Proteins play a key role in every cellular process. It is impossible to understand the functioning of a living organisms without understanding the functioning of proteins. They are macromolecules composed of amino acid residues forming a polypeptide chain. The amino acid sequence determines how the polypeptide chain will fold and what final structure it will reach. However, for many proteins, folding may fail, and instead of the functional structure, proteins reach a misfolded state. Misfolded proteins strongly bind to each other and form insoluble aggregates. To counteract incorrect protein folding and the resulting loss of protein function, the cell possesses several protective systems composed of molecular chaperones. Chaperones are proteins that interact with a polypeptide chain, preventing its abnormal folding and aggregation and guiding misfolded proteins back onto the correct folding pathways.

Some proteins require chaperones not only during folding, but also for the regulation of their function. One such protein is Glucocorticoid Receptor (GR), which binds glucocorticoid hormones, such as cortisol, and activates the production of many proteins. Such reprogramming of the cell in response to a hormone signal, produced by the adrenal cortex, plays critical roles in many processes, e. g., reduces inflammation, stimulates fat breakdown and glucose synthesis, and is very important for fetal development. Decreased GR concentration or its incorrect regulation has implications in many disorders. Therefore, understanding the role of chaperones in folding and regulation of proteins such as GR may help to prevent and treat such conditions.

Chaperones involved in GR folding and regulation belong to two protein families: Hsp70 and Hsp90. These chaperones cooperate not only with each other in the folding of different proteins, called protein substrates, but also with a variety of cochaperones, proteins which regulate chaperones' activity, determine the recognition of different substrates and mediate a substrate transfer from one chaperone to another. One such protein is HOP, a cochaperone necessary for collaboration between Hsp70 and Hsp90.

In the cytosol of a human cell, there are 5 proteins from the Hsp70 family. One of them is HSPA1L, which is very similar to other Hsp70s, both in structure and function, however one research group recently reported its much stronger binding to HOP, suggesting distinctive properties with regard to the cooperation with Hsp90.

Although genes encoding Hsp70 proteins are rarely found to carry mutations that could impair their function, HSPA1L is an exception. A few recent reports described mutations in the HSPA1L gene that are more common in patients with a history of several prevalent conditions than in the healthy population. These conditions include: Spontaneous Preterm Birth, Inflammatory Bowel Disease, Multiple Sclerosis and Rheumatoid Arthritis. Each of them affects millions of people worldwide, causes severe symptoms, leads to thousands of deaths worldwide, and has a well-established inflammatory component, which may suggest an important role of GR signalling in their development. The causal relationship between the mutations in HSPA1L and these medical conditions is unknown, however experiments with cells showed that one of the changes in the HSPA1L gene decreases the GR concentration, which supports the hypothesis of the involvement of HSPA1L in GR regulation jointly with Hsp90.

In the proposed study, I aim to verify the molecular consequences of the clinical mutations in HSPA1L gene that were identified in the patients. I will test, if these mutations affect the HSPA1L structure, the ability to convert the energy from ATP hydrolysis to the chaperone activity, the ability to recover misfolded proteins, the interactions with other chaperones and cochaperones. Furthermore, I will assess whether the HSPA1L protein variants collaborate with the Hsp90 chaperone in folding of proteins that have lost their correct structure and in the regulation of the hormone binding by the Glucocorticoid Receptor.

To address these problems, I will produce all the chaperone proteins, including the clinical HSPA1L variants, and purify them, to reconstitute active chaperone machineries in a well-controlled, *in vitro* setup. Next, their activity will be tested with a variety of biochemical techniques, most of which are routinely used in our laboratory. This strategy may not only verify the hypothesis of the HSPA1L involvement in the Hsp70-Hsp90-dependent folding of protein substrates, but also provide better understanding of the general mechanisms of function of Hsp70 proteins, in particular, the functional interplay between the different Hsp70 family members.

Finally, I will test how the mutations in the HSPA1L gene affect the activity of the Glucocorticoid Receptor in human cells. Impairment in the regulation of GR by chaperones would make the cells non responsive to glucocorticoid hormones. Such an effect can be detected by measuring the cellular levels of proteins that depend on GR. Decreased level of these proteins in cells growing in the presence a steroid hormone would indicate major disruptions in GR signalling.

Taken together, the proposed research will provide broad assessment of HSPA1L as a potential risk factor in medical conditions of a major public concern and may uncover novel and unexplored level of Hsp70 chaperones regulation.