control group (n = 28: 13 men and 15 women) with body mass index (BMI) less than 30 kg/m^2 and without cardio-metabolic disorders; 2) patients with type 2 diabetes mellitus (n = 31: 10 men and 21 women) with BMI exceeding 30 kg/m^2.

The material for the biochemical tests was blood obtained the morning before surgery following overnight fasting by puncturing the ulnar vein in vacuum tubes with a clot-forming activator. The biochemical parameters of the blood were analyzed with a Furuno CA-180 analyzer (Furuno Electric Company, Japan) using DiaSys test systems (DiaSys Diagnostic Systems, Holzheim, Germany).

Biopsies of liver tissue were used as material for analyzing the expression of genes of interest. Total RNA was isolated from a liver biopsy with a volume of approximately 100 µl using ExtractRNA (Evrogen, Russia) according to the manufacturer's protocol. The isolated total RNA was eluted in 50 µl of RNase-free water. The RNA concentration was measured immediately after isolation using an Implen NanoPhotometer N (Implen, Germany). Samples were stored at –80°C until reverse transcription, followed by real-time polymerase chain reaction (RT-PCR).

Universal reverse transcription was performed using the MMLV RT kit (Eurogen, Moscow, Russia) with the addition of RiboCare RNase inhibitor (Eurogen, Moscow, Russia) according to the manufacturer's protocol. HS-SYBR PCR (Eurogen, Moscow, Russia) was used for quantitative PCR. The primer sequences are listed in the text below, the annealing temperature of all primers was 62°C. PCR results were amplified and read using a CFX96 thermal cycler (Bio-Rad, Hercules, CA, USA). After amplification, the melting curves were analyzed to verify the specificity of the reactions.

To normalize the gene expression data, the reference gene *RPLP0* was used as an internal control. The following primer sequences were used:

SIRT1-F: AGGAGCAGATTAGTAGGCGGC,
SIRT1-R: TGGACTCTGGCATGTCCCAC,
V1-F: AGGGCGAGGAGGAGGAAGAG,
V1-R: GTCCAGTCACTAGAGCTTGCA,
V2-F: TTCGCTCTTTTCCTCCGTCC,
V2-R: ACAGAAGGTTATCTGGCTGCT,
V3-F: CTGTGCAGTGGAAGGAAAACA,
V3-R: GATTCCCGCAACCTGTTCCA,
PPAR-γ-F: GATGACAGCGACTTGGCAATA,
PPAR-γ-R: GGCTTGTAGCAGGTTGTCTT,
PPAR-α-F: GCCCTGTCTGCTCTGTGGA,
PPAR-α-R: GCCGAGCTCCAAGCTACTCTT,
PGC-1α-F: TGCTCGGAGCTTCTCAAATATC,
PGC-1α-R: CCCAAGGGTAGCTCAGTTTATC,
MFN2-F: CCAGCGTCCCATCCCTCT,
MFN2-R: TCCACACCACTCCTCCAACA,
DRP1-F: TCTGGAGGTGGTGGGGTTG,
DRP1-R: TGGGTTTTGATTTTTCTTCTGCTAAT,
OPA1-F: ATCTGTGGATGCTGAACGCA,
OPA1-R: GAATCCTGCTTGGACTGGCT,
PRKAA1-F: ACAGAGATCGGGATCAGTTAG,
*PRKAA1-R:*GAGGTCACAGATGAGGTAAGA,
TFAM-F: CGCTCCCCCTTCAGTTTTGT,
TFAM-R: TACCTGCCACTCCGCCCTAT,
RPLP0-F: GGCGACCTGGAAGTCCAACT,
RPLP0-R: CCATCAGCACCACAGCCTTC.

Transcripts cycle of thresholds (Ct) were converted to relative expression values using the $2^{-\Delta Ct}$ method and then transformed to Log_{10} for normalization. Outliers were identified and excluded using the ROUT method (Q=1%). The normality of the data distribution was assessed using the Shapiro–Wilk test. If the data conformed to a normal distribution, the hypothesis of equality of sample means was tested using the Welch's t-test, otherwise the non-parametric Mann–Whitney test was used. The difference in the occurrence of transcript combinations of *SIRT1* isoforms between the groups was assessed using the chi-square test (χ^2 test). Correlations were determined using the Spearman's rank correlation coefficient. Differences were considered significant at $p < 0.05$. Statistical data processing was performed using GraphPad Prism 9.3.1 software.

