Summary of PhD thesis: The role of MYO9A at the neuromuscular junction

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Signals from the brain that stimulate muscle contraction are transmitted by motor nerves from the spinal cord to the appropriate muscle, via a connection termed the neuromuscular junction (NMJ). Defects in this connection can lead to disorders such as congenital myasthenic syndromes (CMS) which are characterised by often devastating fatigable muscle weakness from childhood. This weakness can affect the skeletal muscles causing problems with movement, the respiratory muscles thus disrupting breathing, ocular muscles involved in eye movements and also the bulbar muscles which control eating. Current available treatment for these conditions is limited and often inadequate. In 3 CMS patients from 2 unrelated families we identified mutations in *MYO9A* as causative for their disease. In this study we aimed to study the role of MYO9A at the NMJ, a protein in the unconventional myosin family which are involved in the structure of cells and also act as molecular motors among a number of other functions. We used a nerve cell-line derived from mice, as well as fibroblast (skin) cells from one of the patients and converted them into nerves to allow analysis of the molecular functions of MYO9A. We also made a zebrafish model with reduced amounts of MYO9A for studying its role in the whole organism, as well as obtaining muscle from a MYO9A knockout mouse.

Using the nerve cell-line we discovered that MYO9A plays a role in maintaining the structure of the cells and in forming appropriate nerve projections. We were able to demonstrate that this is due to interactions of MYO9A with RhoA, a protein that mediates many cellular functions, by treating the cells with a drug that inhibits this pathway. We also found similar disruptions to nerve cell shape using the patient-derived nerve cells. Furthermore, both cell types displayed problems with movement of proteins within the cell and their ability to release proteins. This function, termed exocytosis, is critically important for NMJ function as factors need to be released from the nerve to act on the muscle. Therefore, we hypothesised loss of MYO9A may be causing a negative effect at the NMJ due to disruption to release of proteins from the nerve. We performed a study to quantify the proteins released from the nerve cells lacking MYO9A and discovered that an important NMJ protein, agrin, was

not being released from these cells. This was an exciting finding as it may underpin the mechanism of MYO9A-CMS.

Our zebrafish model lacking MYO9A displays impaired swimming during development as well as disrupted NMJ structure. Therefore, we used the fish to test whether absence of MYO9A is linked to less agrin being released. We did this by treating the zebrafish with an agrin-based compound termed ‘NT1654’, made by Neurotune. This compound was able to rescue the movement of fish and many of the structural NMJ defects, suggesting that this was part of MYO9A-CMS mechanism. Preclinical trials with this compound are ongoing in mouse models, to determine whether it may be a potential treatment for patients with certain subtypes of CMS, among other diseases with NMJ involvement. This study highlights a role for MYO9A at the NMJ, the first unconventional myosin motor protein associated with a neuromuscular disease, thus opening a new avenue of proteins and pathways that may be relevant for a range of disorders.

I would like thank Kindness for Kids and all the people who have supported this fantastic charity, for giving me the opportunity to undertake this PhD and carry out research into neuromuscular disease, an area in which I am very passionate. I would also like to thank the patients and their families for their involvement in this work. Having successfully defended my thesis I am now a postdoctoral fellow in Ottawa, Canada, where I will continue my research into rare neuromuscular diseases and testing of new treatments.