1) Genome-wide association study of an African snail vector of schistosomiasis identifies genes associated with resistance to infection by *Schistosoma mansoni*

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Schistosomiasis is a chronic inflammatory disease afflicting hundreds of millions of people worldwide. Schistosomes are blood flukes transmitted by aquatic snails, and thus the disease can be controlled by blocking transmission to snails. Most cases of intestinal schistosomiasis occur in sub-Saharan Africa, where they are caused by *Schistosoma mansoni*, transmitted by the snail *Biomphalaria sudanica* and related species. In contrast to the better-studied neotropical vector, *B. glabrata*, there has been little work on African snails and thus very little is known regarding genetic interactions between these snails and schistosome parasites. Identifying snail genes that confer resistance to infection will facilitate ways to leverage these immune mechanisms and disrupt the parasite's life cycle. To uncover such immunogenetic pathways, we have performed a genome-wide association study (GWAS) on F1 offspring from snails collected from Lake Victoria that were designated as either susceptible or resistant after challenge with locally collected *S. mansoni.* Our GWAS had a two-step design. Initially, we sequenced pools of uninfected (295) and infected (493) snails to determine candidate outlier single nucleotide polymorphisms (SNPs) that showed association with resistance phenotypes. Next, we used these data to design an amplicon sequencing panel to enable a cost-effective GWAS of individual snails. The panel included candidates from our pooled sequencing analysis (poolseq) as well as other candidate loci that have been described in the literature to play a role in schistosome resistance to *B. glabrata*. For the second step of the GWAS, we used the amplicon panel to genotype snails (138 negative and 138 positive) from the poolseq analysis and an independent set of snails (98 negative and 97 positive) as a validation population. Results indicated that several genomic regions were strongly associated with schistosome resistance. In particular, our strongest signal came from a genomic region rich in multiple epidermal growth factor-like genes. In comparison with regions previously tied to schistosome infection in *B. glabrata*, our results suggest a complex suite of pathways to defend against *S. mansoni*. These results provide a first glimpse into genes of the innate immune system of the vector *B. sudanica* and will help inform schistosomiasis control strategies aimed at predicting or manipulating the vector competence of the snail host, particularly in the African communities most severely affected by this disease.

2) Introducing schisto.xyz v2: integrative analysis, interactive visualization, and universal expression exploration for helminth research

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Studying gene expression in helminths is essential for advancing our understanding of parasite biology, identifying targets for drug and vaccine development, monitoring drug resistance, and elucidating host-parasite interactions, ultimately contributing to the control and elimination of helminth infections. Schisto.xyz, a pivotal platform dedicated to integrative bulk RNA-seq analysis of all life stages for *Schistosoma mansoni*, unveils its upgraded version (v2), fortified with expanded datasets encompassing diverse life stage and timepoints (>250 RNA-seq samples), and introducing seven single-cell RNA-seq atlases (>100k cells) for both *S. mansoni* and *S. japonicum* and demonstrated support for spatial omics data. All data can be interactively explored through a fast and highly customizable platform, with the visualization state sharable between researchers via web URLs. The highlight of this update also includes the introduction of a robust search framework, named "Omics Expression Explorer (OmicsEE)", empowering researchers to explore gene expression patterns across multiple datasets effortlessly. Besides schistosome data included on Schisto.xyz, OmicsEE provides versatility for querying omics data from various species, whether sourced online or from locally hosted atlases. In summary, by offering integrative analysis and insights into gene expression dynamics across different life stages and cell type populations, Schisto.xyz facilitate the reusability and interoperability of large-scale datasets and serves as a vital resource for advancing schistosome research and underpinning the development of novel therapeutic strategies. The universal expression explorer framework expands its capabilities to other helminth species, enabling comparative analysis across species to identify conserved and divergent expression patterns, thus facilitating comparative genomic and functional studies.

