The Genetics of Susceptibility to Visceral Leishmaniasis in India

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Ninety percent of the estimated 500,000 annual new cases of clinical visceral leishmaniasis (VL) or Kala-azar caused by protozoa of the Leishmania donovani complex occur in India/Bangladesh/Nepal, Brazil, and Sudan. Clinical VL is fatal if left untreated. Epidemiological studies show that only 10% of individuals infected with the parasite develop clinical disease. Others develop cell-mediated immunity that can be measured as a positive delayed-type hypersensitivity (DTH) skin-test response to Leishmania antigen, and are resistant. Understanding the genetic risk factors that determine susceptibility and resistance to clinical disease will provide important leads for development of improved strategies for therapeutic intervention.

We have studied genetic susceptibility to clinical VL in an Indian population from the state of Bihar, which accounts for 90% of all Indian cases. Initially, 59 multicase families (77 nuclear families; 156 affected offspring; 372 individuals), comprised of 32 Hindu and 27 Muslim pedigrees, underwent a 10cM genome-wide linkage scan (GWLS) using 515 polymorphic microsatellite markers. Multipoint non-parametric analysis provided evidence for linkage at p<0.01 (LOD>0.91) on chromosomes 2, 6, 8, 11, and X, and suggested heterogeneity driven by different religious groups. These regions were further investigated by genotyping 65 additional Indian VL multicase families and 19 additional polymorphic microsatellites to provide a denser 2-5cM refined map. Refined analysis that corrects for overrelatedness in the population retained the evidence for linkage at 2q14.1 (D2S363; singlepoint LOD 2.33; p=5x10^-4), at 6p25.1 (D6S1617; singlepoint LOD 1.00; p=0.016), at 8p23.1 (D8S516; singlepoint LOD 1.52; p=0.004), at 11q14.2 (D11S1780; singlepoint LOD 2.53; p=3x10^-5), and at Xq23 (DXS8055; multipoint LOD 1.58; p=0.004). These results contribute novel population-specific candidate regions that are being further pursued as part of the current TMRC project.

This family-based GWLS was only powered to find major genes. Larger sample sizes are needed to provide sufficient power to identify all of the genes that contribute to susceptibility to this complex disease. For this reason, the focus of the new TMRC project has been to increase the Indian resource to provide 1000 genetically unrelated clinical VL cases and 1000 unrelated controls that have been selected in a competitive bid to undergo a SNP-chip population-based genome-wide association scan (GWAS) in collaboration with the Wellcome Trust Case Control Consortium (WTCCC). The positive results from this GWAS will be validated in a set of 1217 cases (1000 common to the case-control dataset) in families (total sample 3630 individuals) using dense tag-SNP family-based allelic association tests that control for ethnicity and are robust to pedigree or nuclear family clustering. Output from the Indian GWAS will be compared with results from a parallel family-based GWAS of 629 VL cases and 1160 DTH+/900 DTH- individuals also being undertaken in collaboration with the WTCCC. These sample sizes are, for the first time, sufficiently powered to carry out GWAS and hypothesis-driven candidate gene studies with confidence in order to identify global- and population-specific susceptibility genes associated with the VL and DTH phenotypes. The large-scale SNP-chip data for the 1000 Indian controls will also provide a valuable resource for studies of other complex diseases in India.