Final report on the research project:

„Erythrocyte membrane proteins for early diagnosis of type 2 diabetes mellitus"

I. Background, scientific questions of the project

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease affecting more than 400 million people worldwide and accounts for about 90% of diabetes cases. It is a multifactorial disease, which means that the genetic background, lifestyle, hormonal changes, infections, or medications can all play a role in the development of the disease, which makes it difficult to establish an accurate diagnosis and therapy. Due to insufficient insulin production and/or tissue insulin resistance, compensatory high blood sugar levels can cause damage to the heart, blood vessels, eyes, kidneys, and nervous system. Since the symptoms of T2DM are mostly non-specific in the early stages, there are currently no biomarkers available that would be suitable for asymptomatic or early detection of the disease. Although there are several biomarkers, including HbA1c, to characterize already established disease, predicting disease-related complications is still difficult. Many studies deal with the complex genetic background of T2DM, but according to the results of the genome-wide association (GWA) study, polymorphic versions of the human genome are difficult to interpret with predictive value. In addition, environmental and nutritional factors greatly influence the actual phenotypic effects of genetic polymorphisms.

Our working group deals with the complex analysis of red blood cell (RBC) membrane proteins, which enables the combined examination of protein expression patterns, genetic polymorphisms, and laboratory data. For the quantitative determination of protein expression, we used a flow cytometric test panel developed and patented in our laboratory to measure red blood cell membrane proteins. Starting from a small amount of blood that can be taken even from a fingertip, the red blood cell membranes become accessible after a mild fixation, without disturbing effect of the haemoglobin. With the help of the unlabeled monoclonal primary antibodies and the fluorescent labeled secondary antibody that are bound to them (indirect labeling), we can determine the expression pattern characteristic of a given population. In the case of expression levels that differ by at least 10% from the average, some regulatory and/or genetic background can be assumed. With this method, it is easy to find those mutations that characterize the disease or one of its phases.[1]

During our work, in the case of membrane transporters ABCA1, ABCG2, PMCA4b, GLUT1, GLUT3, URAT1, MCT1, we found differences in the progression of the disease or in the related laboratory data.

ABCA1 is a large, highly glycosylated member of the ATP-binding cassette (ABC) membrane transporter family that plays a major role in cholesterol and phospholipid regulation in many tissues, including the liver. ABCA1 is involved in the formation of HDL particles by promoting cholesterol transfer from cells to apolipoprotein A. Decreased ABCA1 expression, particularly in macrophages, may promote atherosclerosis and cholesterol deposition in peripheral tissues and organs. Since HDL level is a risk factor for diabetes, ABCA1 may serve as a diagnostic marker in T2DM.

ABCG2 (BCRP, MXR) is a homodimeric ABC transporter whose main function is the extrusion of harmful compounds and the transport of xenobiotics and endobiotics through the

plasma membrane and the tissue barrier, thus playing a key role in the so-called "chemoimmunity".

This protein is expressed in many tissues, including the gut, the blood-brain barrier, the placenta, and various stem cells. ABCG2 plays an important role in modulating the absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox) properties of various drugs and is also involved in multidrug resistance in cancer cells.

ABCG2 is responsible for the extrarenal (mainly intestinal) extrusion of uric acid, as it is one of the key uric acid transporters in enterocytes, while this transporter is also present and functions in uric acid export in the renal proximal tubules. GWA studies have shown that a reduced expression level and/or function of ABCG2 (e.g., Q141K variant) is associated with an increase in serum uric acid levels, which is an important risk factor for the development of gout. Since several diseases, including hypertension, chronic renal failure, and T2DM are also associated with high serum uric acid levels, ABCG2 may play a role in these cases.

PMCA4b plays a key role in maintaining low cellular Ca^{2+} concentrations in many tissues, especially red blood cells. In certain pathological conditions, such as hereditary hemolytic anemia, diabetes mellitus, the Ca^{2+} level of red blood cells increases due to reduced PMCA activity.

GLUT1 (SLC2A1) is the main glucose transporter in red blood cells and is present in large amounts in many other tissues (e.g., blood-brain barrier, adipocytes, renal cortical mesangial cells). The regulation of GLUT1 expression is influenced by both extrinsic and intrinsic factors, for example, blood sugar, hormones or hypoxia modify the expression level of this protein in different organs or tissues. GLUT3 (SLC2A3) is the main glucose transporter in the central nervous system and this protein is also present in other tissues. There are only limited data on the regulation of GLUT3 expression in T2DM, while it has been hypothesized that epigenetic changes may result in differential GLUT3 expression levels.

The main monocarboxylate transporter of the RBC membrane is MCT1 (SLC16A1), and members of the SLC16A family are proton-dependent monocarboxylate transporters that pump protons and monocarboxylates (such as lactate or pyruvate) as symporters from the extracellular matrix into cells. These proteins play an important role in the Cory cycle, that is, the lactate producing metabolic pathway in muscle or other cells, including red blood cells. Plasma lactate concentration correlates with the rate of glycolysis, which reflects mitochondrial oxidative capacity, and lower oxidative capacity is strongly associated with T2DM.

URAT1 (SLC22A12), a member of the OAT (Organic Anion Transporter) family, plays a key role in uric acid homeostasis, and is present in relatively large amounts in the red blood cell membrane. The relatively high level of circulating uric acid characteristic of humans prevents bone mineral loss and oxidative damage, however, on the other hand it leads to gout, high blood pressure and cardiovascular diseases. Studies with mouse models have reported a decrease in the level of URAT1 in diabetic patients, probably related to insulin deficiency.

During the tender, we searched for an answer to the question whether the examined membrane transporters show a correlation with the disease or some of its phases. Do the already known mutations affect the progression of the disease and/or the laboratory values. Can we find differences in the level of the membrane protein expression that can be traced back to certain genetic background. In the course of our work, we compared the laboratory, protein and genetic

data of 36 newly diagnosed patients who did not receive medication, 61 treated, well-maintained patients and 23 T2DM patients with complications with the data of 59 age-matched controls.

II. Key results of the project

1. Alterations in erythrocyte membrane transporter expression levels in type 2 diabetic patients

Membrane proteins play a key role in many phases of the development of T2DM. Studies related to the expression and function of metabolites or ion transporters, as well as membrane proteins that influence the drug treatment of the disease, may offer new diagnostic tools for the prevention and/or effective treatment of T2DM.

In our published study (Szabó et al, SciRep, 2021)[2], we examined the red blood cell membrane transporters GLUT1 (SLC2A1), GLUT3 (SLC2A3), MCT1 (SLC16A1), URAT1 (SLC22A12), ABCA1, ABCG2 and PMCA4b. Examining the differences between the expression levels of control subjects and the entire T2DM patient group, we found that the expression levels of GLUT1, GLUT3 and MCT1 were significantly lower in T2DM patients, while the expression of URAT1 was significantly increased compared to control subjects. We found no significant differences in the expression of PMCA4b, ABCA1 or Band3 between control individuals and T2DM patients (*Figure 1.*).