3) *Strongyloides stercoralis* - one or multiple pathogens - genomic studies on wild isolates

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More than 600 million people are infected with the nematode *Strongyloides stercoralis*. Also non-human primates, dogs and cats were described as natural hosts. For mor than 100 years it is controversially discussed if the *S. stercoralis* in these animals is really the same species as the one in humans and if these animals serve as a reservoir for zoonotic human strongyloidiasis. This discussion was predominantly based on epidemiology and on biological differences such as different preferences for one of the three life cycles these parasites can undergo or the infection potential in experimental infections. Recently, molecular/genomic investigations of wild populations of *S. stercoralis* added new insights. Our lab has been involved in such studies in Cambodia, China, Thailand, Iran and Bangladesh. Based on the "classic" literature and the molecular/genomic studies by us and others I will argue that: a) Dogs can carry *S. stercoralis* indistinguishable from the ones in humans and should be considered a putative source for zoonotic infection. While zoonotic infection may be epidemiologically relevant in certain settings, it appears unlikely that strongyloidiasis is normally a zoonosis. b) dogs also carry genomically distinct types (species) of *Strongyloides* that are not normally found in humans. A case of introgression of a "human and dog type" mitochondrial genome into the "dog only type" population argues for occasional intermixing. c) Even within human derived *S. stercoralis* there is high within-species genetic diversity. This, along with biological differences, suggest that *S. stercoralis* in humans might also be a complex of closely related species or subspecies with possibly different pathogenetic potential. In order to further investigate this and to study biological differences, controlled experiments are desirable. This requires that different isolates are in laboratory culture. I will present our progress towards establishing a collection of *S. stercoralis* isolates.

4) Oxygen sensation in human-parasitic skin-penetrating nematodes

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To detect and invade a human host, infective larvae (iL3s) of the skin-penetrating nematode *Strongyloides stercoralis* rely upon neuronally detected sensory cues – such as host-derived heat and odorants. Upon host entry, *S. stercoralis* iL3s experience a rapid drop in oxygen (O_2) concentration from atmospheric levels in the soil environment (~21%) to relatively hypoxic levels in the host tissues (~7%). We hypothesize that neuronal detection of ambient O_2 levels sculpts behaviors in *S. stercoralis* iL3s that are essential for parasitism. Yet, O_2 sensation in *S. stercoralis* – and all other parasitic nematodes – remains unstudied. We found that *S. stercoralis* iL3s demonstrate robust locomotory changes upon exposure to acute shifts in O_2 concentration. When exposed to 7% O_2, iL3s exhibit a gradual slowing response that is reversible when worms return to 21% O_2. Similarly, the iL3s of the skin-penetrating nematodes *Ancylostoma ceylanicum* and *Strongyloides ratti* respond to changes in ambient oxygen. We then found that the transition from 21% O_2 to 7% O_2 elicits an acute pause response in *Caenorhabditis elegans* dauers that is absent in all tested species of parasitic nematodes - suggesting that O_2 sensation may serve to reinforce parasite-specific behaviors. To explore the molecular and neuronal bases of O_2 sensing in *S. stercoralis*, we identified four candidate O_2 sensors, each of which are homologous to the O_2-sensing soluble guanylate cyclases (sGCs) found in *C. elegans*. Notably, homologs to a specific subset of *C. elegans* sGCs that detect downshifts in O_2 concentration were absent in *S. stercoralis*, *A. ceylanicum*, and *S. ratti*. Using transcriptional reporters, at least three of the *S. stercoralis* sGCs are expressed in candidate O_2-sensing neurons. Chemogenetic silencing of these candidate O_2-sensing neurons results in blunted O_2-evoked behaviors in iL3s. We are now using calcium imaging to quantify the parasite-specific encoding properties of these candidate O_2-sensing neurons.

5) Dopamine signaling drives skin penetration by the human-parasitic nematode *Strongyloides stercoralis*

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Skin-penetrating parasitic nematodes, including *Strongyloides stercoralis* and hookworms in the genera *Necator* and *Ancylostoma*, infect ~1 billion people worldwide, causing debilitating disease and fatalities. Skin penetration, the process whereby these nematodes invade host skin and enter the body, is a critical step that could be targeted to prevent infections. However, the behaviors that skin-penetrating nematodes execute during skin penetration and the underlying neural and genetic mechanisms are poorly understood. We study this problem using the human-infective nematode *S. stercoralis*. To examine skin penetration, we established an *ex vivo* assay wherein infective larvae are placed on excised rat skin and videorecorded. We found that larvae pushed down on the skin with their heads almost immediately following contact. Thereafter, larvae either initiated penetration by puncturing the skin with their heads or crawled a short distance on the skin. Some punctures led to complete penetration whereas others were aborted. Ultimately, larvae repeatedly pushed, punctured and crawled on skin until penetration was completed. We next investigated the neural mechanisms that underlie skin penetration and we hypothesized that the mechanosensory texture-sensing neurons might be necessary. The free-living nematode *Caenorhabditis elegans* senses texture using the dopaminergic neurons; thus, we chemogenetically silenced the dopaminergic neurons in *S. stercoralis* and found that this either delayed or prevented skin penetration. Similar findings were obtained upon genetic inactivation of dopamine signaling using CRISPR/Cas9-mediated targeted mutagenesis and pharmacological inhibition of dopamine signaling using the dopamine receptor antagonist Haldol. Excitingly, Haldol also delayed or inhibited skin penetration in two other species of skin-penetrating nematodes: the rat-infective nematode *Strongyloides ratti* and the human hookworm *Ancylostoma ceylanicum*. Thus, dopamine signaling likely plays a conserved role in driving skin penetration in multiple species of skin-penetrating parasitic nematodes. Ultimately, our work could lead to the development of topical anthelmintics that prevent infections by inhibiting skin penetration.

