A genetic TRP down the channel to praziquantel resistance

James A. Cotton* and Stephen R. Doyle

Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridgeshire, UK.
CB10 1SA

*Correspondence: james.cotton@sanger.ac.uk (J. A. Cotton).

Keywords: *Schistosoma mansoni*, praziquantel, resistance, Transient Receptor Potential channel

Abstract:

The anthelmintic praziquantel (PZQ) is an essential tool in controlling schistosomiasis, so reports of reduced PZQ efficacy are of great public health concern. Le Clec'h *et al.* recently identified a gene responsible for PZQ resistance in experimentally selected resistant *Schistosoma mansoni*. The importance of this locus in natural infections remains to be established.

The mainstay of schistosomiasis control, praziquantel (PZQ) is a key drug in controlling human helminth infections and an essential component of efforts to reduce the impact of this neglected tropical disease. The scale of treatment with PZQ is enormous, with over 100 million people treated in 2019 [1] – mostly school-aged children in Africa. Despite this, prevalence and infection intensity remain high in many areas [2]. PZQ is still mostly very effective, but there is worrying evidence of falling efficacy in areas that have received many years of treatment, and some infections appear recalcitrant to treatment. These findings could be early signs that schistosome populations are evolving tolerance or resistance to PZQ, which would have a major impact on public health. Understanding the genetic basis of PZQ resistance would allow researchers to identify resistant populations and target alternative interventions to prevent the spread of resistance alleles.

Le Clec'h and colleagues [3] have identified a locus for PZQ resistance using an interesting combination of genetic approaches to demonstrate that genetic variation at or near the target for PZQ [4] is associated with resistance. The key to their approach was the recognition that a resistant strain of *Schistosoma mansoni* showed an unusual dose-response relationship, with some worms behaving like the wild-type drug-sensitive strains while others survived unharmed at very high doses. The authors reasoned that this was likely due to genetic variation for PZQ resistance between worms within this "resistant" population. They developed an assay to measure the recovery of adult worms following PZQ treatment *in vitro*, and used this to identify individual worms at either extreme of PZQ response. Using whole-genome sequencing to compare pools of worms, they identified two genomic regions that are genetically different between worms that are sensitive to PZQ exposure and those that survive high exposure.

These regions represented a fairly long stretch of DNA – over 5 million nucleotides and 115 protein-coding genes – so something was needed to narrow these down. Here, the researchers got lucky. While drug resistance can involve a number of different molecular mechanisms – for example through reducing the amount of drug entering cells or increasing the rate at which drugs are removed or broken down – an obvious place for resistance mutations is in the target for the drug. The target of PZQ has long been unclear, but recent work has shown that PZQ binds to and activates an ion channel called *Sm*.TRPM$_{PZQ}$, related to those that respond to temperature in vertebrates (reviewed in [4]). An accompanying paper uses molecular modeling and mutagenesis to reveal how PZQ binds to *Sm*.TRPM$_{PZQ}$ [5]. The gene encoding this channel lies within one of regions identified, making this likely to be responsible for PZQ resistance in these worms.

Additional evidence confirms the involvement of *Sm.*TRPM$_{PZQ}$ in PZQ resistance. Data from individual worms demonstrate that both a SNP variant within *Sm.*TRPM$_{PZQ}$ and a nearby large indel are associated with PZQ response, and that this trait is recessive. A second approach was to establish schistosome lines enriched for resistant and sensitive alleles at the *Sm.*TRPM$_{PZQ}$ SNP and indel. These two lines showed much greater differences in PZQ response *in vitro* and differed in response *in vivo*, but showed little difference in fitness in either mammal or snail hosts. A final piece of evidence that *Sm.*TRPM$_{PZQ}$ variation underlies the difference in PZQ response is that previously identified small-molecule modulators of *Sm*.TRPM$_{PZQ}$ remove the differential response of these lines. Direct evidence that *Sm.*TRPM$_{PZQ}$ modulates resistance would be desirable, as would some understanding of the likely molecular mechanism. Attempts to silence *Sm*.TRPM$_{PZQ}$ were unsuccessful, and non-synonymous mutations in *Sm*.TRPM$_{PZQ}$ found in the resistant parasites showed no difference from the wildtype in functional assays. However, one final experiment gives a first clue about the likely molecular basis of *Sm.*TRPM$_{PZQ}$-mediated PZQ resistance: *Sm*.TRPM$_{PZQ}$ has significantly lower expression in resistant than sensitive parasites.

Taken with the biochemical evidence that PZQ activates the same channel, there is clearly a strong case that *Sm*.TRPM$_{PZQ}$ is the causal locus involved in PZQ response here, although in the absence of identifying a causal variant, this is not quite definitive. An important next step will be to try to understand the molecular basis of the reduced expression, but more urgent is the need to establish whether variation in either the *Sm*.TRPM$_{PZQ}$ protein or its expression is associated with PZQ efficacy in natural populations. In an initial attempt, Le Clec'h *et al.* find little evidence that mutations in *Sm*.TRPM$_{PZQ}$ present in their resistant line are found in nature. Genome-wide data has also failed to identify variation at this locus linked to drug selection [6]. There are several possible explanations: it is hard to identify variants that regulate protein expression and current data is from a small number of natural populations. Furthermore, if PZQ resistance is restricted to a few 'hot spot' locations, resistance alleles may be rare in nature and existing samples under-powered to detect it.