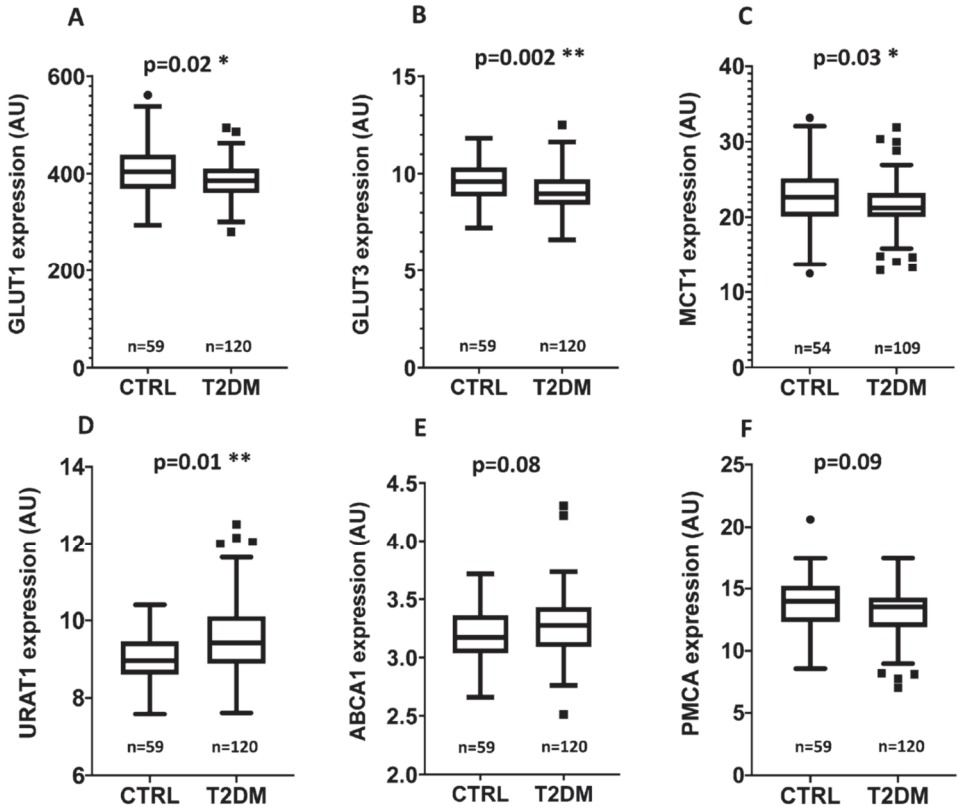

Figure 1. *Expression of the RBC membrane proteins in age-matched control subjects and in T2DM patients, measured by flow cytometry. (**A**) GLUT1, (**B**) GLUT3, (**C**) MCT1, (**D**) URAT1, (**E**) ABCA1, (**F**) PMCA4b. The p values were calculated by the Mann–Whitney test. The number of participants (n) is listed in each panel.*

We also examined differences in protein expression levels between T2DM patients and controls carrying mutations of ABCG2 or PMCA4b and found significant differences. PMCA4b expression was lower in T2DM patients with wild-type haplotype compared to the control individuals, while there was no such difference in patients and controls carrying the minor haplotype. On the other hand, in the case of ABCG2, there was no difference in the level of red blood cell protein expression between control subjects and T2DM patients in individuals carrying the wild-type gene. However, in T2DM patients carrying the Q141K polymorphic variant had significantly lower RBC ABCG2 levels than in control subjects. The ABCG2-Q141K polymorphism discussed in more detail in the next chapter.

In the following analysis, we compared the expression levels of red cell membrane transporters among control subjects, untreated, successfully treated T2DM patients, and T2DM patients with disease-related complications. We found significant differences between GLUT1, GLUT3, MCT1 (all decreased), URAT1 and ABCA1 (both increased) levels when successfully treated patients were compared to the control subjects. This difference was also significant for increases in URAT1 and ABCA1 in successfully treated patients compared to untreated patients. Analyzing the data of T2DM patients with disease-related complications, in these cases can be observed significant decrease in GLUT1 transporter levels, decrease in GLUT3 levels (control versus T2DM complication and successfully treated patients versus T2DM complications), increase in URAT1 and ABCA1 levels (control versus T2DM complication, and untreated patients versus those with T2DM complications). To further analyze and display the differences between T2DM patient groups and control subjects, we created Kernel density estimation diagrams (*Figure 2.*).

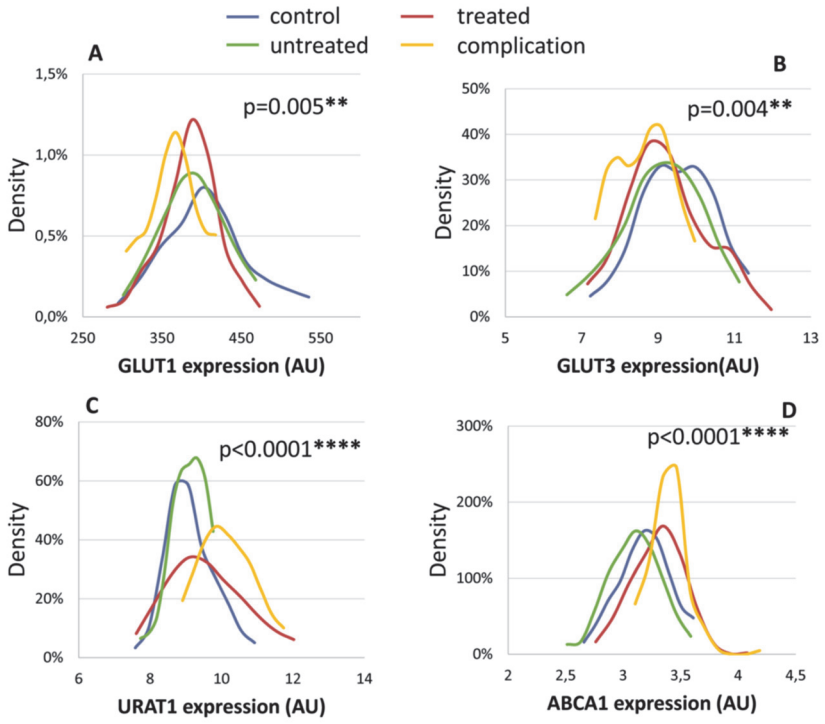

Figure 2. *Kernel density diagrams and the p values obtained for the statistical differences between the protein expression values within the groups. The p values were analyzed by the Kruskal–Wallis test. (**A**) GLUT1; (**B**) GLUT3; (**C**) URAT1; (**D**) ABCA1. Blue: control individuals (n = 59, except in MCT1, n = 54) grey: recently diagnosed, untreated T2DM patients (n = 36, except for MCT1, n = 31), red: successfully managed patients (n = 61, except for MCT1, n = 55), and orange: patients showing treatment resistance or disease-related complications (n = 23)*

Since higher blood glucose levels are associated with T2DM pathology, the next step was to analyze the potential relationship between actual blood glucose levels and the expression of erythrocyte membrane transporters. Based on the recommendations of the American Diabetes Association (ADA), three groups were created based on fasting blood sugar levels, in which blood sugar level below 5.5 mmol/L is considered normal, and increased blood sugar level between 5.6 and 6.9 mmol/L is indicated prediabetes, while level higher than 7 mmol/L indicates the presence of diabetes. Higher HbA1c levels correlated with significantly reduced GLUT1, GLUT3 and MCT1 expression levels in the red blood cell membrane, while URAT1 and ABCA1 expression levels increased in patients with higher HbA1c. This correlation was particularly strong when transporter expression values in subjects with normal HbA1c were compared with transporter expression values in subjects with high HbA1c levels. A small decrease in ABCG2 transporter levels was also observed in this analysis (*Figure 3.*).