6) Genetic and Environmental interactions contribute to immune variation in rewilded mice

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The relative and synergistic contributions of genetics and environment to inter-individual immune response variation remain unclear, despite its implications for understanding both evolutionary biology and medicine. Here, we quantify interactive effects of genotype and environment on immune traits by investigating three inbred mouse strains rewilded in an outdoor enclosure and infected with the parasite, *Trichuris muris*. Whereas cytokine response heterogeneity was primarily driven by genotype, cellular composition heterogeneity was shaped by interactions between genotype and environment. Notably, genetic differences under laboratory conditions can be decreased following rewilding, and variation in T cell markers are more driven by genetics, whereas B cell markers are driven more by environment. Importantly, variation in worm burden is associated with measures of immune variation, as well as genetics and environment. These results indicate that nonheritable influences interact with genetic factors to shape immune variation, with synergistic impacts on the deployment and evolution of defense mechanisms.

7) Title: Multi-omics characterization of the STAT6 regulated micro milieu during *N.brasiliensis* infection

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Hookworms are soil-transmitted parasites that are extremely common sources of infection globally with no effective prophylactic treatment. This is in part due to our incomplete understanding of how hookworms interact, especially at the molecular level (i.e. the level of the 'micro milieu') within the microenvironment of their hosts to mediate their infection. To this end, this project aimed at performing a detailed molecular investigation of host-parasite interaction during murine infection with the model hookworm *N.brasiliensis,* by contrasting the micro milieu of resistant (WT) and susceptible (STAT6 KO) mice. The focus of the project was on characterizing the contribution of understudied regulators of host-parasite interaction, including metabolites, microbes and neurotransmitters in driving the disparate phenotypes observed in WT and STAT6KO mice during infection. Untargeted LC/MS of faeces from infected mice indicated that deficiency of STAT6 drives changes in the metabolism or utilization of various classes of compounds. More specifically, functional enrichment analysis highlighted altered metabolism of catecholamines, pyridoxines xenobiotics and fatty acids. Complimentary to this, bulk RNA-seq analyses of intestinal tissue from infected WT and STAT6 KO mice revealed significant differences in the expression of transcripts coding for enzymes which play critical roles in the metabolism and utilization of such compounds. Additionally, several genes involved in propagation of neuronal signalling pathways were found to be differentially expressed. Furthermore, 16s sequencing of faecal pellets from WT and KO mice indicated an effect of STAT6 on regulating the composition of the microbiome, especially of bacterial species belonging to the family *Lachnospiraceae*. In parallel, transcriptomic analysis of parasites derived from the intestines of either WT or STAT6 KO mice was performed to gain insight into parasite intrinsic adaptation to different host environments. In line with similar studies, it was observed that parasites from WT and STAT6KO mice have altered gene expression profiles, especially with regard to genes involved in catabolism of neurotransmitters, the counteraction of oxidative stress and the reshaping of extracellular matrix proteins. These findings provide novel insight into STAT6-dependent regulation of signaling pathways relevant to the metabolism of diverse compounds, with putative function in shaping gene networks associated with shaping host-parasite interaction. Future studies are aimed at establishing how such observed changes associate with altered immune responses to infection.

