WE ARE LOOKING FOR A MOTIVATED STUDENT FOR A MASTER THESIS PROJECT

We are seeking for a highly motivated student who wants to join our interdisciplinary team for a master thesis in autumn 2018. Our research interests are mainly focused on neurogastroenterologic disorders, resulting impairments of the enteric nervous system (ENS), the "second brain" in our gut, and the elucidation of underlying pathomechanisms.

The enteric neuropathy *Hirschsprung's disease* (HSCR) represents a congenital disorder and the major genetic cause of functional intestinal obstruction in early childhood, characterized by an absence of enteric neurons in the colon (aganglionosis). HSCR is caused by a failure in proliferation, survival, migration or differentiation of neural crest and progenitor cells during embryonic development. It has an oligogenic origin and a sex-dependent penetrance. Currently, just 25 genes are known to be involved in HSCR. The only available treatment represents the complete surgical resection of the aganglionic part of the gut. However, patients often develop post-operative, lifelong complications like enterocolitis, fecal incontinence or obstructive symptoms. Underlying pathomechanisms are still not clarified, hampering patient-specific therapeutic interventions.

To dissect the individual genetic background of HSCR, to assess molecular and functional impairment of the ENS and to discover new candidate genes, an analytical, complementary pipeline was recently established by our team. The study pipeline allows the identification but also the interpretation of genetic findings by the use of tissue-based approaches, combined with the application of human genome-edited cell models mimicking individual pathomechanisms. This gives us insight into consequences of genetic alterations on molecular and functional level. In a pilot study, we identified four novel HSCR candidate genes and generated genome-edited cell clones using the neuroblastoma cell line SH-SY5Y. First results indicate impaired neuronal function in homozygous knock out clones.

In order to verify the potential causality of candidate genes in a more physiological context, the generation of genome-edited cell clones using primary human gastrointestinal cells is envisioned. To assess molecular and functional changes of enteric neurons related to the knockout of the candidate genes, the student will apply complementing approaches. The student will generate genome-edited cell clones using the CRISPR/Cas9 technology. Comparative expression analyses on transcript as well as on protein level investigating non-edited vs edited cell clones will be carried out and functional readouts will be applied. In addition, the state of the art technology nCounter target gene expression analysis is envisioned. The data generated within this thesis will not only contribute to the understanding of the molecular causes of HSCR and its lifelong bowel complaints but may also facilitate the development of novel therapeutic interventions.

TASKS:

- *Genome-editing using the CRISPR/Cas9 technology (cell culture work, FACS, microscopy)*
- *Molecular and functional analyses (immunofluorescence, scratch assay, microscopy)*
- *Comparative expression analyses on mRNA (qPCR, nCounter) and protein level (immunofluorescence, western blot)*

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