Final report on the research project, supported by the Hungarian Scientific Research Fund OTKA, contract nr. NN 108283, PI: Matyas Mink, entitled

"An Animal Model for Novel Human Diseases Associated with Mutations of Type IV collagen *COL4A1* and *COL4A2*"

for the period from 09/01/2013 to 05/31/2018

In this research project we have intended to explore the following specific aims:

Aim #1: Demonstrate congenital muscular dystrophy (CMD) in *col4a1 Drosophila* mutants

Aim #2: Molecular characterization of the *col4a1* mutations in mutant animals

Aim #3: Explore methods to alleviate the effect of the genetic insults; the first step toward therapy

Specific Aim #1

Onset of muscular dystrophy in *col4a1* mutants

Type IV collagen *COL4A1* mutations have recently been identified in patients with intracerebral, vascular, renal, ophthalmologic pathologies and congenital muscular dystrophy, consistent with diagnoses of Walker–Warburg Syndrome or Muscle–Eye–Brain disease. Morphological characteristics of muscular dystrophy have also been demonstrated in *Col4a1* mutant mice. Yet, several aspects of the pathomechanism of COL4A1-associated muscle defects remained largely uncharacterized.

Based on the results of genetic, histological, molecular, and biochemical analyses in an allelic series of *Drosophila col4a1* mutants, we provide evidence that *col4a1* mutations associate with severely compromised muscle fibers within the single-layer striated muscle of the common oviduct, characterized by loss of sarcomere structure, disintegration and streaming of Z-discs, and aberrant integrin expression within the M-discs. This milder phenotypic manifestation occurs at a low penetrance; we observed ectopic assembly of Z-discs by transition from the isotropic (I) band toward the anisotropic (A) band up to the middle of A-band, at the level of M-discs. In these muscle fibers the normal I(Z)-A-I(Z) register of the Z-discs is pushed toward the A(Z)-I-A(Z) pattern.

Features of altered cytoskeletal phenotype include actin bundles traversing over sarcomere units, amorphous actin aggregates, atrophy and aberrant fiber size. Diameters of the muscle fibers in mutant animals was shifted toward smaller values. The ratio of the muscle fibers with diameters below 8 µm and down to 4 µm increased by 12-34 %, whereas the same ratio in

control flies remained 30% with the majority of fiber diameters in the range of 7-8 µm and only 4-6 % of 6 µm as the smallest value. The phenotypic features of the *Drosophila col4a1* mutants allow diagnosis of muscular dystrophy.

The mutant COL4A1-associated defects appear to recapitulate integrin-mediated adhesion phenotypes observed in *Drosophila* by RNA-inhibition of *talin*, *alpha-actinin*, *integrin-linked kinase*, *alpha PS2* and *beta PS* integrin genes. Our results provide insight into the mechanistic details of COL4A1-associated muscle disorders and suggest a role for integrin-collagen interaction in the maintenance of sarcomeres.

Industrially, Z-disc disintegration and streaming, sarcomere loss, irregular integrin expression, actin bundle formation are novel phenotypic elements that associate with *col4a1* mutation and have not yet explored in *COL4A1* mutant patients or in mouse models.

The results are from:

András A. Kiss 1, Nikoletta Popovics1, Márton Kiss1, Zsolt Boldogkői1, Katalin Csiszár2 and Mátyás Mink1§. Type IV collagen is essential for proper function of integrin-mediated adhesion in Drosophila muscle fibers. Manuscript, under review.

Specific Aim #2

Lesions in the allelic series of *col4a1* mutants

We determined the mutation sites within the *Drosophila col4a1* gene. All lesions affect glycine codons, substituted by Asp, Glu or Ser, as summarized in Table 1.

Former designation	*a-30*	*b-9*	*DTS-L2*	*DTS-L3*
Present designation Mutation site	*col4a1*^{G233E} p.G233E GGA->GAA	*col4a1*^{G467E} p.G467E GGA->GAA	*col4a1*^{G552D1} p.G552D1 GGC->GAC	*col4a1*^{G552D2} p.G225D2 GGC->GAC
Former designation	*DTS-L4*	*DTS-L5*	*DTS-L10*	*b-17*
Present designation Mutation site	*col4a1*^{G1025E1} p.G1205E1 GGA->GAA	*col4a1*^{G1025E2} p.G1025E2 GGA->GAA	*col4a1*^{G1043S} p.G1043S GGG->GAG	*col4a1*^{G1393E} p.G1393E GGA->GAA

Table 1. The mutation sites we identified in the *Drosophila col4a1* gene.

