**Project Title:** To examine functional consequences of novel Mendelian Disease Genes identified using next generation sequencing.

**Host School/ Institute** The Children's Hospital at Westmead Clinical School

**Address:** Genetic Metabolic Disorders Research Unit Kids Research Institute Level 3 The Children's Hospital at Westmead, Cnr Hawkesbury Rd & Hainsworth St, Westmead Westmead, 2145

**URL:** [http://www.kidsresearch.org.au/](http://www.kidsresearch.org.au/)

**Personal Supervisor:** Dr Minal Juliet Menezes

**Phone:** 02 9845 1448

**Email:** minal.menezes@health.nsw.gov.au

**Co-Supervisors:** Professor John Christodoulou

**Project Type:** Laboratory Based

**Project Category:** Genetics, Paediatrics/Child Health, Chronic Diseases/Illness

**Project Keywords:**
1. Mendelian disorders
2. Paediatrics
3. Genetics
4. Inborn error of metabolism
5. Next generation sequencing

**Project Description:**

**Background:**
Rare genetic disorders affect 6 – 10% of the population, over half of which have an underlying Mendelian aetiology. Half of these disorders begin in childhood, and continue throughout life, with most being chronically disabling, difficult to diagnose and with significant impacts on health and quality of life for affected individuals and their families. Identification of the genetic aetiology will allow families to come off the protracted diagnostic odyssey, afford them more accurate genetic counselling, and potentially could open up new avenues for targeted therapies to ameliorate or perhaps even halt disease progression. For Mendelian disorders where the disease gene is yet to be identified, next generation sequencing (NGS) technologies have been successfully employed to identify the causative gene with as little as one informative pedigree.

**Aim:**
To be involved in the implementation of functional assays to validate the pathogenicity of a candidate disease gene in a family with a unique Mendelian disorder identified using our established NGS gene discovery pipeline. A combination of in silico, molecular and protein network analyses and relevant wet lab functional studies, to confirm the functional significance of the candidate disease gene will be employed.

**Experimental Strategy:**
Functional and structural analyses to confirm pathogenicity, depending on the precise variations identified, will include:
- PCR and quantitative PCR of mRNA for frameshift or nonsense mutations to examine whether shortened or unstable transcripts are present. For suspected unstable transcripts, studies will look for evidence of nonsense mediated decay
- Western analyses to detect shortened or unstable gene products
- Enzymatic or other relevant functional studies in primary cell lines (lymphocytes or fibroblasts) from affected individuals
- Lentiviral rescue experiments in patient fibroblasts or in cell lines in which the mutation in question has been knocked in, to evaluate the impact of the variation on the functional defect.