Different paths to drug resistance in Leishmania

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Two remarkable discoveries were today revealed by researchers into genome analysis of Leishmania parasites. These results uncovered a surprising level of variation at the genome structure level.

First, they found that the DNA sequence of individual strains of each species populations is almost completely identical. It appears that only a small number of genes may cause different symptoms of infection. Second, the parasite's evolutionary development and success may be driven by a genetic abnormality leading to multiple copies of chromosomes that would kill most organisms. This process leads to multiple copies of chromosomes and genes known as copy number variation. These studies increase our understanding of the process of drug resistance in Leishmania.

Leishmaniasis is a disfiguring and potentially fatal disease that affects two million people each year. There are four main forms of the disease; ranging from skin lesions (cutaneous leishmaniasis), caused by species that include Leishmania mexicana, to a deadly infection of internal organs (visceral leishmaniasis) caused by Leishmania donovani parasites.

In the first study, the researchers generated a high-quality draft genome of L. donovani using a sample taken from an infected patient in Nepal. The team used this as a reference framework to analyse a further 16 isolates from Nepal and India that had different responses to antibiotic medications.

"Our work highlights how genomic research changes our perspectives about these parasites," says Dr Matt Berriman from the Sanger Institute, and a leading author on both studies. "We show that the evolution of these organisms is driven not only by single-letter changes in their genetic codes, but also by larger mutations in the copy numbers of genes and entire chromosomes. The findings have enabled us to discover more about its natural variation and genetic structure which is vital for the further development of effective treatments."

The second study focused on producing a reference genome for *L. mexicana* from a sample taken in Guatemala and comparing it with existing reference genomes for various Leishmania species on the spectrum of cutaneous to visceral disease. Working with colleagues from the University of Glasgow and University of York, the research team discovered that each of the Leishmania species that have been fully sequenced hasroughly 8,000 genes, yet *L. mexicana* has only two genes that are unique to it.

"These findings have important implications for the understanding of parasite variation and the genetic basis of disease. Leishmania has taken a different path to most organisms because of its extensive and highly unusual variation in chromosome and gene-specific copy numbers," says Tim Downing, a lead author on the research from the Sanger Institute. "This variation in the copy numbers of chromosomes and genes provides a new dimension to monitoring the evolution of drug resistance in these parasites."

The presence of more than the standard two chromosomes is generally detrimental to other species, but for Leishmania, it seems to be a beneficial, common occurrence that may be a driver in evolutionary change. One example is chromosome 31, which is present in almost all Leishmania genomes in more than the standard two copies. This unusual variation is likely to be important in the ability of Leishmania to cause

disease.

"We must maintain continuous surveillance to monitor the threat from the on-going emergence of drug resistance. These studies provide the tools to identify and analyse new variants as they emerge," says Jean-Claude Dujardin, senior author from the Institute of Tropical Medicine in Antwerp. "This basic biological difference in the way that drug resistance emerges in Leishmania is essential for tracking strains and resistance.

"We can't simply look for the single-letter changes, but must include structural changes. We have to search differently, more smartly."

The first study enhances the genomic understanding of the most dangerous end of the spectrum of Leishmania species, and also provides clues to the genetic and genomic basis of drug resistance. The second study shows for the first time the scale of abnormal chromosomes in Leishmania species. This is thought to help with the evolution of the species and a possible cause for antibiotic resistance.

The overall picture is of unusual genetic forces equipping the parasites in unexpected ways to circumvent attempts to control them. This research will go a long way to understanding the cause of drug resistance when treating Leishmania.

More information: Downing T, Imamura H et al.(2011) Whole genome sequencing of multiple Leishmaniadonovani clinical isolates providesinsights into the evolution and mechanisms of drug resistance. *Genome Research*. Published online on 28 October 2011 doi:10.1101/gr.122945.111

Rogers MB, Hilley, JD et al. (2011) Chromosome and gene copy number variation allow major structural change between species and strains of

Leishmania. *Genome Research*. Published online on 28 October 2011
doi:10.1101/gr.123430.111