All mutations are G->A transitions, according to the mutagen, ethyl-methane-sulfonate, used to generate these mutants. Interestingly, the *col4a1*^{G552D} and *col4a1*^{G1025E} mutant lines are available in two copies in our allelic series and point to the importance of Gly552 and Gly1025 amino acids. Gly is represented by four codons, GGN; further possible substitutions were the Gly to Ala, Arg, Cys, Trp or Val replacements that we did not find in our allelic series.

The results are from:

András A. Kiss 1, Nikoletta Popovics1, Márton Kiss1, Zsolt Boldogkői1, Katalin Csiszár2 and Mátyás Mink1§. Type IV collagen is essential for proper function of integrin-mediated adhesion in Drosophila muscle fibers. Manuscript, under review.

Novel form of proteolysis in the actinomyosin complex

The *col4a1*^{G233E} allele is within the peptide GFP**G/E**EKGERGD (the G to E substitution in bold), a putative integrin binding site in the COL4A1 protein. The mutation sites in the *col4a1*^{G552D1} and *col4a1*^{G552D2} lines localize in the immediate proximity of the peptide GLPGEKGLRGD that resembles the integrin binding site in the COL4A2 protein in the triple helical model made up of $[COL4A1]_2COL4A2$ protomers. We thus designated these lines integrin binding-site mutants. We recorded allele-specific hydrolysis of myofibrillar proteins in these mutants. Myosin heavy chain devoided the N-terminal methionine and was cleaved into an N- and C-terminal fragment. Similarly, actin cleavage occurred at the KR/GI peptide, matching perfectly

the C-terminal processing site of human insulin, provided by proprotein convertases. A second actin cleavage site occurred at RK/YS C-terminally. The rest of the mutants were free from proteolysis. Muscle protein breakdown as a dynamic process occurs in both physiologic and pathologic conditions and the ubiquitin-26S proteasome system is considered to digest myofibrillar proteins. Initiation of this breakdown seems to be mediated by lysosomal cathepsin, calpain and caspase, as intact proteins of the actinomyosin complex are not substrates of the ubiquitin-26S proteasome. Our results suggest an active role of the type IV collagen in skeletal muscle integrity and provide insight into a novel form of muscle protein breakdown.

Pre-processing of the proteins in actinomyosin complex by cathepsin, calpain or caspase is suspected but demonstration of proteolysis within myofibrillar proteins remains ambiguous. To our knowledge our results are the first data on cleavage of myofibrillar proteins.

The results are from:

Nikoletta Popovics1†, András A. Kiss1†, Tamás Janáky2, Zsolt Boldogkői1, Katalin Csiszár3 and Mátyás Mink1*. Myofibrillar proteolysis in type IV collagen mutants affecting integrin-binding sites. Manuscript, under review.

Systemic nature of the *col4a1* mutations: Actin stress fibers development in epithelial cells of the Malpighian tubules

Mutations in the ubiquitous human basement membrane components *COL4A1* and *COL4A2* cause a multisystem disorder involving nephropathy. Affected patients develop renal

dysfunction and chronic kidney failure with or without hematuria. Mouse *Col4a1* and *Col4a2* mutants recapitulate the human symptoms. In vertebrates, excretion is accomplished by the kidneys and the by the Malpighian tubules in *Drosophila*. The dominant, temperature-sensitive mutation in the *Drosophila col4a1* gene resulted in altered integrin expression and amplified effects of mechanical stress on Malpighian epithelial cytoskeleton, and develop actin stress fibers. These phenotypic elements remained unexplored in mammals carrying *COL4A1* mutation.

The results are from:

András A. Kiss, Nikoletta Popovics, Gábor Szabó, Katalin Csiszár, Mátyás Mink. Altered stress fibers and integrin expression in the Malpighian epithelium of Drosophila type IV collagen mutants. Data in Brief, Volume 7, June 2016, Pages 868-872.