8) The interaction of schistosomes with the maternal microbiota and their respective influences on the offspring immune system

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Schistosomes manipulate the host immune system to ensure their prolonged survival, with bystander effects on the infected host and their progeny including reduced allergic sensitivities and impaired vaccine responses. We propose that these immune alterations in the offspring stem from specific maternal signals that are modified during chronic infection. Given that 40 million women of child-bearing age are infected in Sub-Saharan Africa, it is imperative to delve deeper into these signals. One possible mechanistic angle is the modification of the maternal microbiota, which plays a large role in shaping the offspring immune system and has been investigated during schistosome infection, but not in a fetomaternal setting. Here, we analyse the maternal and offspring stool microbiota from regulatory and Th2 phases of infection by 16s rRNA and whole genome sequencing in a mouse model. We complement this with metabolomic analysis of stool, serum and breastmilk, showing that schistosomiasis alters levels of immunomodulatory short chain fatty acids and bile acids. We will corroborate this data with serum samples from our human mother-child cohort (Helmvit). Finally, to better understand whether the offspring immune system is impacted pre- or postnatally, we carry out a cross-foster experiment and analyse antigen-presenting and costimulatory molecule expression on B cells and dendritic cells in the spleen, mesenteric lymph node and bone marrow as well as frequencies of their stem cell precursors. In the future, the transcriptome of cell types showing the most significant changes will be investigated.

9) The mediatory effect of the gut microbiome in the helminth-associated resolution of cardiovascular disease risk in humans

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Background: Like the epidemiological trends in developed countries, cardiovascular diseases are rapidly increasing in Low- and Middle- Income Countries (LMICs), in parallel with increasing urbanization and rural-urban migration. Hence the need to reinvigorate research efforts aimed at achieving effective strategies that may control and prevent cardiometabolic disease. Preliminary results from Lake Victoria Island intervention study have shown that helminth (*S. mansoni*)-infected individuals had reduced cholesterol and triglycerides levels. Further, comparing rural individuals to those in urban settings (Urban survey) showed increased blood pressures in the latter. The current project is aimed at understanding the mechanisms by which helminth infections and the rural environment may alter cardiovascular disease risk. Our study is predicated on the hypothesis that; by altering the human gut microbiome profiles, helminths and the rural environment may lead to a reduction in cholesterol and blood pressure levels. **Methods:** Firstly, we conducted and published a systematic review and meta-analysis (https://doi.org/10.3389/frmbi.2023.1174034) using a random-effects model for all metrics of microbial diversity, adjusting for age, sex and anthelminthic treatment to explore relationship between helminths and human gut microbiome. Secondly, we aimed at investigating helminth- and urbanisation-induced changes on the human microbiome and metabolome. We performed 16S rRNA sequencing (using V3-V4) and Liquid chromatography- mass spectrometry on the faecal samples that were randomly selected from individuals whose LDL-, HDL- and total-cholesterol, triglycerides levels, blood pressure and *S. mansoni* infection status were known. From LaVIISWA, we randomly selected 40 *S. mansoni* infected samples from intensive arm, and 40 *S. mansoni* infected samples from standard arm and then selected 40 samples that were uninfected. We then selected 80 samples from Urban survey in a ratio of 1:1 for individuals with and those without *S. mansoni* infection. **Results**: From our systematic review and meta-analysis, we report an increase in the alpha diversity (Shannon, observed richness and Chao1 indices) among the helminth infected individuals compared to the uninfected. The second substudy, we illustrate significant differences in both the microbiome and metabolome profiles of *S.mansoni*-infected individuals compared to those without S.*mansoni*-infection, and in the individuals from rural compared to those in to the urban setting. Our correlation analysis showed the different taxa associated with the metabolic risk factors in both populations. Additionally, we conducted extensive mediation analysis to illustrate the mediation role that gut microbes play in helminth-related regulation of the different cardiovascular risk. Using an integrative network biology approach, we also show the link between the metabolome and microbiome changes for each cardiovascular risk factor. We further use pathway analysis to show the cardiovascular-related biological pathways that are significantly enriched by the metabolites found to be differentially abundant in *S. mansoni infected* individuals compared to the uninfected. **Conclusions or interpretation:** Our results show that helminth infection and urbanisation can affect one's microbiome as well as their metabolome. Also, we show, using mediation analysis that S. mansoni urban living affect CVD risk through the gut microbes. This brings us closer to answering the question of whether the helminth-infection and urbanisation effects on host cardiovascular risk are mediated through the gut microbiome changes that these two factors induce. We envisage that understanding the possible roles that helminths and urbanisation may play in altering the relative composition of microbiota and how these changes may contribute to the resolution of cardiovascular risk may set us on a path to identifying beneficial bacteria that may be isolated and cultured for therapeutic use. A paper to illustrate how collaboration has been vital in successfully executing this project has recently been published (https://doi.org/10.1038/s41564-023-01423-w) in NATURE.