Primers were designed for the *SIRT1* isoforms and *SIRT1* total (Fig. 2) so that they were only annealed at isoform-specific sites.

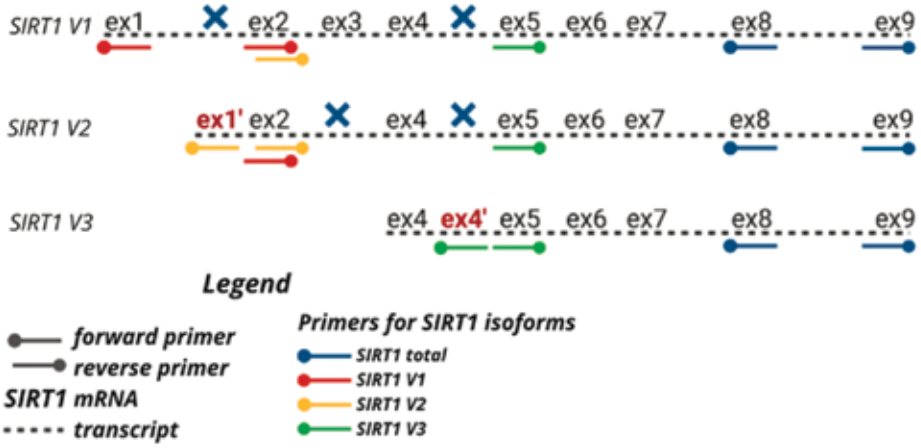

Fig. 2. Design of the primers for the *SIRT1* isoforms. *SIRT1 V1-V3* – *SIRT1* isoforms and specific primers for them; *SIRT1 total* – primers annealed to all *SIRT1* isoforms; ex1–9 – exons of the *SIRT1* gene

RESULTS

A comparative analysis of the clinical and biochemical parameters of the groups involved in the study is shown in Table 1. The control group differed significantly from the study group in terms of BMI, fasting glucose, triglycerides, and HDL. The groups did not differ significantly in other anthropometric and biochemical parameters.

The expression level of the genes of interest in liver biopsies was investigated. We found no significant differences in the mRNA expression of the transcription factors *PGC-1a* (p = 0.1275), *PPARγ* (p = 0.8047), and *PPARα* (p = 0.7927) (Figure 3, a). The expression level of genes associated with MD *TFAM* (p = 0.4188), *MFN2* (p = 0.6295), *OPA1* (p = 0.5149), and *DRP1* (p = 0.7507) also did not differ significantly (Fig. 3, a). The expression level of the AMPK subunit *PRKAA1* (p = 0.1430) and of *SIRT1 total* (p = 0.5609) and isoforms *V1* (p = 0.3166) and *V2* (p = 0.2254) did not change significantly, but the expression of isoform *V3* was significantly increased in patients with T2DM compared to the control group (1.3-fold change; p = 0.0009) (Fig. 3, b).