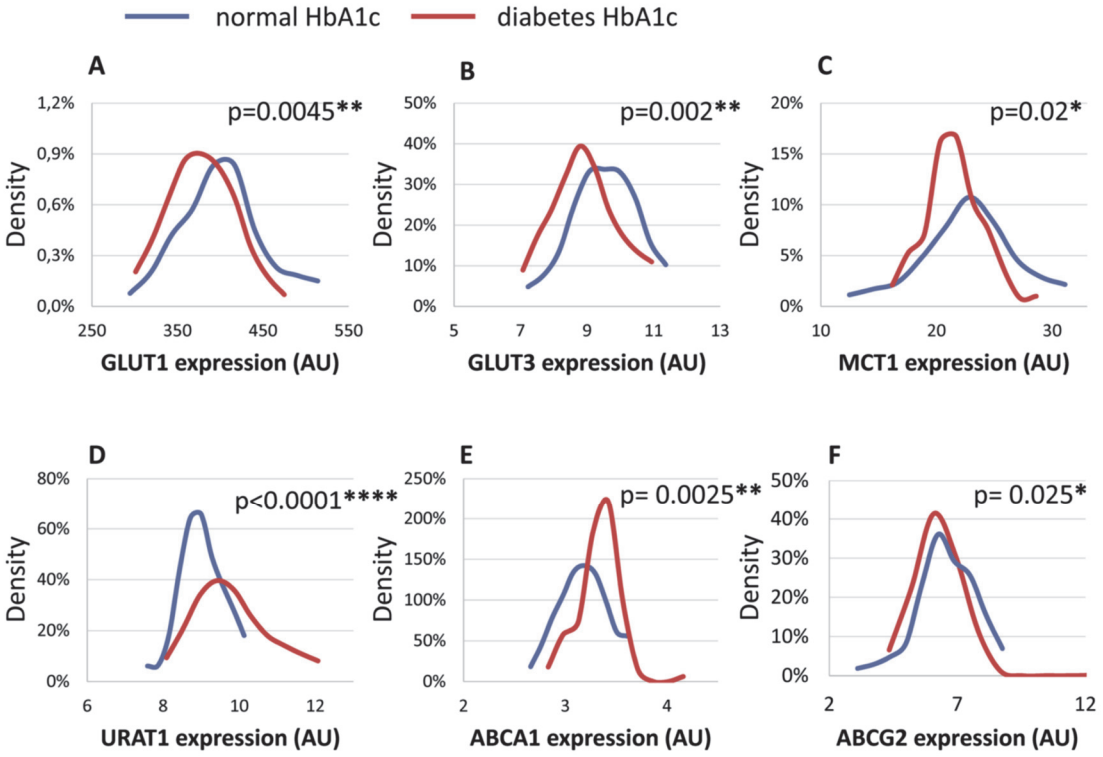

Figure 3. *Kernel density diagrams and the p values obtained for the statistical differences between the protein expression levels within the groups with normal HbA1c (n = 55, except for MCT1, n = 50), versus highly increased HbA1c levels (HIL—n = 64, except for MCT1, n = 59). Blue: normal, < 5.6% HbA1c, red: HIL, > 6.5% HbA1c. The p values were calculated by Mann–Whitney test. Expression levels of (**A**) GLUT1; (**B**) GLUT3; (**C**) MCT1; (**D**) URAT1; (**E**) ABCA1, and (**F**) ABCG2.*

In summary, we discovered new associations between the expression level of the examined membrane proteins and T2DM and its complications, with which a comprehensive analysis at the laboratory, protein and genetic level, similar to gout, is needed to find the most suitable treatments for the patients.

2. Potential role of the ABCG2-Q141K polymorphism in type 2 diabetes and gout

The membrane protein ABCG2 is an important diagnostic marker in the examination of gout, since its reduced expression level and/or function is associated with an increase in the serum uric acid level, which is an important risk factor in the development of gout. Genetic studies have shown a 40-70% heritability of serum uric acid levels, while 20-30% of T2DM patients have high serum uric acid levels. In addition, complications of T2DM, e.g., diabetic nephropathy, retinopathy, and cardiovascular disease are closely related to high serum uric acid levels. GWA and directed molecular genetic studies identified reduced ABCG2 membrane expression levels associated with a relatively common polymorphism (rs2231142, C421A), resulting in the ABCG2-Q141K protein variant.

In our published study (Szabó et al, Plos One, 2021)[3], we compared cohorts of T2DM patients and healthy individuals in terms of HbA1c, blood sugar and uric acid levels, and determined the presence of the ABCG2 leading SNP protein variant (ABCG2-Q141K). We found a significant correlation between metabolic parameters and the ABCG2 polymorphism among diabetic patients. While considering only relatively small cohorts, we found that T2DM patients carrying the ABCG2-C421A SNP had significantly higher blood glucose and HgA1c levels than patients carrying only wild-type alleles. In contrast, there was no such difference in blood glucose or HbA1c levels in healthy control individuals, which correlates with the presence of the ABCG2-Q141K polymorphism. These data suggest that the presence of the functionally impaired ABCG2-Q141K variant, even in adequately treated patients, corresponds to a reduced treatment effect in T2DM patients, both in case of short-term and long-term blood glucose levels. Thus, the presence of this ABCG2 variant may promote the occurrence of disease complications associated with long-term high blood sugar (*Figure 4.*).

