Final report (2017.12.01. – 2022.11.30.)

„A C vitamin kompartimentáció epigenetikai szerepe humán megbetegedésekben”
(“Epigenetic role of ascorbate compartmentalization in human diseases”)
NKFIH – 124442 FK

Vitamin C is best known from its antioxidant properties; however, many other vital functions of the vitamin have already been described. Fe^{2+}/2-oxoglutarate dependent dioxygenases are present in several subcellular compartments and need vitamin C for their activity. In the endoplasmic reticulum, this group of enzymes catalyzes the posttranslational modification of extracellular matrix proteins, while in the nucleus they participate in the modification of epigenetic pattern catalyzing demethylation reactions. The demethylation reactions involve histone demethylations catalyzed by JmjC – domain dioxygenases, and DNA demethylations due to the action of TET (ten eleven translocation) methylcytosine dioxygenases. The role of ascorbate in cytosine and lysine demethylation has been demonstrated in both cellular and animal models. In spite of the well-described role of vitamin C in the nucleus, the transport of the vitamin towards the organelle is a scarcely known process; the intracellular distribution of the compound in mammalian cells is unknown, and the transporters of vitamin C in endomembranes have also been poorly studied. We assumed that either global deficiency, or the inappropriate subcellular distribution of the vitamin may deteriorate the proper functioning of the ascorbate-dependent nuclear enzymes, thus may be an important pathophysiological contributor of vitamin C – related diseases.

According to our previous work, a rare inherited human disease, arterial tortuosity syndrome, is caused by impaired intracellular transport of vitamin C. We already identified this connective tissue disorder – as a vitamin C – related disease, where the lack of GLUT10 transporter on the nuclear and endoplasmic reticulum membrane precludes vitamin C transport towards the affected organelles. Therefore, our assumption was that this disease might serve as a model of organelle-specific ascorbate deficiency. Our other examined condition was scurvy accompanied with a general lack of vitamin C. This disease can be mimicked by knocking out the gulonolactone oxidase gene in mice, catalyzing the last step of vitamin C synthesis.

Our aim was setting up cellular and animal models of the above mentioned two pathological conditions and to study the ascorbate level, the intracellular ascorbate distribution and possibly epigenetic pattern alterations in our model system, as it is logically assumed that the lack of ascorbate in the nucleus may lead to epigenetic modifications in the affected cell lines/animal models. Therefore, we wanted to achieve the following goals: (i) exploring the intracellular distribution of vitamin C, with particular attention to the nucleoplasm; (ii) exploring the relationship between nucleoplasmic vitamin C levels and DNA / histone demethylation; (iii) mapping specific epigenetic modifications of genes that play a key role in the pathomechanism of the two diseases.

Regarding the cellular models of the examined diseases, we have set up fibroblast cultures from arterial tortuosity syndrome patients (lacking GLUT10 transporter) and control subjects

matched in the most important parameters. We have further isolated dermis tissue from control humans at our facility. Fibroblast cells – deriving from control and arterial tortuosity syndrome patients – were kept in vitamin C – free culture medium, and we have characterized

- the subcellular distribution of ascorbate;
- global and site-specific changes in 5-methylcytosine and 5-hydroxymethylcytosine levels;
- effect of ascorbate supplementation in control and arterial tortuosity syndrome fibroblasts;

Global DNA epigenetic analysis of cultured fibroblasts from controls and arterial tortuosity syndrome patients was performed using colorimetric assays and mass spectrometry measurements. We found that DNA demethylation is disrupted in patients' cells, resulting in a different epigenetic pattern that could not be compensated by the addition of vitamin C to the medium; the lower global 5-hydroxymethylcytosine level in arterial tortuosity syndrome fibroblasts could not be significantly modified by the addition of external vitamin C.
For our region-specific epigenetic experiments, we selected candidate genes that have been proven to play a role in vitamin C metabolism and the pathomechanism of the arterial tortuosity syndrome. After selecting the loci to be tested, the methylated DNA was immunoprecipitated, and following RT-PCR methylation-sensitive restriction endonucleases were used. Finally, the samples were subjected to PCR analysis. Among the examined genes, we found a difference in the region-specific epigenetic pattern of PPAR-gamma. The methylation level of this gene was elevated in the analyzed loci of patient cells, while the addition of vitamin C to the medium did not affect the methylation. However, on control fibroblasts, vitamin C was able to significantly reduce methylation levels at the studied loci. Investigation of the (hydroxy)methylation status of specific regions in the PPAR-gamma gene showed that ascorbate addition could stimulate hydroxymethylation and active DNA demethylation at the PPAR-γ gene region in control fibroblasts only. These results were published in Németh et al., Oxid Med Cell Longev, 2019 with my last authorship.

