Do potential new MRGPRX2 ligands induce mastocytes degranulation in human?

In the current project we plan to focus on two groups of drugs, fluoroquinolones (FQ) and neuromuscular blocking agents (NMBA). As both fluoroquinolones and neuromuscular blocking agents are widely used in contemporary medical practice, the safety concerns related to them are a significant health problem and pose a significant challenge to population. Although FQ (e.g. ciprofloxacin, levofloxacin, moxifloxacin) have a favorable safety profile, hypersensitivity reactions, including serious and occasionally fatal hypersensitivity or anaphylactic reactions, are important safety concerns. In the last few decades, there has been an increase in the incidence of hypersensitivity reactions to quinolones. Importantly, reactions are severe up to 70% of cases. In turn NMBA are the most common cause of perioperative immediate hypersensitivity (POH) reactions in many countries over the world. Taking into account that millions of patients undergo general anaesthesia every year POH reactions represent a significant health problem, which affects a substantial part of general population.

The well-recognized mechanism of hypersensitivity reactions to these drugs are mast cells activation proceeding via IgE-dependent pathway, which comprises of allergen matching an drug-specific IgE binding to high affinity IgE receptor (FcεRI) on cell surface. However, the relevant "second route" of mast cells activation, alternative to IgE-dependent pathway, has recently been uncovered to proceed via Mas-Related G Protein-Coupled Receptor-X2 (MRGPRX2). Studies on animal models and *in vitro* experiments on human cell lines demonstrated that FQ and NMBA activate MRGPRX2, but data collected on human beings are lacking. It was shown that single nucleotide polymorphisms (SNPs) may influence MRGPRX2 function, but it has been never explored in drug hypersensitivity patients. Also, bioinformatics modelling of docking and interaction of FQ/ NMBA molecules with MRGPX2 has not been addressed until now. One does not know if (to what extent) the phenomenon of drug-induced MRGPRX2 activation is present in patients hypersensitive to these drugs.

In the project we plan to investigate this concern by two-step approach: 1) quantitative (MRGPX2 level in serum and skin) and 2) qualitative (MRGPRX2 genetic variants) comparison of controls tolerating drugs of interest and patients with anaphylactic events due to FQ or NBMA. Drug causality in patients will be confirmed in routine diagnostic work-up (skin prick tests/ intradermal tests, drug provocation test or incidental re-exposure to drug). The status of drug-specific IgE-dependence of reaction will be evaluated by measurement of drug-specific IgE in blood and/or positive basophil activation test.

To verify our working hypothesis, that a part of anaphylactic reactions due to exposure to FQ or NMBA, are mediated by MRGPRX2 activation, we will address the detailed research questions: 1) If MRGPRX2 serum level is higher in patients then in controls? 2) If MRGPRX2 mRNA expression level in skin is higher in patients then in controls? 3) If the number of $MRGPRX2^+$ skin MC (or the percentage of $MRGPRX2^+$ MC in all MC) is higher in patients then in controls? 4) If there are any unknown MRGPRX2 genetic variants in the patients? 5) If any patients' MRGPRX2 variants demonstrate unique functional features (hyperactivation) upon exposition to drug of interest? 6) How variants identified in genotyping affect FQ- and NMBA-MRGPRX2 interaction? (by employing bioinformatics modelling)

Evidence for occurrence of MRGPRX2-dependent immediate hypersensitivity reaction due to these drugs, supported by direct bioinformatics structural explanation of potential mechanism of action, would be novelty in our understanding of mechanisms of these reactions. Identification of patients harboring distinct SNPs responsible for severe MRGPRX2-dependent anaphylactic events would be a breakthrough with important clinical implications for management recommendations reflecting precision medicine paradigm.