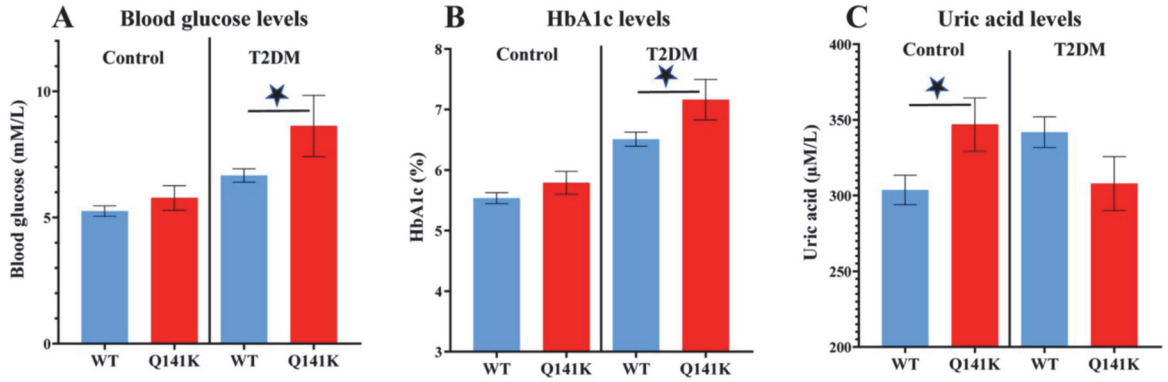

Figure 4. *Blood glucose (A), HbA1c (B) or uric acid (C) levels and the presence of the Q141K variant (both hetero- and homozygotes) in the groups of healthy individuals and T2DM patients. Values are expressed as means ± SE. Blue: ABCG2-wild type, red: ABCG2-Q141K. * indicates a significant difference obtained in the individuals carrying the Q141K polymorphism p<0.05. The p values were calculated by Student's t test.*

In another publication (Palinkas et al, Clinical and Experimental Medicine, 2022) [4], we looked for an answer to the question of what kind of differences would be obtained if ABCG2 is shifted towards the development of generally accepted gout rather than the disruption of sugar metabolism there in the laboratories. In the present case, we examined not only ABCG2-Q141K, but also other mutations affecting the function of the protein (M71V, R236X, R383C).

ABCG2 functional polymorphisms were associated with greater gout susceptibility and clinically severe, early-onset disease. The examined SNPs showed a correlation with reduced protein expression, which could be associated with additional clinical data, such as increased fractional urate excretion and urinary urate excretion values (*Figure 5-6.*). These results demonstrate a clear relationship between ABCG2 dysfunction and renal overload hyperuricemia in clinically defined gout patients.

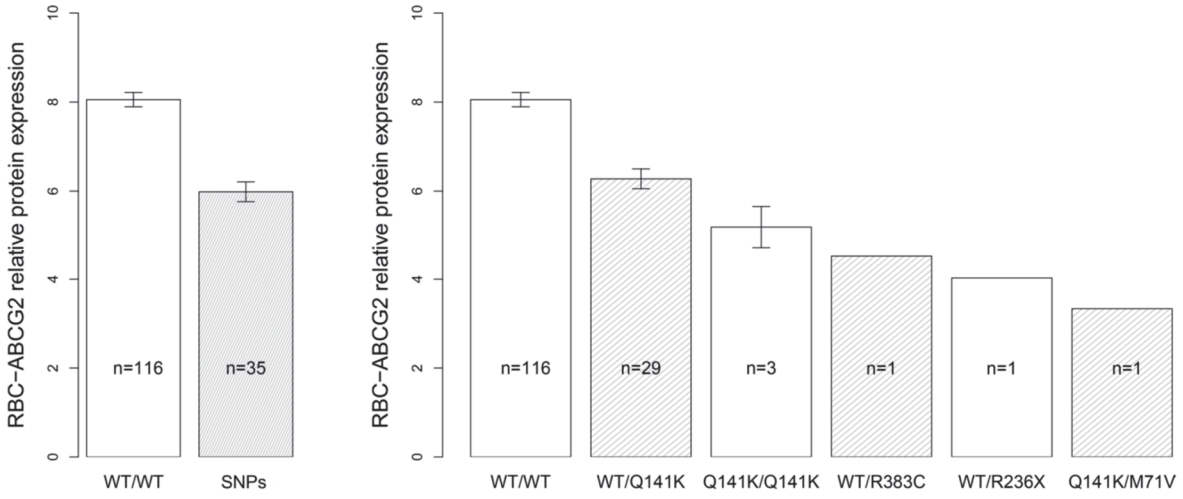

Figure 5. *Correlation of functional ABCG2 polymorphisms and protein expression levels*

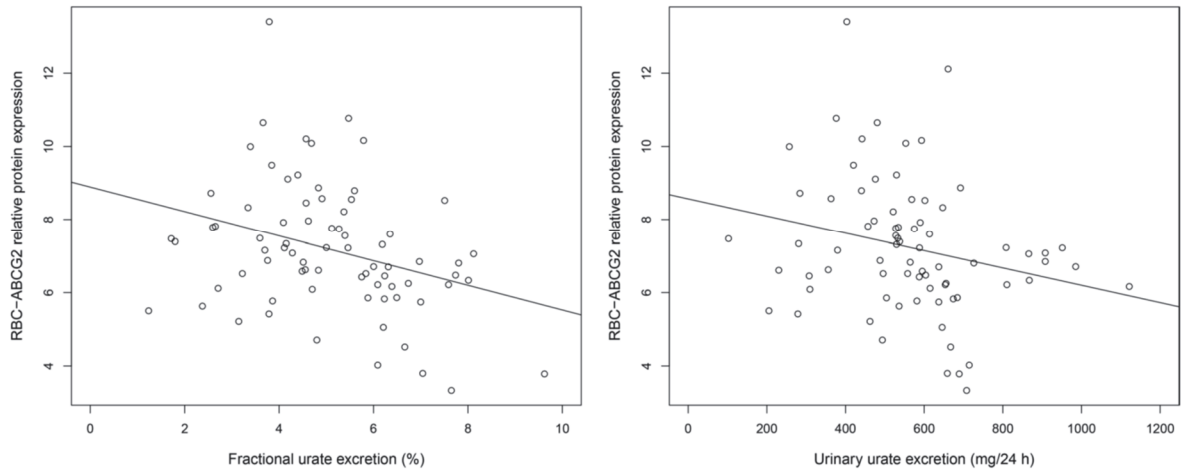

Figure 6. *Correlation of renal-overload hyperuricemia clinical parameters and ABCG2 protein expression*

3. Genetic modulation of the GLUT1 transporter expression - potential relevance in complex diseases

We continued the further investigation of the protein expression patterns measured on red blood cells by characterizing the GLUT1 membrane protein in samples different from the population average. Based on the expression levels, we sequenced the exon and exon/intron boundaries of 3 samples with high, 3 low and 3 average GLUT1 protein levels, where 4 SNPs were identified. rs11282849 (MAF=0.47) is an insertion affecting every second person, so we did not work with it further. SNPs rs11537641 (located in exon 4, MAF=0.19) and rs2229682 (located in exon 5, MAF=0.19) synonymous variants belong to the same haplotype. The rs11537641 SNP was designated as the lead SNP. Variant rs1385129 (MAF=0.22) is a synonymous SNP, located in exon 2 and member of a large haplotype block. Based on literature data, we identified two more SNPs, which may be relevant in the change in the expression level of GLUT1. The rs841848 SNP is in the putative enhancer region of the 2nd intron and is a member of a large haplotype. Variant rs841847 is located in the same enhancer region but is independent of the preceding haplotype (*Figure 7.*).