In the frame of our project, we have successfully set up a novel and unique method to study intracellular distribution of vitamin C. The application of the method was recently described in plants, while we improved, validated and applied it firstly on mammalian cells and tissues. In order to study the intracellular distribution of ascorbate, transmission electron microscopy was used following a treatment with ascorbate antibody and immunogold staining. The method let us obtain direct information regarding the ascorbate distribution of intracellular compartments both in human fibroblasts (either control or isolated from arterial tortuosity syndrome patients) and in various tissues derived from mice.

The method was executed as follows: animals from each genetic group, either kept on vitamin C free diet, or receiving ascorbate were sacrificed, and their liver and brain had been immediately removed; 1 mm × 1 mm × 5 mm strips were cut from the tissues and were fixed with fixation buffer (3.2% methanol-free formaldehyde, 0.32% glutaraldehyde, 1% sucrose, and 0.028% $CaCl_2$ in 0.1 M sodium cacodylate, pH 7.4). From the strips, 1 mm × 1 mm × 1 mm size blocks were cut and transferred to washing buffer (1% sucrose, and 0.028% $CaCl_2$ in 0.1 M

sodium cacodylate, pH 7.4), in which they were washed two times for 10 mins. Before dehydration of the samples, tissue blocks were washed additionally two times for 5 mins in 0.1 M sodium cacodylate buffer, then incubated in OsO_4 0.1 M sodium cacodylate buffer for 1 hour in dark. Lastly, samples were washed two times for 5 mins in distilled water and incubated in 1% uranyl acetate in dark for 1 hour. During dehydration, blocks were washed with different percentage of ethanol (25, 50,70, 80, 96 %) for 15 mins each, finally washed 3 times with absolute ethanol for 30 mins in -20 ^{o}C. The dehydration was followed by embedding the blocks into London Resin Gold (Agar Scientific, UK). We used LRG:abs.ethanol 1:2 for 1 hour, then LRG:abs.ethanol 1:1 for 1 hour, and LRG:abs.ethanol 2:1 for 1 hour, finally pure LRG overnight. Then blocks were washed with LRG plus 1% benzoyl peroxide 2 times for 1 hour and embedded in easy mold with polymerization in UV chamber for 4 days at -20 ^{o}C.

Ultrathin sections (50-60 nm) were collected on nickel grids and incubated with rabbit polyclonal antibody to conjugated Vitamin C (MyBioSource) (1 : 10) in TBS with 1,5% milk overnight at room temperature, followed by 18 nm Colloidal Gold-AffiniPure Goat Anti-Rabbit IgG (1 : 50; Jackson ImmunoResearch Laboratories Inc.) for 4 hours in TBS with 1% BSA at room temperature. Immunolabelled sections were viewed on a transmission electron microscope (JEOL JEM-1011) operating at 80 kV. Electron micrographs were taken with a CCD camera (Morada; Olympus) and iTEM software (Olympus). A total of 20-30 images were randomly taken with 15,000 X magnification of sections from four animals in each group. Samples were evaluated by manually encircling relevant structures in ImageJ (NIH) and calculating their percentage of area relative to total cytoplasm. The distributions of gold particles over relevant structures were also counted. Values were calculated with Mann–Whitney's test for pairwise comparison of non-normal distribution data and nonparametric Kruskal-Wallis tests for multiple comparisons of non-normal distribution data.

