Diabetic nephropathy is one of the most common and serious complications of diabetic kidney disease. However, our knowledge of the causes and mechanisms of this form of nephropathy is still very limited. Disturbance of podocytes function has a central role in the development of proteinuria in diabetic nephropathy. Podocytes are highly specialized cells that wrap around glomerular capillaries, and they comprise a key component of the glomerular filtration barrier. Podocytes consist of three morphologically and functionally different segments: a cell body, major processes, and foot processes. The podocyte cell body gives rise to primary processes that branch into foot processes; in turn, the foot processes of neighboring podocytes establish a highly branched, interdigitating pattern known as the slit diaphragm. Now, it is known that the slit diaphragm protein complex does not only serve as static molecular sieve but rather is a highly dynamic functional protein complex. The most important of these proteins are nephrin and podocin. The permeability of slit diaphragms is constantly regulated, and disturbances in the functioning of the filtration barrier result in renal failure. In diabetic nephropathy, a negative effect on the glomerular filtration is caused by hyperglycemia. Its presence results in increased inflammation of the renal tissue, causing narrowing of foot processes that form slit diaphragms, along with their detachment from the basement membrane. The hyperglycemia and inflammation of the renal tissue also initiate the death of podocytes that do not have an ability to divide and renew. At present, the correlation of the retraction of foot processes with the development of proteinuria is not well understood.

It has been known that increased activity of proteolytic enzymes promotes injury to the renal filtration barrier and renal dysfunction. These enzymes, in addition to the main function of protein degradation, are involved for the cell division, blood clotting or immunological function. Recently, we showed that increased activity of CatC podocytes express and secrete cathepsin C (CatC). We showed that a hyperglycemic-dependent activation of CatC induced cytoskeletal rearrangement and diminished the levels of major slit diaphragm proteins, resulting in increased albumin permeability in podocytes. Cathepsin C is a dipeptide exopeptidase responsible m.in. for activating proteins by cutting off propeptide domains. The most well-known function of CatC is in the activation of immune cell-associated serine proteinases such as neutrophil serine proteinases (NSPs). Excessive activity of these enzymes leads to the development of various types of inflammatory and immunological diseases. Additionally, podocytes have developed several features of immune cells, which allow them to quickly protect themselves against threats and metabolic imbalance. Recently, the existence of pyroptosis was demonstrated in podocytes, which is a type of cell death that had previously been seen only in immune cells. Our preliminary studies revealed the presence of neutrophil serine proteases in podocytes. We hypothesized that the physiological balance between serine proteinases and anti-proteinases is required to maintain proper podocyte barrier function and that this could be disrupted in diabetes. The principal goal of this project is to define the role of neutrophil serine proteases in the development of pathological changes in the renal filtration barrier observed in diabetes, which eventually lead to diabetic nephropathy and kidney failure.

The research project is divided into two parts: *in vivo* and *in vitro*. In the *in vivo* study, we will use ZDSD rat model and knockout $CTSC^{-/-}$ mouse model. Model ZDSD displays type 2 diabetes progression similar to the human disease – prediabetes (8-16 weeks of age), through overt diabetes (>16 weeks of age), to diabetic complications (24 weeks of age). In experiments *in vitro*, we will use kidney glomeruli and podocytes isolated from Wistar rats and immortalized lines of podocytes (mice and human). The functional, biochemical and molecular studies will aim to determine the role of serine proteases in regulation of function and structure of the podocyte. Moreover, we are assured that such an approach will lead to the development of new synthetic tools allowing the global monitoring of proteolytic activity (especially NSP origin) in a broad range of biological materials (lysates, supernatants, and urine samples). Summing up, the interdisciplinary nature of this project should, on the one hand, confer a better understanding of the functional disorders of the glomerular filtration barrier occurring in diabetes and, on the other hand, generate the chemical basis for describing new diagnostic tools for the early detection of diabetic nephropathy.