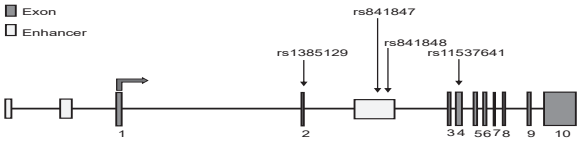

Figure 7. *Structure of human SLC2A1 gene with the 10 exons and the putative enhancers. The 4 examined SNPs are also presented.*

Based on these data, we performed TaqMan analysis on our patient and control samples with the SNPs found. Compared with flow cytometry data, we found that rs1385129, rs11537641 and rs841848 significantly increased GLUT1 expression level compared to rs841847 SNP, which was significantly enriched in samples with low GLUT1 expression. We found no difference between the expression level and the presence of SNPs associated with the disease. A luciferase reporter gene assay was used to examine the actual effect of the SNPs. With the help of this method, we can determine the regulatory role of a given SNP and its associated region on the expression of the protein.

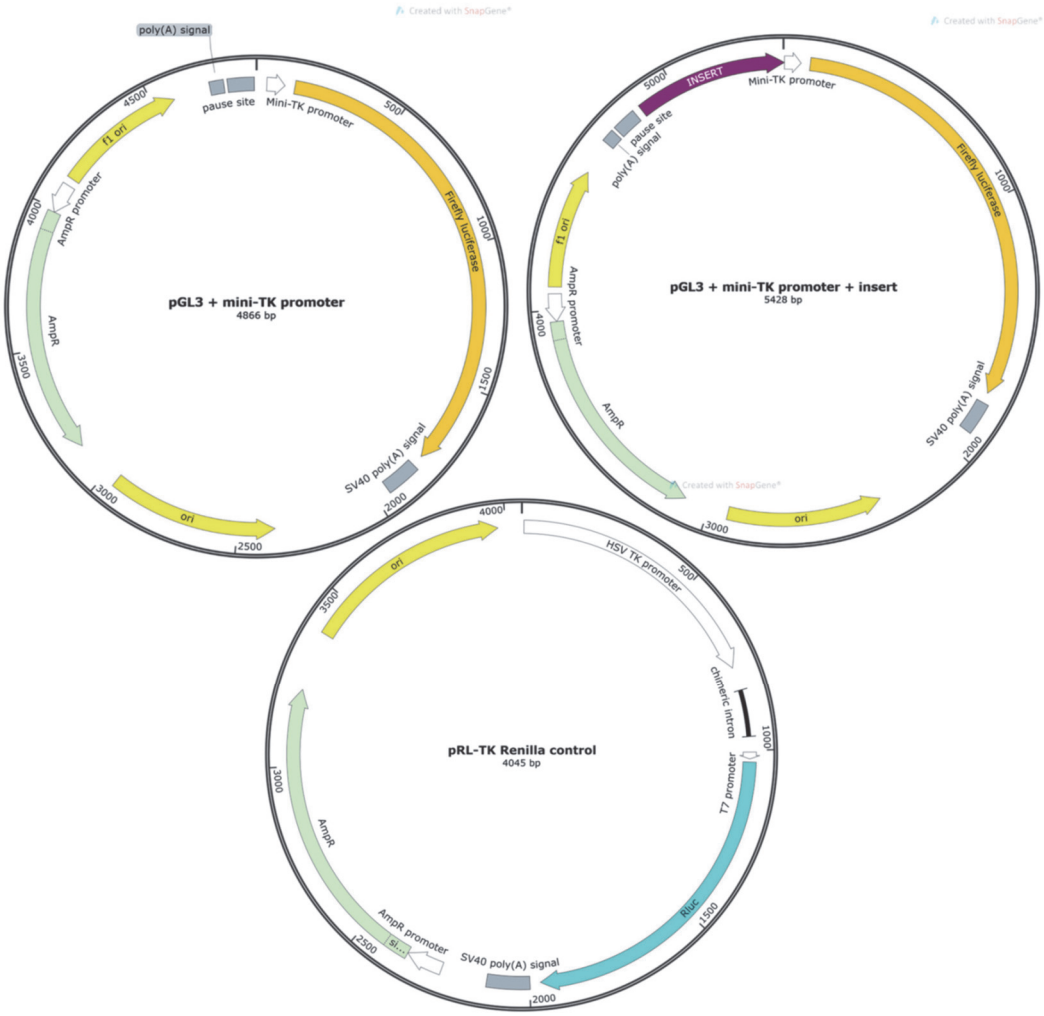

Figure 8. *Vector Map of the modified basic pGL3 vector and the inserts containing rs1385129. rs841847. rs841848 or rs11537641. Vector Map of the pRL-TK (Renilla luciferase vector with HSV-Thymidin kinase promoter).*

First, kidney-derived HEK293T cells were transfected with a luciferase reporter gene containing a wild or mutant insert of the corresponding segments of GLUT1 (*Figure 8.*). We found that, similarly to the red blood cell analysis, rs1385129 significantly increased, while rs841847 significantly decreased luciferase activity compared to the negative control. The rs841848 and rs11537641 did not show the same results as the red blood cell analysis.

We also repeated our experiments with HepG2 cells. This cell line is more relevant for sugar metabolism studies, than the HEK293T, however the results were similar both of two cells. Furthermore, in order to better understand the transcriptional regulation of the gene, we tested the effect of glucose level, insulin and hypoxia on the expression of the luciferase reporter. As a result of our study, we found that in the case of rs1385129, the reduced glucose level had a stimulating effect on the wild allele compared to the construct containing the mutant. In case of the other constructs, the incubation conditions did not show a significant difference between the wild and mutant alleles. However, it is important to mention that hypoxia generally induced the luciferase activity, independent of the SNPs. (*Figure 9*).