Immunologic aspects of the *col4a1* mutations

We have carried out a series of tests using *Drosophila DTS-L3* mutants from our allelic series of *col4a1* mutations with confirmed degeneration of various cell types and lowest survival rate among the *col4a1* mutant lines. Results demonstrated epithelial cell degeneration in the gut, shortened gut, enlarged midgut with multiple diverticulae, intestinal dysfunction and shortened life span. Midgut immunohistochemistry analyses confirmed altered expression and distribution of basement membrane components integrin PSI and PSII alpha subunits, laminin gamma 1, and COL4A1 both in larvae and adults. Global gene expression analysis revealed activation of the effector AMP genes of the primary innate immune system including

Metchnikowin, Diptericin, Diptericin B, and *edin* that preceded morphological changes. The fusion gene *Attacin::GFP* midgut expression pattern further supported these changes. An increase in reactive oxygen species production and changes in gut bacterial flora were also noted and may have further enhanced an immune response. The phenotypic features of *Drosophila col4a1* mutants confirmed an essential role for type IV collagen in maintaining epithelial integrity, gut morphology and intestinal function and suggest that aberrant structure and function of the COL4A1 protein may also be a significant factor in modulating immunity. These aspects are unexplored yet in mammalian *COL4A1* mutants.

The results are form:

Kiss, Márton, András A. Kiss, Monika Radics, Nikoletta Popovics, Edit Hermesz, Katalin Csiszár, and Mátyás Mink. Drosophila type IV collagen mutation associates with immune system activation and intestinal dysfunction. Matrix Biology 49, 120–131, 2016.

4-hydroxy-2-nonenal alkylated and peroxynitrite nitrated proteins in *col4a1 Drosophila* mutants

The clinical manifestations of the COL4A1/A2 mutations are systemic affecting many tissues and organs among these the kidneys. In order to uncover the cellular and biochemical alterations associated with aberrant type IV collagen, we have explored the phenotype of the Malpighian tubules, the secretory organ and insect kidney model, in *col4a1* collagen gene mutants of the fruit fly *Drosophila melanogaster*. In Malphigian epithelial cells of *col4a1*

mutants, robust mitochondrial fusion indicated mutation-induced stress. Immunohistochemistry detected proteins nitrated by peroxynitrite overlapping with enlarged mitochondria and increased level of membrane peroxidation assessed by elevated amount of proteins alkylated by 4-hydroxy-2-nonenal that also localized to sites of fused mitochondria. Nuclei within the Malpighian epithelium showed TUNEL-positivity suggesting cell degradation. Results collectively demonstrated that *col4a1* mutations affect the epithelia, and consequently, secretory function of the Malpigian tubules and provide mechanistic insight into col4a1 mutation-associated functional impairments not yet reported in human patients or in mouse models with mutant COL4A1.

The results are form:

András A. Kiss,1 Nikoletta Popovics,1 Zsolt Boldogkői,1 Katalin Csiszár,2 and Mátyás Mink. 4-Hydroxy-2-nonenal Alkylated and Peroxynitrite Nitrated Proteins Localize to the Fused Mitochondriain Malpighian Epithelial Cells of Type IV Collagen Drosophila Mutants. BioMed Research International, Volume 2018, Article ID 3502401, 2018.

Ophthalmologic aspects of the *Col4a1* mutations in mouse model – results of the international cooperation

Ocular anterior segment dysgenesis (ASD) describes a spectrum of clinically and genetically heterogeneous congenital disorders affecting anterior structures that often lead to impaired vision. More importantly, 50-75% of patients with ASD develop early onset and aggressive glaucoma. Although several genes have been implicated in the etiology of ASD, the underlying

mechanisms remain elusive. Type IV collagen alpha 1 (COL4A1) is an extracellular matrix protein and a critical component of nearly all basement membranes. COL4A1 mutations cause multi-system disorders in patients, including ASD (congenital cataracts, Axenfeld-Rieger's anomaly, Peter's anomaly and microphthalmia) and congenital or juvenile glaucoma. Here, we use a conditional Col4a1 mutation in mice to determine the location and timing of pathogenic events underlying COL4A1-related ocular dysgenesis. Our results suggest that selective expression of the Col4a1 mutation in neural crest cells and their derivatives is not sufficient to cause ocular dysgenesis and that selective expression of the Col4a1 mutation in vascular endothelial cells can lead to mild ASD and optic nerve hypoplasia but only on a sensitized background. In contrast, lens-specific expression of the conditional Col4a1 mutant allele led to cataracts, mild ASD and optic nerve hypoplasia, and age related intraocular pressure dysregulation and optic nerve damage. Finally, ubiquitous expression of the conditional Col4a1 mutation at distinct developmental stages suggests that pathogenesis takes place before E12.5. Our results show that the lens and possibly vasculature play important roles in Col4a1-related ASD and that the pathogenic events occur at mid-embryogenesis in mice, during early stages of ocular development.

The results are from:

Disease Models & Mechanisms (2017) 10, 475-485 doi:10.1242/dmm.027888.