Regarding the examination of ascorbate distribution, we have found that ascorbate accumulates in the nucleus of mammalian cells. We found that the nuclear level of vitamin C in cells from patients was significantly lower, which can be attributed to a vitamin C- deficiency localized specifically to the nucleus. If we compared the ratio of nuclear/cytoplasmic ascorbate levels, it was significantly decreased in fibroblast cells deriving from arterial tortuosity patients. The lack of a functioning nuclear ascorbate transporter (i.e. GLUT10) in arterial tortuosity syndrome therefore possibly plays a role in the development of this condition and may result in the defective function of nuclear ascorbate-dependent enzymes involved in the formation of the epigenetic pattern (see above) (Németh et al., Oxid Med Cell Longev, 2019).

Regarding the animal models of our project, we have received the mouse models of arterial tortuosity syndrome and scurvy; heterozygous gulonolactone-oxidase knockout and GLUT10 knockout mice were obtained from our Flemish collaborators (Prof. Paul Coucke, Ghent University) and were maintained at our animal facility, at the SE-Basic Medical Science Center. Homozygous GLUT10 knockout and homozygous gulonolactone-oxidase knockout were generated. However, as mice are able to synthesize ascorbate, knocking out GLUT10 alone wouldn't represent properly the human disease arterial tortuosity syndrome. Therefore, double knockout (gulonolactone oxidase + GLUT10 knockout) mice were also generated, maintained and bred in T2 cages at our facility.

(The GLUT10 constitutive knockout model harbored a LacZ/Neo selection cassette replacing a sequence ranging from exon 2 to the beginning of exon 5, oriented in the same transcriptional direction than the GLUT10 gene. Gulonolactone-oxidase constitutive knockout model harbored a Neo selection cassette replacing a sequence comprising exon 3 and 4, oriented in the opposite transcriptional orientation than the gulonolactone-oxidase gene).

The mouse rooms have a 12-hour light-dark cycle and the air temperature is 19-22 degrees with the humidity set to 50%. The rooms have a negative pressure compared to the corridor and the outside to prevent the escape of pathogens. The mice live in social housing, a maximum of 7 mice/cage, the cages are H:330 x W:160 x D:137 mm in size. The animal house provides the litter material ABEDD MAXI Chips (LTE E-004 L-15). For environmental enrichment, toilet paper cores are placed in each cage. Drinking water and food for mice (SSNIFF Specieldiäten S8189-S095) were available *ad libitum*. The health of the animals is checked daily, and veterinary care is provided at the animal house.

The mice were bred as follows: heterozygous GLUT10 mice were crossed to obtain 25% GLUT10 $^{-/-}$, 25% GLUT10 $^{+/+}$ and 50% GLUT10 $^{+/-}$ genotypes. GLUT10$^{-/-}$ mice were crossed then to maintain the GLUT10 knockout strain. The breeding and maintenance of this strain was similar to that of wild type, and the mice did not show any phenotypical alterations. We also bred the GLUT10 $^{+/+}$ mice to obtain wild type mice, which were used as a control group for experiments. For generating gulonolactone-oxidase knockout strain, we crossed the gulonolactone-oxidase $^{-/-}$ offspring of the heterozygous gulonolactone-oxidase mice. The reproduction rate of this strain was significantly slower due to the smaller litter size, and the survival rate of the pups was largely decreased.

GLUT10 $^{-/-}$ and gulonolactone-oxidase $^{-/-}$ mice were crossed to obtain mice heterozygous for both genes. After genotyping the pups of the cross, we selected the double knockout (double knockout: gulonolactone oxidase $^{-/-}$ and GLUT10 $^{-/-}$) mice and we crossed them to maintain the double knockout strain. During breeding, we faced with severe technical difficulties, as the extremely low reproduction rate and the closure during the COVID19 pandemic hindered us obtaining and breeding double knockout mice in the originally planned time frame.

