Résumé du projet de thèse *(1 page maximum, en anglais)*
Indiquer la participation de chaque co-directeur et structure dans la gestion du projet. Please indicate explicitely the specific contribution of each supervisor to the PhD project.

We are proposing a comprehensive analysis of the role of *CHRFAM7A*, a ***human-specific*** gene in novel, *in vitro*, biomedical disease models, based on the use of ***human induced pluripotent stem cells (hiPSC)***. *CHRFAM7A* is present in humans for the last 200,000 years, but ***not*** in non-human primates, after a fusion between the *CHRNA7* nicotinic acetylcholine receptor (nAChR) α7 gene, and *ULK-4*, encoding a serine/threonine kinase (*1*). The resulting protein is thought to integrate into the canonical nAChR pentamer, but due to its lack of ACh binding sites, reduces receptor signaling. The importance of this human-specific, additional level of AchR signaling modulation/tuning has become clear in human genetic studies linking variation in *CHRFAM7A* to schizophrenia (SZ) (*2*, *3*). These have identified ***copy number variation (CNV)*** and a two base-pair coding variation (*2*) in SZ. Upregulation of *CHRFAM7A* was observed in SZ brain samples (*4*). Further studies found genetic association with the presence of a two base-pair coding deletion (***2bp variant***) leading to a further truncation of the protein. A further key finding: Expression patterns of the *CHRFAM7A* and *CHRNA7* gene transcripts were quantified (*5*) in the prefrontal cortex (PFC) of 380 SZ subjects and 325 comparison subjects collected from fetal state to old age. The main findings were that the ratio of *CHRFAM7A* over *CHRNA7* transcript was significantly increased in SZ. Increased *CHRFAM7A/CHRNA7* ratios are also found in immature (incl prenatal) PFC samples, suggesting an abnormal persistence of neurodevelopmental expression pattern in the (adult) cortex. Together these data argue that the CHRFAM7A is an important modulator of SZ susceptibility and may significantly contribute to the heterogenous drug responses targeting the CHRNA7 nAChR in SZ. The study thus has fundamental as well as clinical implications for personalizing treatments. **Our main aims are: 1. model *CNV* and the** delta2bp variant **of** *CHRFAM7A* **with** isogenic **hiPSC-derived cortical organoids and microglia; 2. study the consequences in biomedical disease models.**

Experimental plans

The genetic manipulation will be supervised by **Matthias Groszer**: We are using human induced pluripotent stem cell (hiPSC) lines from the Sanger Centre collection. They are fully sequenced at the genomic and transcriptomic level and corresponding proteomics data are published. All genetic engineering and potential genetic drifts during long-term cultures can therefore be controlled for with respect to the starting material.

- A *CHRFAM7A* ***knock-out (KO)*** will be obtained using a CRISPR/Cas9 strategy in two iPSC lines.
- Generation of isogenic *CHRFAM7A*delta2bp ***knock-in (KI)*** hiPSC.
- Generation of isogenic *CHRFAM7A*delta2bp ***over-expression*** hiPSCs: We already generated lentiviral expression vectors (LVs) and validated them in NPCs. This need is based on human genetic findings that implicate the delta*2bp* variant in SZ(*3*).

The analysis will be supervised by **Uwe Maskos**. Our approach will employ recent methodological advances, including the transplantation of hiPSC-derived microglia into brain ***organoids*** (*6*)(*7*). We will use **two-photon imaging of organoids** to follow the morphological and functional maturation of the human neurons. Single-cell sequencing (10XGenomics) will identify corresponding changes in the transcriptome. CHRFAM7A is expressed in neurons and microglia, the latter are important for synaptic pruning in neurodevelopment. Thus hiPSC-derived microglia with different CHRFAM7A genotypes will be transplanted into the developing organoid. The consequences on neuronal and microglial function will be evaluated using KI of the transgenic calcium indicator GCaMP7b (*8*), where we have already generated the line.

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