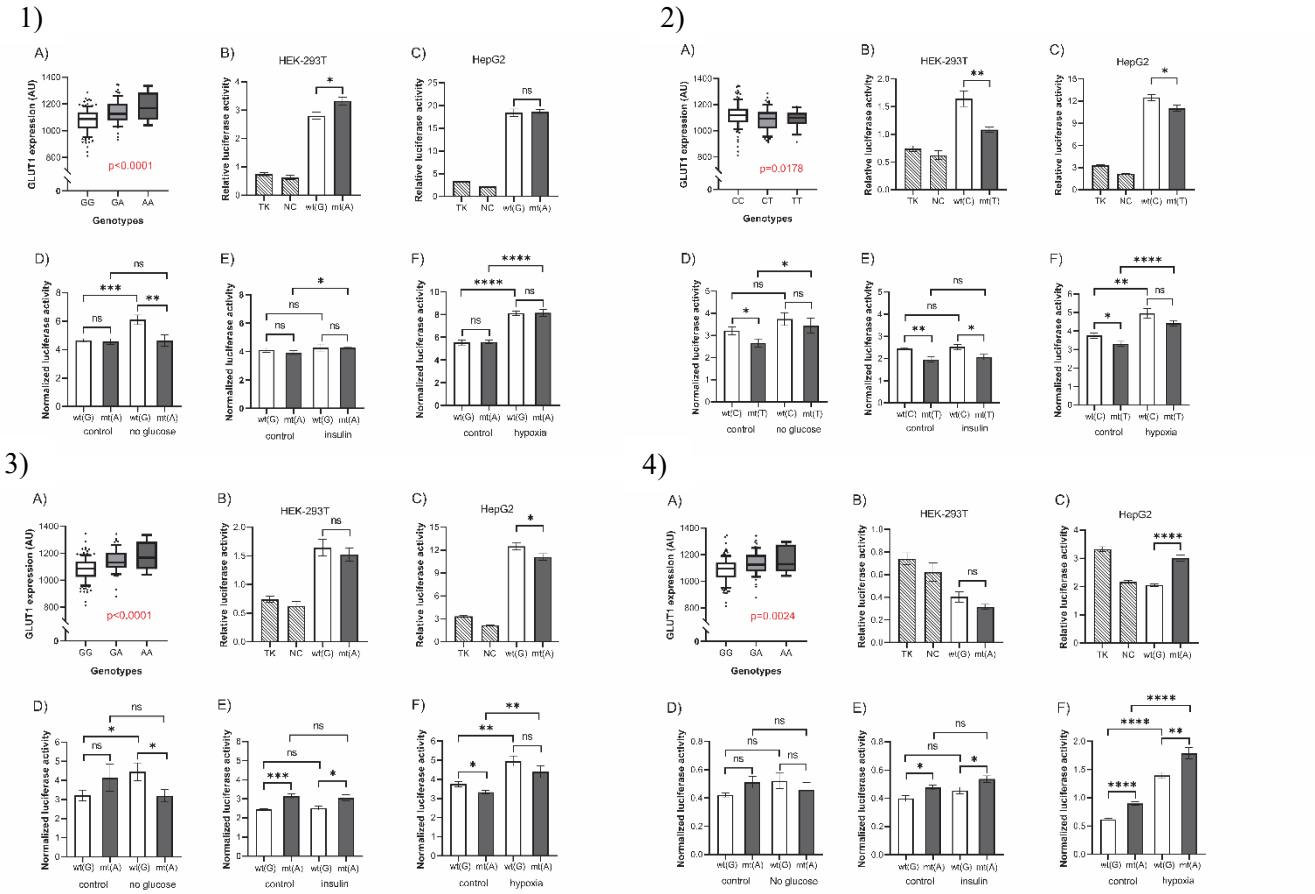

Figure 9. *The effects of rs1385129 (**1**), rs841847 (**2**), rs841848 (**3**) and rs11537641 (**4**) on GLUT1 expression* 1) *luciferase activity. The association between the expression of GLUT1 membrane protein and the genotypes of variant (Kruskal–Wallis test, p<0.05) (A). Each boxplot represents samples with different genotypes, in the range of 10-90 percentile, while dots represent samples out of range. Values are expressed as means ± SD. The p-values of the luciferase experiments (B-F) were calculated using the Welch t-test. The stars represent significance at different levels: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. In case of the treatments, data were normalized to the results of the "empty" vector. wt (G): wild type allele; mt (A): mutant allele; TK: "empty" vector without insert; NC: negative control (random sequence); ns: not significant; AU: arbitrary unit.*

The key results of this study are under publication, submitted to Scientific Report, in 2022: Kulin et al., „Genetic modulation of the GLUT1 transporter expression - potential relevance in complex diseases".

4. Development of new functional assays for ABCB1 multidrug transporter

In the framework of the GLUT1 subtopic, we dealt with transport studies, when we experienced a surprising dye accumulation in the behavior of the TO-PRO ™ -3 dye used as a membrane impermanent live/dead marker. In our published study (Szabó et al, IJMS, 2022) [5], we examined two new fluorescent indicator dyes, which enable the selective examination of ABCB1 in a microarray system. TO-PRO™ -1 and TO-PRO™ -3 (TPs) were originally proposed as indicators of dead cells and as the most sensitive probes for nucleic acid detection (*Figure 10.*).

