Structure and selection: Insights into the evolution of host-parasite interactions of Tetraspanin 23 in *Schistosoma turkestanicum*.

Eldridge, C.J.L.[1 2], Juhasz A[3 4]., Majoros, G.[5], Emery, A.M.[2], Cook, R.T.[1], Kidd, D.[1], Lawton, S.P.[1 6]

[1] Kingston University London, Surrey, UK.
[2] Natural History Museum, London, UK.
[3] Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, 10 Liverpool, L3 5QA, UK.
[4] Institute of Medical Microbiology, Semmelweis University, H-1089 Budapest, Hungary.
[5] University of Veterinary Medicine, Budapest, István u. 2, 1078 Hungary
[6] Epidemiology Research Unit (ERU) Department of Veterinary and Animal Sciences, 13 Northern Faculty, Scotland's Rural College (SRUC), An Lòchran, 10 Inverness 14 Campus, Inverness, IV2 5NA, UK.

Schistosoma turkestanicum is a widespread Asian veterinary parasite of domestic livestock and a causative agent of cercarial dermatitis in humans. Since its discovery in Hungary in 2010 the population of *S. turkestanicum* from the Gemenc region has been of particular interest as an undisturbed schistosome population that has escaped anthelmintic treatment with praziquantel. Currently there are no vaccines to protect against schistosomiasis and it has been hypothesised that high levels of parasite diversity in host populations is responsible for the current lack of protective efficacy in vaccine candidate trials. One promising vaccine candidate Tetraspanin-23, an immune evasion protein that has hypothesised non-immune IgG binding function in the large extracellular loop region (LEL), has so far had limited success in clinical trials. In this study protein function and site selection analyses were combined with inter-host population sequencing of the Tsp-23 gene to study host-parasite interactions and selective pressures that may influence population diversity at this locus. Tetraspanin-23 is highly conserved across the schistosome phylogeny and analysis of Tsp-23 orthologs predicted a single site under diversifying positive selection in the LEL region. Further sequencing of individual *S.turkestanicum* worms taken from five red deer hosts in the Gemenc, Hungary identified evidence of balancing selection at the same site. In addition structural and antigenicity score prediction identified this site to be associated with structural antigenic variation both between species and between individuals within host populations. Interestingly this site was predicted on the outer region of the LEL and not in the FC binding domain which was found to be entirely conserved in the population sample and predicted to be under purifying selection from between species selective site analysis. This suggests that host-parasite interactions with the Tsp-23 LEL region may be occurring at two levels, a conserved FC binding motif that functions to bind to the less variable non-immune IgG FC region in the host as well as diversifying positive selection

acting on the exposed region of the LEL which is physically interacting with the more variable components of the host immune system. With the numerous strategies of immune modulation and evasion employed by schistosomes at the tegument surface this study on Tsp-23 suggests that protein function is likely an important factor in the assessment of vaccine candidate suitability for schistosomes.