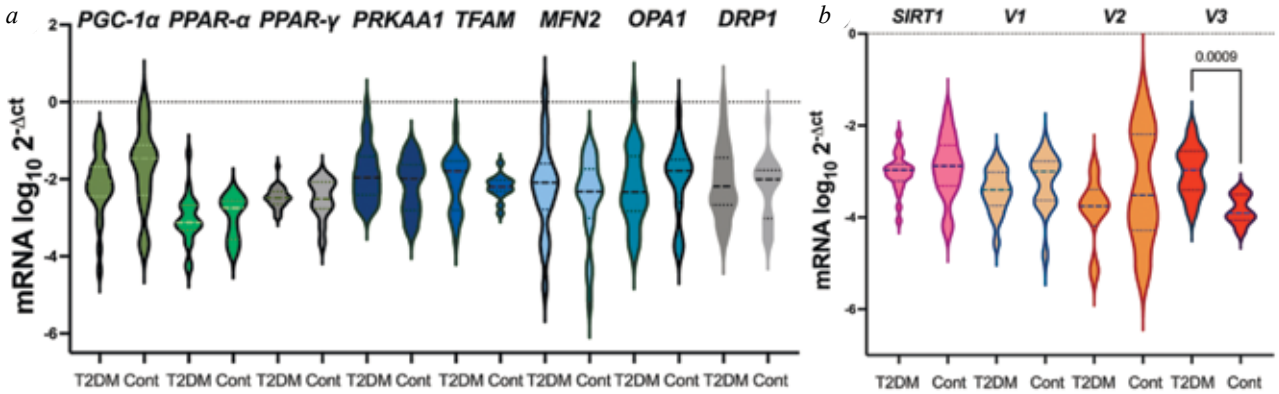

Fig. 3. Expression level of genes of interest: *a* – Genes related to mitochondrial homeostasis *PGC-1a, PPARα, PPARγ,* and *PRKAA1* and MD *TFAM, MFN2, OPA1,* and *DRP1*; *b* – *SIRT1* and its isoforms. Statistical analysis was performed using the Shapiro-Wilk test, the unpaired Mann–Whitney test and the Welch's t-test. Abbreviations: Cont – conditionally healthy donors, T2DM – patients with type 2 diabetes mellitus.

Table 1

Clinical and Kiochemical Parameters of the Studied Groups, *M* ± *SD*

	Control group, n = 28	T2DM, n = 31	p-value
BMI, kg/m^2	24.12 ± 3.78	49.49 ± 10.61	<0.0001**
Age, years	51.07 ± 13.43	47.22 ± 7.52	0.2009**
Sex (men / women)	13 / 15	10 / 21	0.2965***
Fasting glucose, mmol/l	4.56 ± 0.54	7.45 ± 1.98	<0.0001*
Total cholesterol (TC), mmol/l	5.27 ± 1.10	5.30 ± 0.91	0.9066**
Triglycerides (TG), mmol/l	1.17 ± 0.44	2.01 ± 1.09	<0.0001*
High-density lipoproteins (HDL), mmol/l	1.44 ± 0.37	1.23 ± 0.73	0.0036*
Low-density lipoproteins (LDL), mmol/l	3.18 ± 0.84	2.97 ± 0.71	0.5544*
Alanine aminotransferase (ALT), mmol/l	17.85 ± 13.87	22.45 ± 15.07	0.2010*
Aspartate aminotransferase (AST), mmol/l	24.27 ± 15.23	21.00 ± 8.36	0.2040*

* the analysis was performed using the unpair ed Mann–Whitney test, ** the analysis was performed using the Welch's t-test, *** the analysis was performed using the Fisher's exact test

When investigating the expression of *SIRT1* isoforms, it was found that *SIRT1* isoforms occurred not only individually, but also in various combinations. The occurrence of transcript combinations of *SIRT1* isoforms was analyzed in different groups (Fig. 4).

It was found that the *V2* isoform did not occur individually in patients with T2DM, while, in contrast, the *V3* isoform alone was only found in patients with T2DM, and the combination of *V2* + *V3* isoforms was not found in either patients or healthy individuals.

None of the *SIRT1* isoforms was detected in 15 individuals. The combination of isoforms differed significantly between patients with T2DM and the control group ($p = 0.0047$) (Fig. 4). The processes that control the expression patterns of the *SIRT1* isoforms have not yet been investigated.

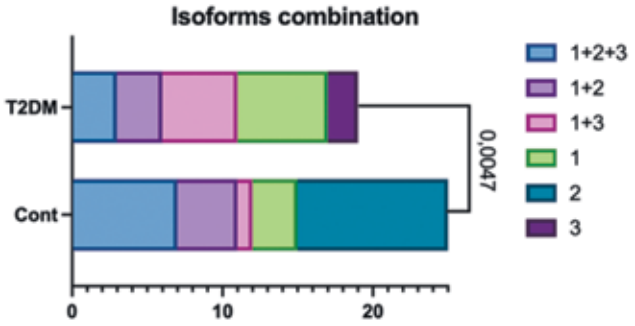

Fig. 4. Analysis of the combination of isoforms depending on the study group. The statistical analysis was performed using the χ^2 test.

