Diabetic nephropathy (DN) is a major microvascular complication of diabetes, which remains the leading cause of renal failure requiring renal replacement therapy. Despite the intensive studies on the pathogenesis of DN the mechanisms underlying the development of this complication are still unclear. DN is clinically manifested by progressive proteinuria, as a result of the dysfunction of podocytes – cells, which constitute the external layer of glomerular filtration barrier. It is widely accepted that injury to podocytes is a major culprit of the disturbance of the renal filtration and a hallmark of DN. There are various mechanisms of podocyte damage, which mainly results from persistent hyperglycemia. One of the most common feature of injured podocytes and kidney in diabetes is dysregulated lipid metabolism. It is characterized by an impaired cholesterol metabolism, increased lipid uptake and synthesis, intracellular lipid droplets accumulation, and imbalance of biologically active sphingolipids. All these processes induce oxidative stress, inflammation and cell death, leading to the dysfunction of glomerular filtration barrier and renal failure.

An intricate crosstalk exists between the lipid metabolism and mitochondrial dynamics (biogenesis, organelles fusion/fission, and mitophagy) in podocytes, though, less is known about regulation mechanisms of reciprocal lipid-mitochondria crosstalk, especially in diabetes. Lipidomic analyses of urine samples from obese patients revealed an increased level of odd-chain fatty acids in patients with renal disease, which can be produced from catabolism of branched chain amino acids (BCAA), valine, leucine, and isoleucine. Elevated levels of plasma BCAA have been also linked with obesity and type 2 diabetes. This suggests that BCAA or derivatives of their turnover can be master regulators of lipid metabolism in diabetic kidney.

β-Aminoisobutyric acid (BAIBA) is a BCAA metabolite and non-protein amino acid produced in mitochondria and secreted by skeletal muscles upon regular exercise. It has been shown that BAIBA regulates carbohydrate and lipid metabolism in adipocytes, increases insulin sensitivity in skeletal muscle, and protects osteocytes from reactive oxygen species-dependent apoptosis. However, nothing is known about potential role of BAIBA signaling in podocytes and its possible effects on mitochondrial and lipid homeostasis in these cells in the context of DN. Our initial studies showed that podocytes express receptor for BAIBA (Mas-related G protein-coupled receptor type D; MRGPRD), and BAIBA treatment significantly improves cellular respiration, ATP production and mitochondrial biogenesis. To the best of our knowledge, this is the first experimental evidence of MRGPRD presence and its responsiveness to BAIBA stimulation in podocytes. Moreover, we present a novel regulatory mechanism of mitochondrial dynamics in podocytes, which involves MRGPRD receptor stimulation by L-BAIBA and its effects on mitochondrial biogenesis. Therefore, **the main goal of this project is to further explore the molecular mechanisms of BAIBA effects on lipid metabolism and mitochondrial functions in podocytes and kidneys in diabetes**. We will use immortalized human podocytes to elucidate of the role of BAIBA receptor in normo- and hyperglycemic environment. In these cells we will study BAIBA metabolism by analyzing the expression levels and activities of the main enzymes involved in its production. Some previous studies demonstrated a positive impact of BAIBA on lipid metabolism, which encompasses synthesis and degradation of lipids, their transmembrane transport and storage. However, no studies has been done yet to elucidate possible effects of BAIBA on lipid metabolism and insulin sensitivity in podocytes. To address this issue, we will analyze the expression levels and/or activities of some key players of lipid transport (e.g., cholesterol efflux transporter ABCA1, LDL transporter CD36), fatty acid β-oxidation (e.g., PPARα, ACADM, ACOX1/2), and lipolysis (e.g., perilipin 5, ATGL) in podocytes cultured in high glucose concentration (HG) and treated with BAIBA. We will also analyze various mitochondrial parameters in podocytes cultured in HG±BAIBA: respiratory efficiency (by measuring oxygen consumption rates), size and shape of mitochondria (using bioinformatics analyses of confocal images of fluorescently stained mitochondria), mitochondrial quantity (measurements of mitochondrial DNA level by Real-Time PCR), expression of mitochondrial biogenesis markers (PGC-1α and TFAM) and marker of mitophagy (PINK1).

We will also investigate the possibility of improvement of renal injury using BAIBA in animal models of diabetes, which can indicate a new therapeutic strategy for DN. Additionally, taking into account the beneficial effects of physical activity in DN treatment and prevention of diabetes onset, exercise-induced BAIBA may serve as a mediator of positive clinical outcomes in DN, i.e., by triggering mitochondrial biogenesis and due to its antioxidative properties. Metformin (MTF), a popular anti-diabetic drug, has been also shown to enhance renal mitochondrial dynamics and attenuate hyperglycemia-induced mitochondrial reactive oxygen species production. Despite numerous studies on diabetic kidneys and podocytes, the molecular mechanisms of MTF are still unclear. Considering similar effects of MTF and BAIBA at the cellular level, in this project we will try to define potential reciprocal mechanisms of both agents in the context of renal function in diabetes, which would be of high clinical importance. Finally, we will elucidate the diagnostic usability of BAIBA detection in plasma and urine in patients suffering from diabetes. All these studies will not only explain the role of BAIBA and its receptor in podocyte biology in physiological conditions, but also will show the significance of its signaling in the development of DN. Additionally, the potential of BAIBA alone and combined with MTF to treat or prevent DN will be defined, along with elucidation whether BAIBA can be an indicative biomarker in diagnosis or prognosis of diabetic kidney disease.