Black fever beats drugs by adding just two DNA bases to its genome

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In *eLife* today (22 March), Wellcome Trust Sanger Institute scientists show how the parasite responsible for the neglected tropical disease Black Fever (visceral leishmaniasis) can become resistant to drug treatment. Studying the whole genomes of more than 200 samples of Leishmania donovani revealed that the addition of just two bases of DNA to a gene known as LdAQP1 stops the parasite from absorbing antimonial drugs.

While antimonials are no longer the first-line treatment for the disease, the discovery does show that whole-genome sequencing of L. donovani parasites could be used to study and track the emergence of resistance to frontline drugs - alerting health workers to potential hot spots of resistance.

Black Fever is the second most deadly parasitic disease after malaria, affecting nearly 300,000 people every year and killing up to 50,000. The parasite is mainly found in the Indian subcontinent, where up to 80 per cent of the disease occurs. To best understand how the parasite evolves and track the spread of drug resistance, researchers need a way to survey and monitor the parasite's population structure. Unfortunately standard techniques to do this have proved fruitless because the strains of L. donovani parasite are so genetically similar.

Dr James Cotton, senior author of the study from the Sanger Institute said: "If you want to control visceral leishmaniasis, you need to understand what is going on at the geographic epicentre of the disease,

and you need to be able to see changes at the level of individual DNA bases in the parasites' genomes. Until now studies have been limited to looking at small regions of the parasite's DNA or at what happens in the laboratory. To truly understand what is happening in the real world, we analysed the whole genomes of more than 200 samples from parasites captured in India, Nepal and Bangladesh over almost a decade."

Exploring the genetic landscape of L. donovani at such depth and breadth yielded new insights into the parasites' ability to develop drug resistance, and its evolutionary history. In particular, the researchers found that the insertion of just two bases of DNA into the genome of approximately 35,000,000 bases helped the parasite to overcome antimonial drugs.

Dr Tim Downing, one of the paper's first authors from the Sanger Institute and National University of Ireland Galway said: "We discovered that many of the parasites that were resistant to antimonial drug treatment had just two additional DNA bases in the gene LdAQP1, which produces an aquaglyceroporin protein. This insertion produces a scrambled version of this protein that can no longer move small molecules - including antimonials - across its cell membrane. These strains of L. donovani are likely to be resistant because they cannot take in the drugs."

Black Fever - "kala azar" in Hindi - is second largest life-threatening parasitic disease after malaria. Spread through the bites of sandflies, the parasites enter the internal organs such as the liver, spleen and bone marrow, making them inflamed and swollen. The infection produces fever, weight loss, fatigue and anaemia and is fatal if left untreated.

Professor Jean-Claude Dujardin of the Institute of Tropical Medicine Antwerp and the University of Antwerp, senior author of the study and leader of the consortium that ran the study said: "This study perfectly

illustrates the relevance of collaboration between large sequencing centres like the Sanger Institute and clinicians and scientists involved in the battle against the most neglected diseases. Thanks to the acquired knowledge, it will be our turn now to beat Black Fever 2-0 by providing local health authorities with performance monitoring tools, and guiding research and development for new and more efficient anti-parasitic drugs."

More information: Imamura H et al. Evolutionary genomics of epidemic visceral leishmaniasis in the Indian subcontinent. *eLife* 2016, published online at 8am 22 March

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