The results of the correlation analysis are shown in Figure 5. It is worth noting that *SIRT1 total* did not show significant correlations with the genes of interest (*PGC-1a, PPARγ, PPARα, TFAM, MFN2, OPA1,* and *DRP1*), which only confirms the need to study the expression of the isoforms separately. In contrast to the *SIRT1 total*, *SIRT1* splicing isoforms correlated differently with genes related to mitochondrial homeostasis.

The expression level of the *V1* isoform correlated significantly with the expression level of *PGC-1a, PPARα,* and *PPARγ* ($r = 0.73$, $r = 0.73$, and $r = 0.45$, respectively; $p < 0.05$) and with *PRKAA1* ($r = 0.50$, $p < 0.05$) as well as with the expression level of the MD genes *MFN2, OPA1,* and *DRP1* ($r = 0.45$, $r = 0.50$, and $r = 0.45$, respectively; $p < 0.05$). The expression level of the *V2* isoform correlated positively with the expression level of *PGC-1a* ($r = 0.69$, $p < 0.05$), as well as with *PRKAA1* ($r = 0.58$, $p < 0.05$) and the MD genes *MFN2, OPA1,* and *DRP1* ($r = 0.74$, $r = 0.65$, and $r = 0.57$, respectively; $p < 0.05$). In contrast, the expression level of the *V3* correlated significantly only with the expression level of *MFN2* ($r = 0.53$, $p < 0.05$). In addition, a strong positive correlation was found between *V3* and FBG levels ($r = 0.68$, $p < 0.05$).

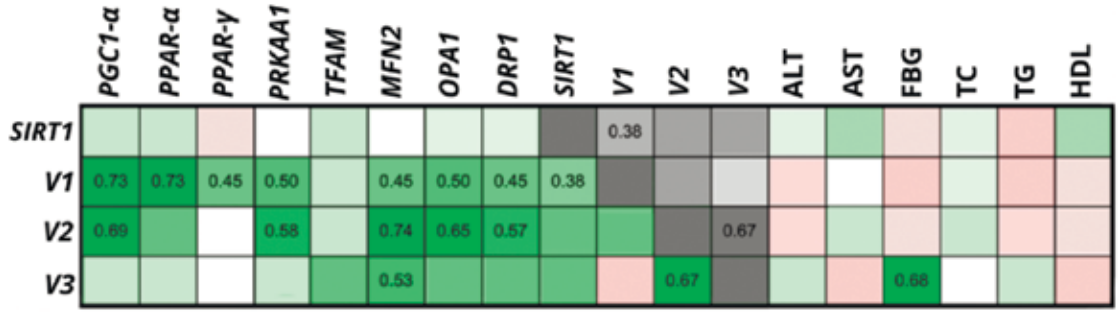

Fig. 5. Correlation matrix. Spearman's rank correlation coefficient, only significant correlations are shown ($p < 0.05$). Abbreviations: ALT – alanine aminotransferase; AST – aspartate aminotransferase; FBG – fasting blood glucose; TC – total cholesterol; HDL – high-density lipoproteins; TG – triglycerides

DISCUSSION

In this work, the expression of the histone deacetylase *SIRT1* in liver samples from patients with T2DM and obesity and apparently healthy donors was comprehensively investigated for the first time, taking into account the different *SIRT1* splicing isoforms and their relationship to the expression of the main signaling-associated genes.

The transcription factors *PGC-1a, PPARγ, PPARα,* and *TFAM*, which are associated with mitochondrial biogenesis and ATP synthesis, as well as the MD genes *MFN2, DRP1*, and *OPA1* were studied in detail in the context of T2DM and its comorbidities. For example, it was shown that the expression of *PGC-1a* was suppressed in the skeletal muscle of patients with T2DM [36, 37].

In mouse models of hepatic steatosis, it was shown that the level of *PGC-1a* expression and production in the liver decreased significantly, resulting in suppression of the expression of the mitochondrial transcription factor *TFAM* [21], and the level of *PPARα* expression also reduced significantly, while the level of *PPARγ* expression increased [38].

Using a cellular model of hepatic steatosis (HepG2 + oleic acid) *in vitro*, it was found that the production of *SIRT1* and *PGC-1a* decreased significantly, while a decrease in *SIRT1* levels directly suppressed mitochondrial division due to a decrease in MFF protein levels [39]. It was found that in the oxidative stress model

(HepG2 + tret-butylhydroperoxide), the expression of *TFAM, DRP1*, and *MFN2* was significantly reduced [40]. Consistent with these results, the expression and production of *MFN2* in the liver were reduced in a rat model of insulin resistance [41].

