Identification and Characterization of Variant Surface Glycoprotein (Vsg) Genes with Diagnostic Potential for Trypanosoma Brucei Rhodesiense

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Human African Trypanosomiasis (HAT) is present in 36 sub-Saharan African countries. The disease is easier to treat in early stage than late stage when treatment with melarsoprol is responsible for significant fatality. Therefore there is need to develop a diagnostic test for early detection before progression of the disease into the late stage. Currently available tests have poor sensitivity. Thus highly sensitive methods are needed for the detection of the parasite in human blood. The surface coat of bloodstream trypanosomes including the flagellum is composed predominantly of Variant Surface Glycoprotein (VSG). African trypanosomes have evolved a complex mechanism for sequentially expressing only one VSG from a repertoire of several hundred to thousand VSGs. Some VSGs occur more frequently than others. The objective of this study was to evaluate the diagnostic potential of VSGs frequently encountered in early stage of T. b. rhodesiense infections using blood samples from Kenya and Uganda. VSGs 3 and 4 that were previously found to be the most frequently encountered VSGs in early stage of T. b. rhodesiense infections were chosen as putative diagnostic VSG candidates in this study. Sequence alignment of VSGs 3 and 4, showed high similarity between the two VSGs hence common primers were designed and used to amplify both VSG 3 and 4. 25% of 24 blood samples from Kenya and 23% of 43 blood samples from Uganda were positive for both VSG 3 and 4 in Polymerase Chain Reaction (PCR) using hotstar Taq polymerase. While a detection of 95.8% and 86% among blood samples from Kenya and Uganda respectively was observed in PCR using phusion Taq polymerase. The VSG 4 open reading frame was cloned into pRSET-A expression vector and expressed in Escherichia coli BL21 cells. Recombinant crude VSG 4 protein was detected by anti-VSG antibodies using positively documented human serum samples. Purified recombinant protein had a molecular weight of 55KDa which was the expected size of VSG 4 protein. BLASTn of VSGs 3 and 4 against the T. brucei databases revealed their top orthologs to be a putative atypical VSG, on chromosome 6.
of *T. brucei* TREU927 (geneDB: Tb927.6.5450 and GenBank accession number XM840543-National Center for Bioinformatics Information NCBI) and *T. evansi* strain KETRI-JN394 clone 1A VSG messenger RNA (GenBank accession number AF317931-NCBI). However no significant matches were found on alignment of the predicted amino acid sequences of VSGs 3 and 4 with the amino acid sequences of the other *Trypanozoon* VSGs already in the public domain databases. This shows that the protein expressed is specific only to *T. b. rhodesiense*. From the results of this study it can be concluded that the primers used in this work which were designed from the sequences VSGs 3 and 4 may be used to detect *T. b. rhodesiense* infections in humans in molecular assay and the VSG 4 protein can be a good diagnostic antigen in serological assays since it is cheaper than molecular assay.