Characterization of clozapine and risperidone mechanism of action at the cell nucleus level

The aim of the project is a detailed description of the molecular mechanism of action of clozapine and risperidone by systematic studies of drug-induced alteration in the level of nuclear and nuclear intrinsically disordered proteins derived from selected rat brain structures and human neuronal and astrocyte cell lines. The second purpose is characterization the effect of silencing of *SmarcA5* and *Foxp1* genes on the whole proteome and nuclear subproteome of human neuronal and astrocyte cells as well as selected rat brain structures. Furthermore, the abilities of clozapine and risperidone to abolish the gene silencing induced alteration in the protein profile will be tested. Additionally, hypotheses driven targeted proteomic experiments will be performed to examine the working hypotheses concerning the effect of risperidone and clozapine on the canonical Wnt and AICD signaling.

Statistically 1 person per 200 suffers from schizophrenia as worldwide prevalence of schizophrenia is about 0.5–0.7%. Life expectancy of schizophrenia patients is even 20 years shorter than in the general population. 11-year follow-up of mortality in patients with schizophrenia (30 803 men and 36 078 women) published in the Lancet [1], showed that clozapine is associated with a substantially LOWER mortality than ANY other antipsychotic drugs (APDs). Very recent statistics from 17 countries shown however, that clozapine is still underutilised [2] although not only the general omission to prescribe clozapine, but also a delay in initiating clozapine treatment can put patients with treatment-resistant schizophrenia at a disadvantage [3]. Among the reasons for clozapine underutilisation are concerns regarding side-effects as likelihood of occurrence of very serious side effects is high. Thus, there is a strong requirement for more efficient and especially safer APDs. **A reasonable way to accelerate the development of new antipsychotic drugs is deeper understanding the molecular mechanism of action of these currently used and particularly clozapine**. The pharmacological profile of clozapine is extremely complex as it binds many different types of surface receptors. However, molecular mechanism of clozapine action remains unclear and it is still not know which biochemical pathways are responsible for clozapine superior therapeutic effect and with are related to adverse effects.

Comprehensive proteomic approach is particularly advisable and justified for tackling intracellular APDs mechanism of action as ensure the global insight into thousands of proteins. In this project we would like to focus our interest on nuclear proteins as there is growing body of evidence for the robust cross-talk between synapse and nucleus accomplished by synapto-nuclear protein messengers, which regulates synaptic plasticity via transcriptional regulation. Our previous studies provided us very limited insight into nuclear regulatory proteins because they exist in very low abundance in the cell. However, obtained results allow us to hypothesize about the impact clozapine and risperidone on nuclear AICD and canonical Wnt signalling pathways. Our preliminary data concerning nucleus points that clozapine and risperidone influence on nuclear transport, chromatin and transcription regulation. We would like to explore the most interesting result on the functional level through the investigation of the effect of *SmarcA5* and *Foxp1* genes silencing and examination of ability the clozapine and risperidone to abolish silencing effect.

In the project we will use very advanced mass-spectrometry-based methodology; quantitative shotgun technique for “discovery” studies in which we will be looking for proteins regulated by drugs or gene silencing and parallel reaction monitoring (PRM) method for validation of the results of shotgun studies and for hypothesis-driven analyses. Gene silencing will be performed with modified CRISPR/Cas9 technology allowing for neuron-specific gene targeting. Studies on SH-SY5Y cells differentiated to neurons and NT2 cells differentiated to neurons or astrocytes maybe very valuable in faced of interpretation of the results obtained for brain structures (prefrontal cortex, hippocampus, striatum, thalamus). Results will be also validated on the mRNA level with qRT-PCR technique and functionally using ImageStream method.

According to our knowledge, there are no investigations on the effect of any antipsychotic drug on nuclear proteome. Therefore, the planned studies are innovative and will allow to broaden the knowledge on the molecular mechanism of action of clozapine and risperidone.

[1] *Lancet*, vol. 374, pp. 620–627, 2009; [2] *Acta Psychiatr. Scand.*, pp. 1–15, 2017; [3] *Pharmacopsychiatry*, vol. 49, no. 6, pp. 254–259, Oct. 2016.