An investigation into *Leishmania* genome plasticity in response to disruption of sphingolipid biosynthesis

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Leishmania parasites cause devastating diseases in tropical areas around the world. With a lack of vaccines, treatment relies entirely on drugs such as amphotericin B which have several limitations, including severe side effects and emerging resistance. These are major concerns worldwide which have led to the use of genetic-based approaches for the identification of targets essential for parasite survival where can be exploited for drug development.

Genetic approaches in *Leishmania* species have relied heavily on homologous recombination, however whole genome sequencing of a serine palmitoyltransferase (SPT, the first enzyme in sphingolipid biosynthesis) knockout in *L. major* identified surprising non-targeted deletions. The putative functions of these encoded proteins of the deleted genes led us to consider that they may have compensated for the loss of SPT. Recent advancements in genetic technology include CRISPR-Cas9, this was employed to investigate this phenomenon further in the tractable *L. mexicana* model. Notably, deletion of SPT was not possible in the parental background suggesting an essential function. However, loss of the SPT locus was achieved when the genes encoding ceramide synthase (an upstream enzyme in biosynthesis) were knocked out first. This provided proof of principle that compensatory deletions may facilitate the loss of essential genes, a finding that was further investigated with respect to the non-targeted genes lost in the *L. major* SPT knockout. In an alternative mode of genome plasticity, CRISPR-Cas9 mediated deletion of sphingosine kinase (SK) in *L. mexicana* resulted in targeted deletion and a phenotype resembling that reported for homologous recombination driven SK knockout in *L. major* (Zhang et al, 2013). However, further analyses demonstrated that the gene encoding SK was maintained in a different chromosomal location, leading us to conclude that it was essential.

Taken together these data demonstrate that genetic knockouts, especially those obtained via homologous recombination, need reanalysis due to the genome plasticity *Leishmania.*