Editing of HNF1A gene in human induced pluripotent stem cells with CRISPR/Cas9 for disease modeling of endothelial (dys)function in maturity onset diabetes of the young (MODY)

Diabetes is a global health issue affecting children, adolescents, and adults. According to the World Health Organization, approximately 180 million people worldwide currently have type 2 diabetes and this number is estimated to double by 2030 year. With up to 75% of mortality in diabetic patients arising from vascular diseases, this is an issue of utmost social importance. Many studies have established that endothelial dysfunction (ED) is not only initiator, but could also be an important factor in the progression of vascular diseases. ED could develop as diabetes consequence or as recently shown could precede the development of diabetes. Maturity onset diabetes of the young (MODY) is caused by a mutation in a single gene and leads to diabetes under the age of 25. The mutations are inheritable and any child have 50% chance of getting the same mutation as a parent. The mutations in *HNF1A* gene (leading to MODY3) causes about 70% of MODY cases. Diabetes is induced by lowering the amount of insulin. However, the clinical expression in MODY3 patients is quite variable even within the same family. Some can develop hyperglycemia, whereas others can be normoglycemic at the same age. It was shown that patients with MODY3 have diabetic microvascular complications, related to endothelial dysfunction (ED), however it is not clear whether these complications are result of the hyperglycemia or due to the genetic mutation in *HNF1A* gene. Therefore, an improved understanding of the mechanisms and causes of ED could provide new approaches for MODY3 patient management.

In the current project we aim to investigate the role of *HNF1A* mutation (causing MODY3 diabetes) in endothelial cells and study its effect on the basic endothelial functions like vasodilation, vasoconstriction, active state and others. Our approach includes usage of novel techniques for human disease modeling like induced pluripotent stem cells and CRISPR/Cas9 gene editing. The generation of isogenic pairs of disease-specific and control iPSC lines that differ exclusively at the disease-causing mutation would be used to control the individual variances and define the subtle disease-relevant differences in monogenic diseases. It is worth mentioning that the combination of iPSCs and genome editing provides an unprecedented opportunity to study the fundamental principles of cell biology. Additionally, well-established methods for assessment of the endothelial function would be applied, together with important experiments in shear stress conditions, which recapitulate the blood flow conditions *in vivo*.

The results of the current project should reveal the possible connection between *HNF1A* gene mutation and ED. Taking this into account other treatments that may improve endothelial function systemically could be applied earlier and thus provide protection from further diabetes-mediated vascular events.