Project Title: Receptor identification of AAV capsids used in gene therapy	**Code: CENT1**

Host School / Institute: Centenary Institute	**Address:** Centenary Institute, Royal Prince Alfred Hospital Grounds

Certificates & Clearances required: No

Primary Supervisor: Dr Charles Bailey

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Co-Supervisor/team: Professor John Rasko, Gene & Stem Cell Therapy Program, Centenary Institute

Project Type: Laboratory based

Project Category: Genetics; Gene Therapy

Skills / Attributes of a successful student: The successful student should be motivated and enthusiastic, and have a capacity to think critically and independently. They should have a realistic expectation to achieve publishable results in the eight-week project timeframe.

Project Keywords: Aene therapy; AAV; receptor; Haemophilia

Project Description: Recombinant adeno-associated virus (rAAV) has gained widespread use as a gene delivery vector for corrective gene therapies due to its lack of association with any human disease and its ability to safely and efficiently deliver a genetic payload into a broad range of tissues. Current rAAV modalities have not provided the necessary high-level human hepatocyte transduction efficiencies and humoral neutralisation properties necessary for targeting liver-specific diseases in diverse patient groups. World-first clinical trials, conducted by a team of clinicians including CI Rasko, used rAAVs carrying the Factor IX gene to successfully infuse haemophilia B patients (George, et al., 2017). However, limitations were imposed by the host immune system due to the rejection of AAV –transduced hepatocytes by capsid-specific CD8+ memory T cells (Manno, et al., 2006). Recent efforts have improved the transduction efficiency of rAAV vectors by engineering capsids with higher affinity or cell-specific tropism and increased resistance to neutralising antibodies. Indeed, a third avenue for increasing AAV-mediated therapeutic efficacy remains unexplored: modulation of the receptors to increase AAV capsid entry. It is this third approach with vast potential that we will focus on in this project. We will use a combination of phylogenetic, structural, biochemical, genetic strategies to functionally characterise the cell surface determinants that permit entry of human hepatotropic AAV capsids. The overall goal is to test the hypothesis that modulation of receptor expression can improve gene transfer efficiency in clinically relevant circumstances.

Research topics suitable for a summer research project include:
1. Molecular modeling of AAV capsids interacting with its receptor
2. Biochemical modulation of hepatotropic AAV capsid binding to cells
3. Mutagenesis studies of KIAA0319L to identify motifs important for trafficking
4. Screen for pharmacological agents that increase receptor expression

Skills/Tools: Mammalian cell culture, retroviral gene transfer, cell biology assays, CRISPR/Cas9 gene editing, flow cytometry, Western blotting, immunofluorescence, RT-qPCR, homology modeling.
Publications: Data generated by the student will directly contribute to a publication.