Once we obtained all genetic groups of the animals, we set up two types of diets: a vitamin C-free diet (standard mouse food does not contain vitamin C, because the animals are able to produce it) and a vitamin C-containing diet. In the latter case, mouse food was supplemented with 300 mg vitamin C / kg and vitamin C was also added to the animals' drinking water (1.5 g / l). The vitamin C-free diet *versus* control diet was applied to the animals for 2 and 4 weeks respectively, after which the animals were sacrificed and processed. Vitamin C level of the animals was measured by the colorimetric iron-dipyridyl method and with HPLC technique from blood samples or liver/brain homogenates of the respective animals (Figure 1 shows ascorbate level of each genetic group of animals in their brain tissue).

The gulonolactone-oxidase knockout and double knockout strains had slower reproduction and smaller body weight compared to GLUT10 knockout and wild type mice. The litter size of gulonolactone-oxidase knockout and double knockout mice was an average 4-5 pups, while the wild type had an average of 7 pups. The death ratio before the first 30 days was also higher in gulonolactone-oxidase knockout and double knockout mating cages compared with the wild type or GLUT10 mating cages. Figure 2 shows the differences in the body weight among the male mice of each strain.

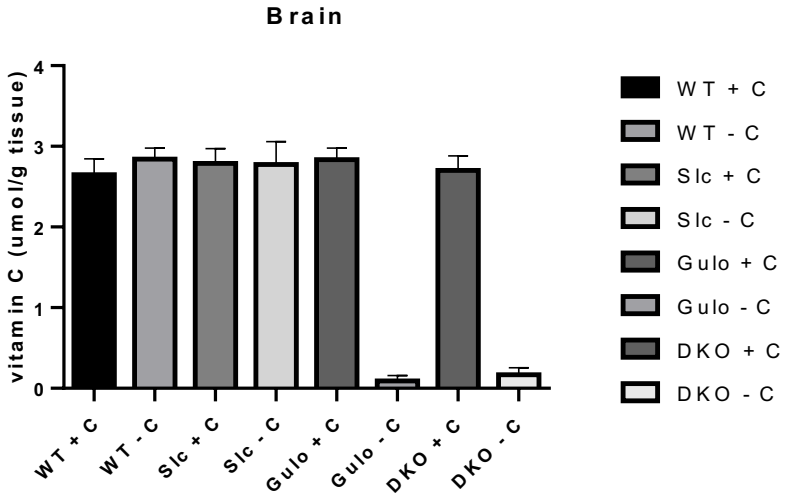

Figure 1. HPLC analysis of brain homogenates isolated from each genetic group of mice, either kept on ascorbate-free diet or supplemented with vitamin C, showed severely decreased ascorbate level in models of scurvy or arterial tortuosity syndrome.

In order to analyse the redox homeostasis, we have examined the GSH level and reduced vs oxidized glutathione ratio of isolated endoplasmic reticulum samples of each strain. Redox imbalances of the animals were further detected by Ellmann method and lipid peroxidation assay. DNA was isolated from the liver, brain, skin, and aorta of the animals and we carried out global epigenetic analysis. We have found a significant growth restriction of double knockout animals even on control diet, while the simple knockout and controls were developing normally. No differences in DNA methylation levels were found in GLUT10 knockout animals, ascorbate levels were similar to controls, and liver ultrastructure was maintained according to electron microscopic analysis, and vitamin C content was similar to controls. Gulonolactone-oxidase knockout mice showed altered redox state in the liver when scorbutized: redox state was shifted towards the oxidative direction and the analysis of global DNA showed a different epigenetic pattern is scorbutized mice respect to those receiving ascorbate-containing diet.

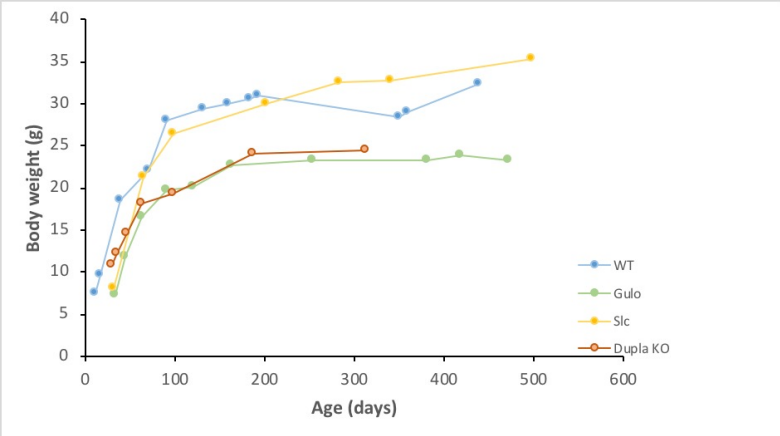

Figure 2. Body weight of representative animals from each strain was measured consequently during breeding. Gulonolactone-oxidase (Gulo) and double knockout strains showed lower body weight throughout their life cycle respect to wild type (WT) or GLUT10 knockout animals (Slc)