Specific Aim #3

Explore methods to alleviate the effect of the genetic insults; the first step toward therapy

We identified a group of drugs that alleviate effectively the symptoms linked to type IV collagen mutations. We followed three markers including the life span, sarcomere structure of the striated muscle and the onset of intestinal dysfunction. All four drugs tested so far increased the life expectancy of the mutant animals up to the level of wild-type ones. The disrupted sarcomere structure was re-capitulated in the mutants following drug treatments. The third marker, intestinal dysfunction set later on, comparably with the wild-type control animals. All observations are statistically significant at $p<0.05$.

In a genetic interaction test overexpression of the lysyl oxidase gene *Dmloxl-1* has been proved beneficiary regarding the life span of the mutants, according to our expectations.

We have proven that the drugs tested act epigenetically, measured by the enhancement of position effect variegation.

As there is some interest for the results we achieved by the biotech industry we are going to patent the results in the future.

Theses achieved by the aid of the project

Dissertation to achieve the title Doctor of the Hungarian Academy of Sciences

Mátyás Mink: Genotype-phenotype relationships in model organisms with emphasis of *Drosophila col4a1* mutants. Manuscript.

PhD theses completed by the aid of the project

Márton Kiss: "Emésztőrendszeri diszfunkció és humorális immunválasz kapcsolata IV-es típusú kollagén mutációkkal *Drosophila*-ban", the candidate defended his thesis successfully.

András A. Kiss completed his research and wrote his PhD thesis entitled: Oxidative, nitrosative and alkylative stress in Drosophila type IV collagen mutants. Public defense is expected soon.

Diploma theses completed by the aid of the project

Kiss András Attila, Biology MSc student, OTDK thesis: „Az izom differenciálódást és a szarkomer képződést szabályozó IV-es kollagén peptid domének azonosítása interallélikus komplementáció révén", 2015

MSc thesis: „A IV-es típusú kollagén mutációk recesszív fenotípusának izom manifesztációi", 2015

Nikoletta Popovics, Biology BSc student: “Transzlációs tanulmány a *Drosophila col4a1* mutációk okozta tünetek enyhítésére”, Szeged, 2014

Nikoletta Popovics, Biology MSc student prepared her diploma thesis entitled: “A *Drosophila* IV-es kollagenopátia terápiája”, Szeged, 2016

Mónika Radics, Biology MSc student prepared her diploma thesis entitled: A IV-es típusú kollagén mutációjának immunológiai vonatkozásai *Drosophila*-ban”, Szeged, 2016

Gábor Szabó, Biology BSc student wrote his diploma thesis entitled: “*Drosophila col4a1* mutáns Malpighi epithél sejtjeiben megjelenő stressz fibrillumok és helytelen integrin kifejeződés”, Szeged, 2016

All four BSc/MSc students contributed to our research aims and are authors in our research papers and conference abstracts.

Dissemination of the results

A. Research articles

András A. Kiss, Nikoletta Popovics, Gábor Szabó, Katalin Csiszár, Mátyás Mink. Altered stress fibers and integrin expression in the Malpighian epithelium of Drosophila type IV collagen mutants. Data in Brief, Volume 7, June 2016, Pages 868-872. RGIF: 1.43. http://dx.doi.org/10.1016/j.dib.2016.03.059

Kiss, Márton, András A. Kiss, Monika Radics, Nikoletta Popovics, Edit Hermesz, Katalin Csiszár, and Mátyás Mink. Drosophila type IV collagen mutation associates with immune system activation and intestinal dysfunction. Matrix Biology 49, 120–131, 2016. IF: 7.4.

http://dx.doi.org/10.1016/j.matbio.2015.09.002

Mao Mao, Marton Kiss, Yvonne Ou, and Douglas Gould. Genetic Dissection of Anterior Segment Dysgenesis caused by a Col4a1 mutation. Disease Models & Mechanisms (2017) 10, 475-485. doi:10.1242/dmm.027888. IF: 4.691.

