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WHOLE EXOME SEQUENCING DIAGNOSIS OF X-LINKED MOESIN-ASSOCIATED IMMUNODEFICIENCY AND DEVELOPMENT OF A CRISPR/CAS9 RESCUE PHENOTYPE STRATEGY

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Aims: We aimed to use next generation sequencing to identify the molecular aetiology of a primary immunodeficiency disorder present with persistent lymphopaenia and neutropaenia, and to develop an *in vitro* rescue phenotype strategy.

Methods: Whole exome sequencing was performed on a proband and his parents on the Ion Torrent platform at a read depth of 20X with target base coverage of 94.63%. Trio analysis was performed on the Ion Reporter Suite where variant annotation identified included SNPs and indels for each exome library. Confirmation of the causative role of the candidate gene was performed by qPCR and Western Blot analyses on the proband, family members and healthy control. We will further develop an *in vitro* proof-of-principle rescue phenotype strategy to correct the causative mutation in proband lymphocytes using CRISPR/Cas9 ribonucleoprotein (RNP) technology.

Results: Data filtering and *in silico* prediction tools identified a damaging and disease-causing single base mutation in X-chromosome gene *MSN* (c.511C>T p.Arg171Trp), not identified previously in gene variant databases. The mutation was validated in all family members by Sanger sequencing, confirming the proband was hemizygous X-linked recessive (-/T) and had inherited the affected T allele from his non-symptomatic carrier mother (C/T). Western blot has demonstrated the absence of moesin (MSN) protein in proband lymphocytes, compared with normal expression in lymphocytes from the healthy control, father and mother. qPCR identified significantly lower levels of MSN mRNA transcript expression in the proband compared to the healthy control in whole blood ($P = 0.02$) and in lymphocytes ($P = 0.01$). These results confirmed MSN deficiency in the proband, directly causative of his immunodeficient phenotype.

Expected results: We expect that proband primary lymphocytes transfected with custom designed guide RNAs, wild type donor sequence and Cas9 v2 protein, will undergo homology directed repair of the ^{R171W}MSN mutation with limited off-target effects, with rescue phenotype confirmed by qPCR/Western Blot.

Biography

Gabrielle Bradshaw has a BSc (Hons) in Molecular Medicine and Haematology from Wits University, South Africa, a Master's in Medical Research/Genomics from Griffith University, Australia, and is currently a final year PhD Candidate at Queensland University of Technology in Brisbane, Australia. She is a qualified Medical Laboratory Scientist (Nat. Dipl in Biomedical Technology from University of Johannesburg, South Africa with experience working full time for the National Health Laboratory Service in Johannesburg, and part-time in the Molecular Genetics pathology laboratory at the Royal Brisbane and Women's Hospital for Queensland Health. She has so far published 3 first-author papers in her research area of non-Hodgkin lymphoma and primary immunodeficiency, and she has an interest in Personalised Medicine, Diagnostic Genomics, and Gene Therapies to treat Cancer and Congenital Diseases.

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