Upon the examination of expression levels of epigenetic-related proteins, interesting, though yet preliminary data were obtained. Protein expression levels of various enzymes participating in epigenetic modification was tested with Western blot analysis on isolated the protein samples from brain and liver of mice with different genetic background and diet groups. In brain homogenates, the expression levels of TET1 and Ezh2 were similar, and we haven't found any difference in the phosphorylation pattern of Akt, Stat3 and ERK1/2. In liver samples however the phosphorylation of Stat3, Akt and ERK1/2 was increased in case of ascorbate deficiency in gulonolactone-oxidase and double knockout mice. These initial data suggests that lack of ascorbate– either localized to the nucleus or affects the whole cell – might be an important contributor in epigenetic modifications by altering the activity/ expression level of certain enzymes involved in epigenetic modifications.

Ultrastructure of liver and brain, and intracellular ascorbate distribution was also analysed with transmission electron microscopy by the above described method in the liver of mice with four different genetic background (wild type, GLUT10 knockout, gulonolactone-oxidase knockout, double knockout), either kept on ascorbate-free diet or received ascorbate supplementation. We have embedded liver samples from all groups for immunostaining, and representative images have been taken. We have found significant differences in the total ascorbate level of different groups of animals (Figure 2). Gulonolactone-oxidase knockout and double knockout mice showed significantly lower lever of ascorbate when kept on ascorbate-free diet, while WT animals had higher ascorbate levels. We have also analysed the images to obtain data on the intracellular distribution of ascorbate. The intracellular organelles, especially in those species kept on ascorbate-free diet, contained such a low level of ascorbate that it was not possible to analyze accurately. Therefore, the endoplasmic reticulum, peroxisomes and mitochondria were not evaluated separately. Nuclear ascorbate content was calculated and compared to the cytosolic one. Cytosolic ascorbate content covered the vitamin content for all of the organelles – except nucleus.

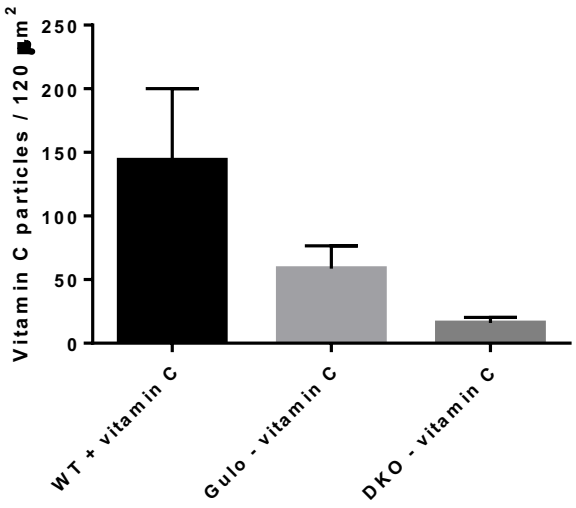

Figure 3. Total number of immunogold particles—representing ascorbate—was assessed and are shown in some representative groups of animals. A significantly lower level of ascorbate was detected in double knockout (DKO) mice after 4 weeks of vitamin free diet compared to wild type mice. Data are the mean ± SD of 3 images.