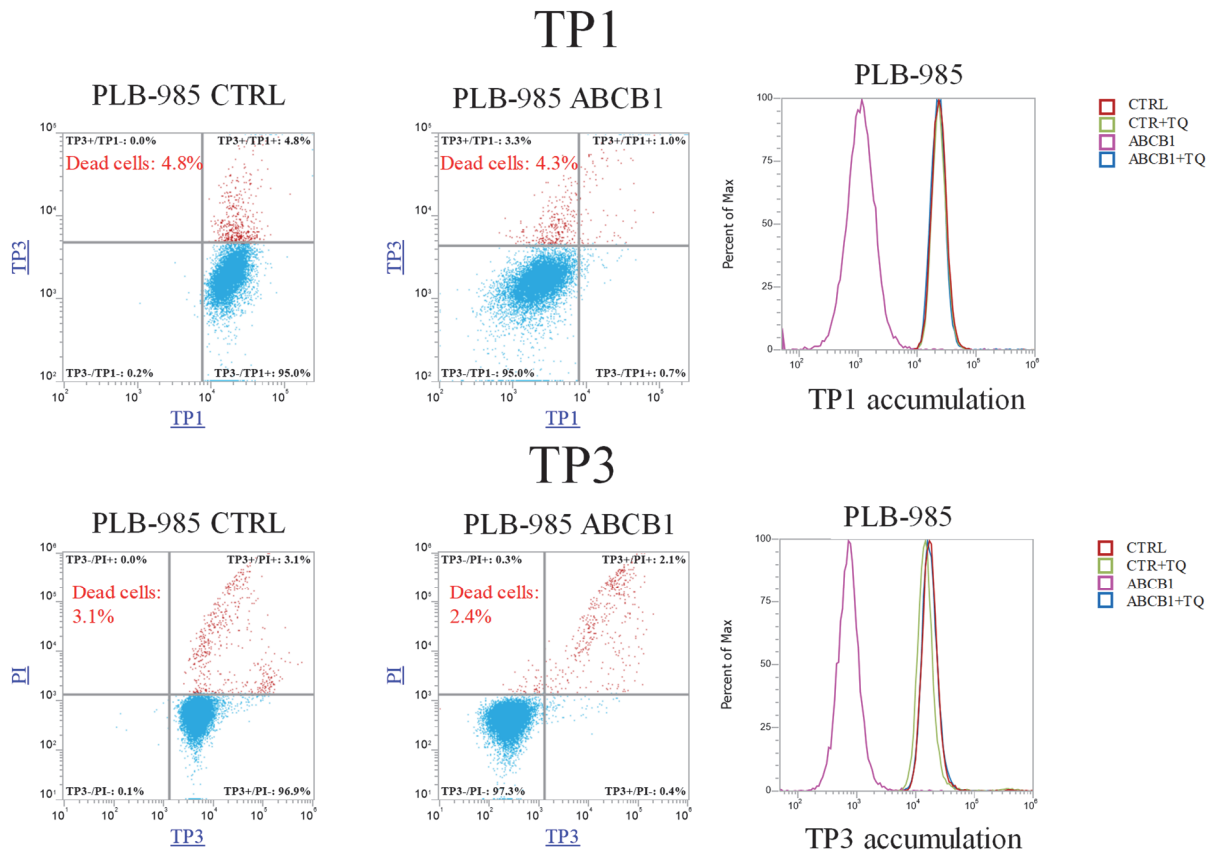

Figure 10. *Uptake of 500 nM TP1 or 100 nM TP3 in PLB-985 control and ABCB1-expressing cells was measured after incubation for 24 h, at 37 °C. TP3 fluorescence was measured by Attune NxT flow cytometer with red laser (638 nm) in the RL1 channel (emission filter: 670/14 nm). Dead cells were identified based on PI staining (488 nm blue laser, emission filter: 695/40 nm). TP1 signal was measured by Attune NxT flow cytometer with blue laser (488 nm) in the BL1 channel (emission filter: 530/30 nm). Dead cells were identified based on TP3 staining (added immediately before measurement). Dead cells are labeled red, live cells are labeled blue. Experiments were repeated at least three times, the result of one representative experiment is shown.*

Our results reveal a new, yet undiscovered, application for these compounds. These carbocyanine monomer nucleic acid dyes slowly enter living cells and accumulate in nucleic acid-containing organelles *(Figure 11.*). We found that in cells expressing a functional ABCB1 transporter, the fluorescence of cellular TPs was much lower after a one-day incubation period.

ABC multidrug transporters are important players in cancer multidrug resistance and general xenobiotic elimination. These transporters have a variety of substrates, including fluorescent dyes, but in transporter assays it is important to use dyes with well-established specificity and low toxicity.

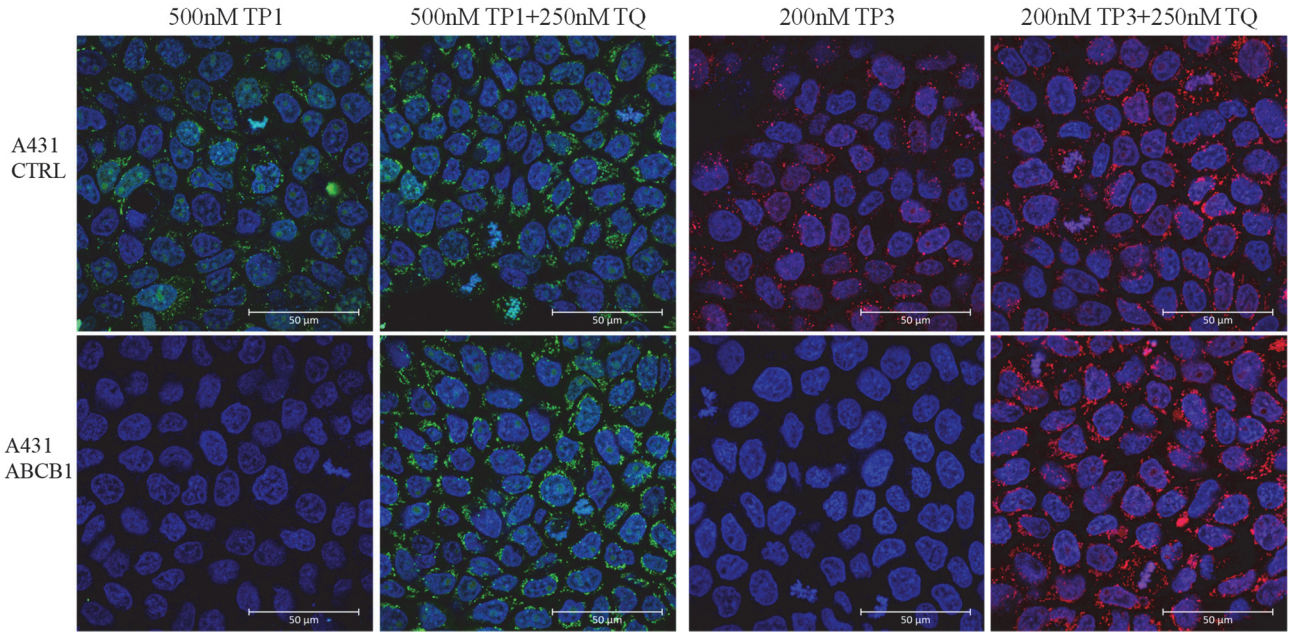

Figure 11. *Fluorescent TP1 or TP3 accumulation in human A431 cells, examined by confocal microscopy. Effects of ABCB1 protein expression and the specific inhibition of the transporter function by Tariquidar. Cellular fluorescence was followed by confocal microscopy. TP1 fluorescence (green) or TP3 fluorescence (red) was examined after 24 h of the addition of 500 nM TP1 or 200 nM TP3 to the medium, either in the absence or presence of the transporter inhibitor (250 nM Tariquidar for ABCB1). The nuclei were counterstained with DAPI.*

TP1 and TP3 are the first dyes that are only substrates of the ABCB1 transporter, because the accumulation of these dyes was not affected by the presence of ABCC1 or ABCG2, while a specific P-glycoprotein inhibitor, tariquidar, significantly increased the fluorescence level of cells expressing ABCB1. Based on these data, TP1 or TP3 uptake assays, supplemented using selective transporter inhibitors, offer new, highly sensitive, highly stable, microplate-based, potentially high-throughput assays for investigating the functional properties of the ABCB1 multidrug transporter (*Figure 12*.).

