DEVELOPMENT OF A NEXT-GENERATION SEQUENCING PLATFORM FOR RETINAL DYSTROPHIES, WITH LCA AND RP AS PROOF OF CONCEPT

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BACKGROUND AND AIM OF THE PROJECT

Human retinal dystrophies such as retinitis pigmentosa (RP) or Leber Congenital Amaurosis (LCA) are hereditary disorders that lead to degeneration of the photoreceptors and/or the retinal pigment epithelium, resulting in irreversible blindness. Their genetic heterogeneity together with the large number of unknown genes hampers the establishment of a molecular diagnosis in families with LCA and RP[1]. An accurate molecular diagnosis is essential, however. From a clinical perspective, a molecular diagnosis does not only facilitate genetic counseling and reproductive decisions, but is also a prerequisite for possible future gene therapy. Successful *RPE65* gene therapy trials in human constituted a breakthrough in the treatment of early inherited retinal dystrophies[2]. As these are likely to be gene-specific, robust screening strategies of known disease genes and efficient gene identification programs are urgently needed.

The aim of the current study is (1) To optimize a next-generation sequencing (NGS) platform for retinal dystrophies, with LCA and RP as a proof of concept; (2) To screen patients with LCA and RP who lack a molecular diagnosis with this platform, aimed at establishing a molecular diagnosis

DEVELOPMENT OF THE PROJECT

Optimization of a next-generation sequencing (NGS) platform for LCA and RP

We dispose of genetic material from several hundreds of patients with LCA and RP. Prescreening of all 14 LCA genes and 47 known RP genes in our families has not been feasible using conventional Sanger sequencing, as this is expensive, laborious and has a low throughput. Currently, new sequencing technologies, the so-called next-generation sequencing (NGS) technologies allow high-throughput and cost-efficient sequencing of (parts of) genomes (3). A NGS panel will be developed for all 14 and 47 as yet known RP and LCA genes respectively. If the proof of concept will prove to be successful for LCA, a similar workflow will be followed for RP.

For validation of the two platforms 12 LCA and 12 RP patients with known mutations will be screened respectively.

Screening of LCA and RP patients who lack a molecular diagnosis with NGS platform

A patient cohort consisting of 30 LCA patients and 30 patients with sporadic RP, who tested

negative using conventional routine testing, will be subjected to the respective NGS platforms. As identification of a large number of variants is anticipated by this approach, appropriate software will be used for data analysis. Following this approach, all variants will be ranked following their potential pathogenic effect. Relevant variations will be confirmed using Sanger sequencing. Novel mutations will be subjected to segregation analysis in all available family members and they will be screened for in 200 ethnically matched healthy control individuals.

REFERENCES

(1) Retinal Information Network (RetNet). Available from: *http:www.sph.uth.tmc.edu/retnet*.

(2) Maguire AM, Simonelli F, Pierce EA et al. – Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008; 358(21): 2240-8.

(3) Metzker ML- Sequencing technologies – The next generation. Nat Rev Genet. 2010; 11(1): 31-46.