Ultrastructure of subcellular organelles of the respective animal groups were also analysed by transmission electron microscopy (see representative image on Figure 3). In spite of our earlier observation, that scorbutisation might cause ultrastructural anomalies in the affected animal models, we were not able to observe such anomalies here in our current model system. Endomembranes and other intracellular structures preserved their original shape and organization

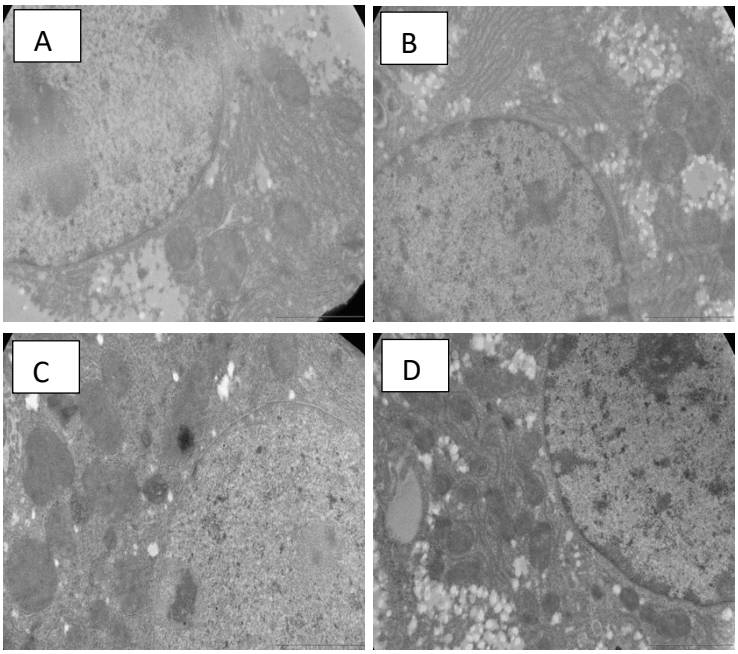

Figure 4. Subcellular distribution of ascorbate was assessed by transmission electron microscopy. After the 4 weeks of diet ascorbate-free, mice were sacrificed, liver blocks were fixed and sections were analyzed. Representative images from the liver sections from each group are shown: (A) wild type, ascorbate supplemented, (B) GLUT10 knockout, ascorbate supplemented (C) gulonolactone-oxidase knockout, ascorbate-free diet, (D) double knockout, ascorbate-free diet. Scale bar is 2 μm.

The presence of GLUT transporter on plasma membranes is well-known and described, but the occurrence of GLUT isoforms in intracellular compartments – especially on endomembranes - is sparsely studied. We have described GLUT10 being present in the endomembrane system and responsible for glucose – and vitamin C – transport towards the luminal compartment. These results were summarized in a paper, where we have detailed the so far proven ways of glucose transport on the endomembrane system and the transporters responsible for them. The paper contains the most extensive *in silico* analyses of intracellular localization of GLUT transporters till nowdays, and was published in the special issue ("Channels and transporters") of *Int J Mol Sci* (IF: 4,183) with my last-authorship.

Arterial tortuosity syndrome is our most important model disease where we are studying the compartment-specific roles of vitamin C and its function in disease pathomechanism. We revised the current knowledge on arterial tortuosity syndrome and the role of GLUT10 within the compartmentalization of ascorbate in physiological and diseased states in a review paper published in *Antioxid Redox Signal* (IF: 5,828) with my last-authorship. The paper was written together with our Flemish collaboration partners who provided us some of the knockout mice.

Besides the nucleus, GLUT10 transporter is also present on the endoplasmic reticulum, where it might be important in driving ascorbate-mediated luminal processes, such as the post-

translational modifications of certain proteins. Mevalonate pathway is crucial for implementing post-translational modifications in the lumen effectively. We showed that inhibition of this pathway impaired the somatic haploid state on a haploid cellular model, presumably through the induction of endoplasmic reticulum stress (Yaguchi et al., *Cell Struct Funct,* 2020.).