Our study showed that the expression level of all listed genes remained unchanged in the liver of patients with T2DM and obesity.

There are few such data on studies performed on human liver tissue (for T2DM or its comorbidities, especially steatosis and steatohepatitis).

Thus, in the English-language literature, no information was found on the nature of the expression of the *PRKAA1, TFAM, MFN2, DRP1,* and *OPA1* genes in the human liver in T2DM. At the same time, the expression of *PGC-1a* was found to be significantly decreased in the liver tissue of patients with obesity and T2DM [42]. The same pattern of expression changes was observed in hepatic steatosis [43], which was associated with an increase in the degree of methylation of the promoter of this gene. In another study, *PPARα* levels in human liver tissue negatively correlated with the degree of steatosis and the presence of steatohepatitis and positively correlated with adiponectin level [44]. In contrast, *PPARγ* levels were elevated in individuals with NAFLD [45]. The reasons why the expression of these genes did not change in our study are not entirely clear and require further investigation.

However, unique results were obtained for *SIRT1*. Previously, it was shown in animal models that *SIRT1* expression significantly decreased in T2DM or its concomitant diseases [46, 47]. In general, the role of *SIRT1* in insulin resistance in the liver is defined as protective as it enhances the regulation of gluconeogenesis and mitochondrial biogenesis [48]. Data on *SIRT1* expression in human liver are limited, but suggest that the pattern of *SIRT1* expression is generally similar to cellular and mouse models of T2DM, with a decreasing trend [49, 50]. We have shown that the expression of *SIRT1* in the liver did not change in patients with T2DM and obesity compared to the control group. However, this does not apply to the individual *SIRT1* isoforms. Thus, *V3* expression was significantly increased in patients with T2DM ($p = 0.0009$). In addition, *V3* expression level correlated positively with FBG levels ($r = 0.68, p < 0.05$), indicating a fundamental, completely unexplored relationship of this subunit with the pathogenesis of T2DM.

Differences in the expression patterns of *SIRT1* transcripts were found between the study groups. In both groups, a combination of all three isoforms was most frequently observed. However, the *V2* isoform was only expressed alone in the control group, while the *V3* isoform was only found individually in patients with T2DM. In general, the occurrence of transcript combinations of the *SIRT1* isoforms differed significantly between the control group and the study group (χ^2 test, $p = 0.0047$).

The correlation analysis data showed that *SIRT1 total* did not correlate significantly with the genes of interest. This is an unexpected and interesting result that only confirms the need to study the *SIRT1* splicing isoforms separately. At the same time, the individual *SIRT1* isoforms showed positive correlations with many genes of interest. Most importantly, the *SIRT1* isoforms had slightly different correlation signatures. Nuclear *V1* and cytoplasmic *V2* isoforms were similar in their association with MD gene expression levels (positively correlated with *MFN2, DRP1,* and *OPA1*) and with *PGC-1a* and *PRKAA1*, but differed in their association with the transcription factors *PPARγ* and *PPARα*: *V1* significantly correlated with them, whereas *V2* did not. At the same time, *V3* showed a correlation only with *MFN2*, which further underlines the presumed special function of *V3* in the liver.

Thus, the *SIRT1* isoforms may represent novel links in the regulation of mitochondrial homeostasis in T2DM. It is particularly important to study the isoforms separately as they have different expression patterns and probably regulate metabolic processes in the cell in different ways, including mitochondrial homeostasis.

CONCLUSION

SIRT1 V1, *V2*, and *V3* splicing isoforms were stably expressed in the liver of patients with T2DM, and the expression level of *SIRT1 V3* was significantly increased in the liver of patients with T2DM. Studying the *SIRT1* splicing isoforms thoroughly is necessary in the context of the molecular mechanisms of disruption/maintenance of mitochondrial homeostasis as it is likely that *SIRT1* isoforms regulate this process in different ways. The resulting correlation map can be used for further selective studies of interactions between *SIRT1* isoforms and mitochondrial homeostasis proteins.

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