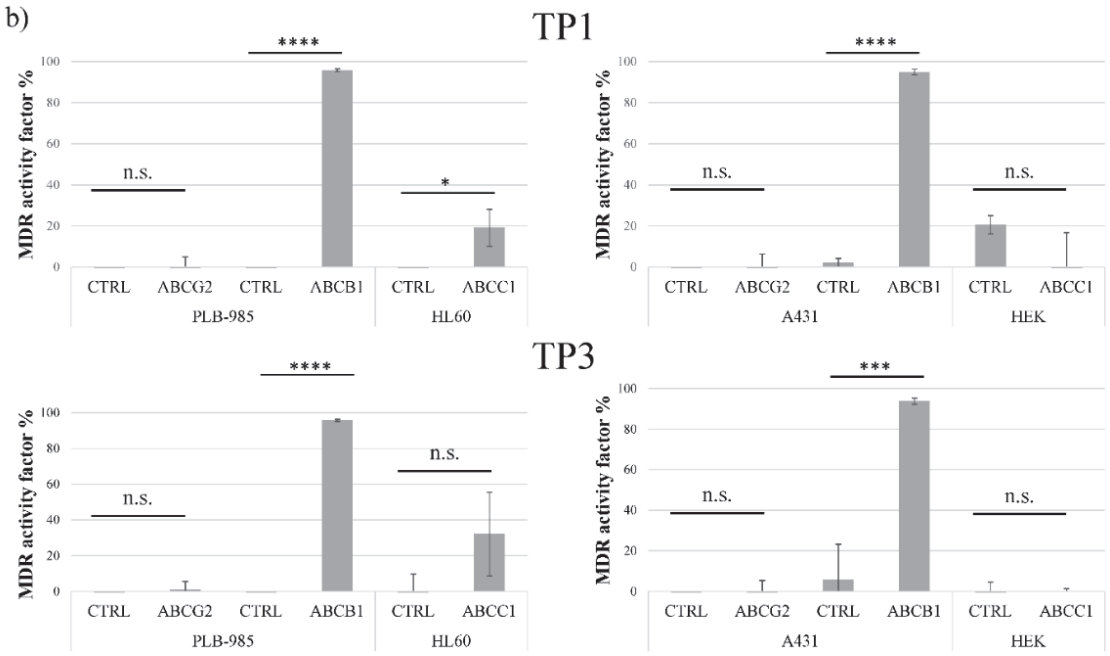

Figure 12. *MDR activity factors calculated by measuring inhibitor-sensitive TP1 or TP3 accumulation in various human cells, expressing ABCG2, ABCB1 or ABCC1. Upper row: TP1 measurements, lower row: TP3 measurements. The specific inhibitors used were 2.5 μM KO143, ABCB1 by 250 nM TQ, and ABCC1 by 10 μM IM. SD +/- values are indicated. Statistical analysis was performed by Student's t-test. n.s. not significant * $p < 0.05$ *** $p < 0.001$ **** $p < 0.0001$ ($n = 3$).*

The test also provides an effective selection method for extracting cells expressing the ABCB1 transporter. In addition to this publication, we applied for patent protection in a Hungarian patent application (P2200328) with the extension of diagnostic applications.

III. Summary of the results

In the course of the project, flow cytometric analysis was performed on the blood samples of 36 newly diagnosed, untreated patients, 61successfully treated and 23 treated with disease-related complications type 2 diabetes patients, and 59 age-matched control subjects. Based on the results obtained, transporters GLUT1, GLUT3, MCT1, URAT1, ABCA1, ABCG2 and PMCA4b showed a significant difference between the patient and the control group in terms of expression levels measured on the red blood cell membrane protein. As the disease progressed, a significant changes were shown in the levels of membrane proteins GLUT1, GLUT3, URAT1 and ABCA1. During the analysis of HgA1c laboratory data, a significant change in GLUT1, GLUT3, MCT1, URAT1 and ABCA1 protein levels were found between normal and elevated HgA1c values. The PMCA4b haplotype conferred mild protection against the disease. The ABCG2 Q141K polymorphism investigated in gout can plays an important role in the manifestation of the disease due to the significant increase in blood sugar levels. Furthermore, by examining the polymorphisms of ABCG2, we found that the genetic modification causing a decrease in the protein level can cause the early development of gout. Four polymorphisms of the GLUT1 membrane protein were identified and characterized. We found that three single-nucleotide polymorphisms significantly increase and the last one decreases GLUT1 levels. These results were also confirmed by a luciferase activity assay. As a further discovery, we demonstrated that TO-PRO1 and TO-PRO3 dyes are previously unknown, sensitive, specific substrates of the ABCB1 multidrug transporter.

IV: Communications in international journals as a result of this NKFIH/OTKA project:

(1) Várady Gy, Szabó E, Kulin A, Zámbó B, Mózner O, Pálinkás M, P. G. and S. B. Potential Diagnostic Application of the Quantitative Assessment of Red Blood Cell Membrane Protein Expression. *Thromb. Haemost. Res.* **2019**, *3* (2), 1024.

(2) Szabó, E.; Kulin, A.; Korányi, L.; Literáti-Nagy, B.; Cserepes, J.; Somogyi, A.; Sarkadi, B.; Várady, G. Alterations in Erythrocyte Membrane Transporter Expression Levels in Type 2 Diabetic Patients. *Sci. Rep.* **2021**, *11* (1), 2765. https://doi.org/10.1038/s41598-021-82417-8.

(3) Szabó, E.; Kulin, A.; Mózner, O.; Korányi, L.; Literáti-Nagy, B.; Vitai, M.; Cserepes, J.; Sarkadi, B.; Várady, G. Potential Role of the ABCG2-Q141K Polymorphism in Type 2 Diabetes. *PLoS One* **2021**, *16* (12), e0260957. https://doi.org/10.1371/journal.pone.0260957.

(4) Pálinkás, M.; Szabó, E.; Kulin, A.; Mózner, O.; Rásonyi, R.; Juhász, P.; Nagy, K.; Várady, G.; Vörös, D.; Zámbó, B.; Sarkadi, B.; Poór, G. Genetic Polymorphisms and Decreased Protein Expression of ABCG2 Urate Transporters Are Associated with Susceptibility to Gout, Disease Severity and Renal-Overload Hyperuricemia. *Clin. Exp. Med.* **2022**. https://doi.org/10.1007/s10238-022-00848-7.

(5) Szabó, E.; Kulin, A.; Jezsó, B.; Kucsma, N.; Sarkadi, B.; Várady, G. Selective Fluorescent Probes for High-Throughput Functional Diagnostics of the Human Multidrug Transporter P-Glycoprotein (ABCB1). *Int. J. Mol. Sci.* **2022**, *23* (18), 10599. https://doi.org/10.3390/ijms231810599.

Submitted, under review:

Kulin et al., Genetic modulation of the GLUT1 transporter expression - potential relevance in complex diseases, Scientific Report, 2022.

Patent application:

Selective fluorescent probes for measuring multidrug transporter activity**.** Inventors**:** Edit Szabó, György Várady, Balázs Sarkadi, Anna Kulin, Bálint Jezsó and Nóra Kucsma - under approval, date of the announcement: August 12, 2022, No. P2200328.

Personal notes on the conditions of this research project

The NKFIH-OTKA grant provided an important support for the works described in this project, while the conditions of the use of this support were less than optimal. Due to the coronavirus epidemic, research was completely stopped for half a year (because of the home office), and later we were no longer able to work with fresh blood samples due to the possible risk of infection by the virus. In addition, in the middle of the project, due to the attempted transfer of the Academy Institutes to a governmental body, again all new enrollments, supply orders and external contracts were blocked for about six months. Due to the increased inflation, the costs of project have increased significantly. The planned, originally free database management programs (e.g. OpenClinica) became paid and unaffordable. On the positive side, this project supported to obtain the successful diploma of one MSc student. She won a prize at the biological national scientific student conference.