Ancillary related to our project, we have examined and characterized the occurrence of glucose-6-phosphatase system in the intestinal endoplasmic reticulum of different species. This system is strongly related to the maintenance of luminal redox environment by contributing to the production of reducing equivalents, therefore may influence the vitamin C – related processes of the endoplasmic reticulum. The components of the glucose-6-phosphatase system were analyzed in homogenates or microsomal fractions prepared from the small intestine mucosae and liver of rats, guinea pigs, and humans. Protein and mRNA levels, as well as glucose-6-phosphatase activities, were detected. Our results demonstrate that the gluconeogenic role of the small intestine is highly species-specific and presumably dependent on feeding behavior (e.g., fructose consumption) and the actual state of metabolism. These results were published in *Int J Mol Sci* (IF: 4,183) with my last-authorship.

In summary, our findings represent the first example for the role of vitamin C transport in epigenetic regulation, and suggest that arterial tortuosity syndrome might be considered as a compartmentalization disorder. Based on our results we can strongly assume that diseases disturb ascorbate compartmentalization do exist, and are caused by hindered ascorbate transportation or increased ascorbate consumption into an organelle (in case of arterial tortuosity syndrome, into the nucleus). Therefore, the lack of vitamin C is localized into a certain compartment, which effects the disease development leading to generalized symptoms. Further studies with clinical orientation might be necessary to understand better ascorbate subcellular compartmentalization in health and certain pathological conditions.

Our results were published in the following research papers in international journals (I'm the last, corresponding author in four of them):

1. Németh CE, Nemoda Z, Lőw P, Szabó P, Horváth EZ, Willaert A, Boel A, Callewaert BL, Coucke PJ, Colombi M, Bánhegyi G, **Margittai É.** Decreased Nuclear Ascorbate Accumulation Accompanied with Altered Genomic Methylation Pattern in Fibroblasts from Arterial Tortuosity Syndrome Patients. Oxid Med Cell Longev. 2019; 2019:8156592.
2. Varga V, Murányi Z, Kurucz A, Marcolongo P, Benedetti A, Bánhegyi G, **Margittai É.** Species-Specific Glucose-6-Phosphatase Activity in the Small Intestine-Studies in Three Different Mammalian Models. Int J Mol Sci. 2019 Oct 11;20(20). pii: E5039.
3. Lizák B, Szarka A, Kim Y, Choi KS, Németh CE, Marcolongo P, Benedetti A, Bánhegyi G, **Margittai É.** Glucose Transport and Transporters in the Endomembranes. Int J Mol Sci. 2019; 20(23). pii: E5898.

4. Boel A, Veszelyi K, Németh CE, Beyens A, Willaert A, Coucke P, Callewaert B, **Margittai É**. Arterial Tortuosity Syndrome: An Ascorbate Compartmentalization Disorder? *Antioxid Redox Signal* 2021 Apr 10; 34(11): 875-889.
5. Yaguchi K, Sato K, Yoshizawa K, Mikami D, Yuyama K, Igarashi Y, Banhegyi G, **Margittai É**, Uehara R. Mevalonate Pathway-mediated ER Homeostasis Is Required for Haploid Stability in Human Somatic Cells. *Cell Struct Funct*. 2021 Feb 19; 46(1): 1-9.

Beside these, currently a handout is being prepared containing the data we obtained so far on the animal model systems.

Presentations on national and international conferences, meetings during the reported period:

