Genome Wide Search for Visceral Leishmaniasis Susceptibility Genes in Sudanese Population

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Sudan is one of the major foci of visceral leishmaniasis (VL).

Familial clustering and ethnic differences suggest genetic factors may be involved in the infection. In this study a two stages genome-wide scan was employed using two independent sets of families from two villages (El-Rugab and Um-Salala) inhabited by the Masalit ethnic group and located in the endemic area -eastern Sudan.

In the first stage (= scan1) 400 highly polymorphic microsatellite markers were typed in 220 individuals from 38 multicase pedigrees (= scan1 families).

In scan 1, the multipoint analysis performed provided evidence for linkage of VL susceptibility to 5 regions on chromosome 1p22, 1q31.3, 5q34-35.3, 6q27 and 13q 31 (logarithm of the odds LOD $0.0002 < p < 0.05$) for linkage.

Stratification of scan1 families by village (after the grid tightening strategy was employed in which 35 additional markers were added close to regions that gave suggestive evidence for linkage of VL susceptibility in scan1) revealed village-specific peaks for linkage: in Um Salala at 1p22 and 5q34 ($p = 1.6 \times 10^{-4}$, $p = 0.047$ respectively), in El Rugab at 1q31.1, 5q35.3 and at 6q27 ($p = 0.007$, $p = 0.002$, $p = 8.95 \times 10^{-5}$).

To confirm linkage; family set 2 (=scan2 families: 21 nuclear families, 48 affected sibs) were genotyped across these regions.

Analysis of scan1+2 families stratified by village demonstrated a major gene on 6q27 (LOD score 3.07; $p = 8.6 \times 10^{-5}$) in El-Rugab only. A broad region of linkage on chromosome 1 also resolved into two clear peaks upon stratification by village: on 1p22 (LOD score 1.19; $P = 0.009$) for Um-Salala and on 1q31.1 for El-Rugab (LOD score 1.25; $p = 0.008$). These results indicate that VL susceptibility might be complex inheritance and that population substructure could be vital in the implication of the disease in different populations.

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Microarray Analysis of Gene Regulation in *Streptococcus pneumoniae* Upon Penicillin Exposure

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Background: Streptococcus pneumnoiae is a major cause of morbidity and mortality, with presenting invasive infection such as lobar pneumonia, bacteremia and meningitis. The emergence of penicillin resistant strains since the 1970s has been life threatening. In order to curb this problem, a better understanding of the mechanisms of antibiotic resistance is essential. In this study, the Affymetrix gene chip array was used to study a wider range of genes that may play a role in the development of antibiotic resistance.

Methods: 3 confirmed strains of *S. pneumoniae* (known MIC: Sensitive, Intermediate and resistant to Penicillin) were used. Total RNA of 3 strains were extracted using the hot acid phenol method. cDNA synthesis, fragmentation, labeling and hybridization were carried out according to manufacturer's protocol (Affymetrix). Labelled cDNAs were hybridized onto respective gene chip expression arrays masked with probes representing the known genome of S. pneumoniae. The strains were further treated with penicillin prior to extraction of RNA to elucidate the effect of antibiotic in the expression of genes. Scanning and analysis was done using the GCOS software. To further confirm that the induction of gene expression was specific for penicillin or other blactam drugs which involves inhibition of cell wall synthesis, the strains were exposed to other antibiotics such as cefotaxime, and ceftriaxone, and the relative mRNA expression were measured using real-time PCR.

Results: Significant differences within the gene expression of the genome were observed among the 3 categories of strains; Penicillin Sensitive *S. pneumoniae* (PSSP), Penicillin Intermediate *S. pneumoniae* (PISP) and Penicillin Resistant *S. pneumoniae* (PRSP). Functional genes with significant expression levels include genes encoding transport, transcription regulation, two component signal transduction, ribosomal proteins and cell surface proteins. Genes which are involved in the biosynthesis of the cell wall envelope showed to have significant expression levels upon penicillin stress. These genes include the genes encoding the penicillin binding proteins, choline binding proteins and D-alanylation of cell wall. Exposure with the other b-lactam drugs showed variation in the expression which was induced by the specific drugs.

Conclusion: The penicillin binding proteins (PBPs), encoding the formation of the cell wall proteins and the choline binding proteins (CBPs) had significant levels of expression, which correlated with the initial antimicrobial susceptibility of the strains. This shows that antibiotic stress has an effect on the bacterial physiology and gene regulation. Understanding the mechanisms of antibiotic resistance may lead to a proper management of pneumococcal infections.

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