András A. Kiss,1 Nikoletta Popovics,1 Zsolt Boldogkői,1 Katalin Csiszár,2 and Mátyás Mink. 4-Hydroxy-2-nonenal Alkylated and Peroxynitrite Nitrated Proteins Localize to the Fused Mitochondriain Malpighian Epithelial Cells of Type IV Collagen Drosophila Mutants. BioMed Research International, Volume 2018, Article ID 3502401, 8 pages. IF: 2.476.

https://doi.org/10.1155/2018/3502401

András A. Kiss 1, Nikoletta Popovics1, Márton Kiss1, Zsolt Boldogkői1, Katalin Csiszár2 and Mátyás Mink1§. Type IV collagen is essential for proper function of integrin-mediated adhesion in Drosophila muscle fibers. Manuscript, under review.
https://www.biorxiv.org/content/early/2018/05/09/318337

Nikoletta Popovics1†, András A. Kiss1†, Tamás Janáky2, Zsolt Boldogkői1, Katalin Csiszár3 and Mátyás Mink1*. Myofibrillar proteolysis in type IV collagen mutants affecting integrin-binding sites. Manuscript, under review.

Sum of IFs of the published papers: 15.997; upon acceptance of our two manuscripts a significant raise can be expected.

B. Conference abstracts

Ildikó Valkonyné Kelemen1, Márton Kiss1, András Kiss1, Mónika Radics1, Katalin Csiszár2, Mátyás Mink1. An animal model for novel human diseases associated with mutations of type IV collagen COL4A1 and COL4A2. Hungarian Molecular Life Science, Siófok, 5-7 April 2013.

http://2013.hunlifesci.hu/doc/hunlifesci_book_web.pdf

M. Mink1, M. Radics1, I. Kelemen-Valkony1, N. Popovics1, A.A. Kiss1, M. Kiss1, K. Csiszar2. Glycine Substitutions within COL4A1 Cause Systemic and Allele-specific Phenotypes in Drosophila. 1st MBE (Matrix Biology Europe) conference, 21 - 24 June 2014, Rotterdam, The Netherlands. www.mbe2014.eu

András Attila Kiss1, Ildikó Kelemen-Valkony1, Márton Kiss1, Nikoletta Popovics1, Gábor Szabó1, Katalin Csiszár2 and Mátyás Mink1. Type IV collagen domains identified via interallelic complementation regulate muscle differentiation and sarcomer formation. Hungarian Molecular Life Science, Eger, 27-29 March 2015. http://hunlifesci.hu/wp-content/uploads/2015/03/hunlifesci_book_final_web.pdf

Ildikó Kelemen-Valkony1 , Katalin Csiszár2 , Mátyás Mink1: CONGENITAL MUSCULAR DYSTROPHY IN DROSOPHILA TYPE IV COLLAGEN MUTANTS: RAPID PROGRESSION ASSOCIATED WITH ALTERED INTEGRIN BIDING SITES WITHIN COL4A1, http://hunlifesci.hu/wp-content/uploads/2015/03/hunlifesci_book_final_web.pdf, 2015

Márton Kiss1 , Mónika Radics1 , Nikoletta Popovics1 , Katalin Csiszár2 , Mátyás Mink1: OVEREXPRESSION OF ANTIMICROBIAL PEPTIDE GENES AND INTESTINAL DYSFUNCTION IN DROSOPHILA TYPE IV COLLAGEN MUTANT, http://hunlifesci.hu/wp-content/uploads/2015/03/hunlifesci_book_final_web.pdf, 2015

Ildikó Kelemen-Valkony1, Márton Kiss1, András Attila Kiss1, Mónika Radics1, Nikoletta Popovics1, Katalin Csiszár2, Mátyás Mink2. Genotype-phenotype correlations in Drosophila col4a1 mutants: Humoral immune response, muscular dystrophy with various progression, centronuclear/myofibrillar myopathy, and options of treatment. Hungarian Molecular Life Science, Eger, 27-29 March 2015.

http://hunlifesci.hu/wp-content/uploads/2015/03/hunlifesci_book_final_web.pdf

Nikoletta Popovics1, András Attila Kiss1, Katalin Csiszár2 and Mátyás Mink1. Osmolytic treatment mitigates severe myopathy associated with IV collagen mutations in Drosophila. Hungarian Molecular Life Science, Eger, 27-29 March 2015.

http://hunlifesci.hu/wp-content/uploads/2015/03/hunlifesci_book_final_web.pdf

András Attila Kiss*, Nikoletta Popovics*, Márton Kiss*, Katalin Csiszár† and Mátyás Mink. Biomarkers associated with Drosophila type IV collagen col4a1 mutations as therapeutic tools of collagenopathy. Matrix Biology Europe (MBE) 2016, ATHENS, GREECE, JUNE 11 – 14.

http://www.mbe2016.upatras.gr/attachments/article/89/CONFERENCE%20PROCEEDINGS.pdf