- **Margittai É**, Németh CE, Nemoda Zs, Lőw P, Bánhegyi G (2018) C-vitamin kompartimentáció és epigenetika a kanyargós artéria szindróma pathomechanizmusában *48. Membrán-transzport Konferencia,* Sümeg
- **Margittai É** (2018) EGCG promotes autophagy-dependent survival via influencing the balance of mTOR-AMPK pathways upon endoplasmic reticulum stress. The Final *COST Action BM 1307 Meeting*, Athens, Greece
- **Margittai É** (2018) Compartmentalization of Vitamin C in health and disease Seminar at Hokkaido University, Sapporo, Japan
- **Margittai É** (2019) Shaping endoplasmic reticulum redox homeostasis – role of the thioredoxin system. *The first Hungary-Japan joint meeting on Cellular/Molecular Dynamics* Sapporo, Japan
- **Margittai É**, Németh CE (2019) A C vitamin kompartimentáció élettani vonatkozásai. *Intézményi ÚNKP Rendezvény, Semmelweis Szalon*.
- **Margittai É** (2019) Compartmentalization of ascorbate in health and disease. *49. Membrán-transzport Konferencia*, Sümeg
- **Margittai É** (2019) Epigenetic role of ascorbate compartmentalization in human diseases. *Molekuláris Élettudományi Konferencia 2019.*
- **Margittai É** (2019) Kutatói és módszertani szemináriumok a kutatási program keretében. Semmelweis Egyetem, Klinikai Kísérleti Kutató Intézet.
- Varga V, Murányi Zs, Kurucz A, Marcolongo P, Benedetti A, Bánhegyi G, **Margittai É** (2020) Species-specific glucose-6-phosphatase activity in the small intestine - Studies in three different mammalian models. *PhD Tudományos Napok 2020*, Budapest
- Veszelyi K, Varga V, Nemeth CE, Besztercei B, **Margittai É** (2021) Parallel occurrence of reduced pyridine nucleotids and oxidized proteins in the endoplasmic reticulum lumen-a possible absence of electron transfer chains. *FEBS OPEN BIO* (11) 488-488.
- Veszelyi K, Varga V, Németh CE, Besztercei B, **Margittai É** (2021) The role of thioredoxin / thioredoxin reductase system in the redox homeostasis of the endoplasmic reticulum. *SFRR-E 2021*, Belgrade, Serbia (online)
- Veszelyi K, Varga V, Németh CE, Besztercei B, **Margittai É** (2021) Redox Homeostasis of the Endoplasmic Reticulum –Absent Electron Transfer Chains in the Lumen? *Hungarian Molecular Life Sciences 2021*, Eger

- Varga V, Veszelyi K, Németh CE, **Margittai É** (2021) The epigenetic role of ascorbate in the pathomechanism of arterial tortuosity syndrome. *Hungarian Molecular Life Sciences 2021*, Eger
- Veszelyi K, Varga V, Németh CE, Besztercei B, **Margittai É** (2021) A tioredoxin/tioredoxin-reduktáz rendszer lehetséges hiánya az endoplazmás retikulum lumenében, *50. Membrán-Transzport Konferencia*, Sümeg
- Varga V, Veszelyi K, Németh CE, **Margittai É** (2021) Az aszkorbát epigenetikai szerepe a kanyargós artéria szindróma patomechanizmusában. *50. Membrán-Transzport Konferencia 2021*, Sümeg
- Veszelyi K, Varga V, Németh CE, Besztercei B, **Margittai É** (2021) The Possible Absence of the Thioredoxin / Thioredoxin Reductase System in the Lumen of the Endoplasmic Reticulum. *PhD Tudományos Napok*, Budapest
- Veszelyi K, Varga V, Németh CE, Besztercei B, **Margittai É** (2022) The Role of Thioredoxin / Thioredoxin Reductase System in the Redox Homeostasis of the Endoplasmic Reticulum – A Possible Absence of Electron Transfer Chains. *PhD Tudományos Napok 2022*, Budapest
- Varga V, Veszelyi K, Németh CE, **Margittai É** (2022) The epigenetic role of ascorbate in the pathomechanism of arterial tortuosity syndrome *EEHD 2022*, Brüsszel, Belgium
- Veszelyi K, Németh CE, Varga V, **Margittai É** (2022) Epigenetic alterations in TRP32-silenced HeLa cells. *EEHD 2022*, Brüsszel, Belgium
- Varga V, Veszelyi K, Németh CE, Péter L, **Margittai É** (2022) The epigenetic role of ascorbate in the pathomechanism of arterial tortuosity syndrome. *Magyar Élettani Társaság Vándorgyűlés 2022*, Budapest
- Veszelyi K, Varga V, Németh CE, Besztercei B, **Margittai É** (2022) A tioredoxin/tioredoxin-reduktáz rendszer lehetséges hiánya az endoplazmás retikulum lumenében. *Magyar Élettani Társaság Vándorgyűlés 2022*, Budapest
- **Margittai É** (2022) – Romhányi emlékelőadás: Az endoplazmás retikulum redox rendszereinek világa. *51. Membrán-Transzport Konferencia 2022*, Sümeg