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EDCas9-mediated rescue of clustered deep-intronic variants in *ABCA4*

Either for a Master's thesis, traineeship or Erasmus exchange, we are looking for a motivated, dedicated and results driven Master's student with interest in genome editing technologies applied to inherited disorders.

Background: Stargardt disease is an autosomal recessive inherited retinal disorder caused by biallelic mutations in *ABCA4*. Deep-intronic variants (DIVs), resulting in aberrant mRNA splicing, have been associated with an increasing number of cases. Two clusters of DIVs have recently been characterized in intron 30 and 36. Upon splicing, they determine the retention of intronic sequences in the mature mRNA transcript, which ultimately results in premature stop codon formation. We aim to investigate a novel bioengineered CRISPR/Cas9 variant (EDCas9) with the aim to rescue such DIVs. Patient-derived photoreceptor precursor cells (PPCs) are used as an *in vitro* model for strategy assessment. Ultimately, the most performant gRNA-EDCas9 combinations will be delivered via AAV particles in PPCs and possibly organoids.

Planned experiments: The student will take over the validation of selected gRNA-EDCas9 combinations in patient-derived PPCs. This will involve iPSCs handling, differentiation and generation, either via reprogramming or CRISPR/Cas9 knock in, transfection and or/transduction of differentiated PPCs, downstream analysis for gDNA, mRNA and protein. In parallel, novel CRISPR/Cas9 constructs will be designed, cloned and established.

Techniques involved: Molecular cloning, mammalian cell culturing (including iPSCs), differentiation protocols, transfection methods (lipofection and electroporation), PCR, nucleic acid isolation and manipulation, bacterial cloning work, CRISPR/Cas9, reprogramming of human primary cell lines, Sanger sequencing, agarose electrophoresis and chip electrophoresis, NGS library preparation, flow cytometry.

Techniques probably involved: immunohistochemistry, western blot, lentivirus and AAV.

Where: Molecular Genetics Laboratory (Wissinger Lab), Institute for Ophthalmic Research, Centre for Ophthalmology, University of Tübingen, Germany.

Starting Date: March 2022 for at least 6 months.

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