András A. Kiss1, Szabolcs Zahorán2, Nikoletta Popovics1, Márton Kiss1, Monika Radics1, Edit Hermesz2, Zsolt Boldogkői1, Katalin Csiszár3 and Mátyás Mink1. Chronic inflammation, oxidative-nitrosative stress, mitochondrial dysfunction, improper integrin expression, loss of sarcomeres and proteolysis of myofibrillar proteins allow diagnosis of muscular dystrophy in

Drosophila type IV collagen col4a1 mutants. 6th FEBS Advanced Lecture Course FEBS - MPST 2017. The Abstract was selected to oral presentaion by the Organizers.

http://www.febs-mpst2017.upatras.gr/attachments/article/130/FEBS%202017.pdf

András A. Kiss1, Szabolcs Zahorán2, Nikoletta Popovics1, Márton Kiss1, Monika Radics1, Edit Hermesz2, Zsolt Boldogkői1, Katalin Csiszár3 and Mátyás Mink1. The systemic phenotype of col4a1 Drosophila mutants demonstrate chronic inflammation, intestinal dysfunction, premature aging and compromised secretory epithelia. ASMB Workshop 2017 on Basement Membranes July 12-14, 2017 Nashville, TN USA. The Abstract was selected to oral presentation by the Organizers.
http://www.asmb.net/files/asmb_2017_workshop_on_basement_membranes_program_final.1.pdf

András A. Kiss 1, Nikoletta Popovics 1, Zsolt Boldogkői 1, Katalin Csiszár 2 and Mátyás Mink 1. Aberrant integrin expression, myofibrillar proteolysis, membrane peroxidation, protein histidine alkylation and mitochondrial dysfunction contribute to muscular dystrophy in type IV collagen Drosophila mutants. Gordon Conference on Collagen (GRC) 2017, July 16 – 21; New London, NH, US. https://www.emedevents.com/c/medical-conferences-2017/collagen-gordon-research-seminar-grs-2017

András A. Kiss 1, Nikoletta Popovics1, Márton Kiss1, Zsolt Boldogkői1, Katalin Csiszár2 and Mátyás Mink1*. Proper function of integrin-mediated adhesion in muscle fibers relies on type IV collagen. Matrix Biology Europe 2018, Celebrating 50 years of FECTS Meetings, The University of Manchester, UK, 21st - 24th July 2018.

The animal model of type IV collagenopathy

We collected a dozen of phenotypic elements, or biomarkers that associate directly with the *col4a1* mutations. These include the reduced lifespan, intestinal dysfunction, elevated synthesis of antimicrobial peptides and the oxidant peroxinitrite, actin stress fibers in epithelia, mitochondrial fusion, membrane peroxidation, loss of sarcomere structure, atrophy of muscle fibers, erroneous integrin expression, Z-disc disintegration, Z-disc streaming. These markers or phenotypic elements are novel, not yet recorded in the cognate mouse mutants or human patients.

Creation of jobs by the aid of the project

Ildikó Kelemen-Valkony received salary in a value of 2 FTEs. She completed her research and generated sufficient data for a PhD thesis, although she has not initiated her scientific qualification. Given my PhD students did not receive stipend at the Faculty of Sciences and Informatics, University of Szeged, distributed by the Biology Doctoral School, the only source of their fellowship was the OTKA project in a value of 3 FTEs. The BSc and MSc students received fellowship in a value of ~1 FTE. Altogether the project created 6 FTEs.

Honors, awards

The PI was elected to contact person and member of the Presidium of the Organization Matrix Biology Europe at the 1st MBE (Matrix Biology Europe) conference, 21 - 24 June 2014, Rotterdam, The Netherlands.

András A.Kiss won the support at the New National Excellence Program, contract nr. UNKP-17-3-I-SZTE-35, 2017-2018.

András A. Kiss won a travel grant to participate on the Conference Matrix Biology Europe 2018, Celebrating 50 years of FECTS Meetings, The University of Manchester, UK, 21st - 24th July 2018.

Cost effectivity of the project

The value of the OTKA NN 108283 project equals to ~90.000 Euros. We consider the four published papers and the two to-be-published manuscripts the most important results of the project. On average, a paper costs ~15.000 Euros. Beyond the financial support of the project we did not enjoy any source provided by the Institution until September 2016, as we moved to Medical Faculty of Szeged University.

Acknowledgements

The Hungarian participants and our American cooperation partners are extremely thankful to the Granting Agency OTKA. The support enabled us to continue our research, publishing

papers, training young scientists and to present our results to the international and home scientific communities.

Szeged, 31 May 2018

Matyas Mink, PI