This study shows that variation at or near *Sm*.TRPM$_{PZQ}$ is associated with resistance, and shows how genome-wide approaches can efficiently identify regions of the genome under drug selection [7]. However, this is in a single laboratory-selected resistant line, where PZQ selection pressure was applied under laboratory conditions on larval stages not normally exposed to PZQ. This procedure might select for different genetic variation to PZQ treatment in patients. Furthermore, the selected line is from Brazil, and it is not clear if the mechanism will be conserved with the genetically divergent *S. mansoni* in East Africa where there are most reports of reduced efficacy. One possibility is that reported PZQ treatment failure is not due to resistance, and PZQ resistance is not present in natural populations. Much work remains to identify whether *Sm*.TRPM$_{PZQ}$ or some other locus is involved in PZQ resistance in the wild. Nonetheless, the two recent papers represent significant advances. A few years ago, we had only an uncertain picture of how PZQ killed schistosomes [4], but we now have an idea of the mechanism and a

strong candidate to guide the search for resistance to this drug. Le Clec'h and colleagues also show how parallel lines of research can enrich each other; beyond prior knowledge of the target, the genetics work takes advantage of cellular assays involving heterologous expression of *Sm*.TRPM$_{PZQ}$, known small-molecule modulators for validation and an understanding of the interaction with PZQ to interpret variation data. Knowledge that *Sm*.TRPM$_{PZQ}$ can mediate PZQ resistance gives new importance to understanding the interaction between drug and protein.

Acknowledgments

We thank Dr Gabriel Rinaldi for comments on this article. We are supported by the Wellcome Trust via their core funding of the Wellcome Trust Sanger Institute [grant 108413/A/15/D] and a UKRI Future Leaders Fellowship [MR/T020733/1] to SRD.

Declaration of interests

The authors declare no competing interests.

References

1 World Health Organization (2021) *Weekly Epidemiological Record. Schistosomiasis and soil-transmitted helminthiases: progress report, 2020*, 96

2 Mawa, P.A. *et al.* (2021) Schistosomiasis Morbidity Hotspots: Roles of the Human Host, the Parasite and Their Interface in the Development of Severe Morbidity. *Front. Immunol.* 12, 635869

3 Le Clec'h, W. *et al.* (2021) Genetic analysis of praziquantel response in schistosome parasites implicates a transient receptor potential channel. *Sci. Transl. Med.* 13, eabj9114

4 Park, S.-K. and Marchant, J.S. (2020) The Journey to Discovering a Flatworm Target of Praziquantel: A Long TRP. *Trends Parasitol.* 36, 182–194

5 Park, S.-K. *et al.* (2021) Mechanism of praziquantel action at a parasitic flatworm ion channel. *Sci. Transl. Med.* 13, eabj5832

6 Berger, D.J. *et al.* (2021) Whole-genome sequencing of Schistosoma mansoni reveals extensive diversity with limited selection despite mass drug administration. *Nat. Commun.* 12, 4776

7 Doyle, S.R. and Cotton, J.A. (2019) Genome-wide Approaches to Investigate Anthelmintic Resistance. *Trends Parasitol.* 35, 289–301

8 McVeigh, P. (2020) Post-genomic progress in helminth parasitology. *Parasitology* 147, 835–840

9 Anderson, T.J.C. *et al.* (2018) Genetic Crosses and Linkage Mapping in Schistosome Parasites. *Trends Parasitol.* 34, 982–996
10 Castelletto, M.L. *et al.* (2020) Recent advances in functional genomics for parasitic nematodes of mammals. *J. Exp. Biol.* 223,

Box 1. Forward vs Reverse Genetics in schistosomes

Advanced techniques to manipulate gene sequence or expression have become well established in protozoan parasites, with even genome-scale reverse-genetics screens being increasingly accessible. The complex lifecycle, multicellular organisation and large, repetitive genomes of schistosomes – and other parasitic worms – makes reverse genetics approaches more difficult [8]. RNA interference varies in the efficiency and longevity of knockdown between targets and lifecycle stages, while any approach to stable transgenesis requires the transforming agent to reach germline cells. *In vitro* cultivation of most helminths is only possible for certain life stages and often only transiently, so the expense, inconvenience and ethical issues of keeping and infecting hosts makes experiments with live worms more challenging. However, some aspects of helminth lifecycles make forward genetics convenient. The alternation of clonal expansion and sexual reproduction in schistosomes is a case in point [9]. Having lots of genetically identical clonal individuals allows replicated characterisation of a single genotype – even if destructive characterisation is required, viable material remains available – making experiments like the marker-assisted selection experiment used by Le Clec'h and colleagues possible. The nematode parasite *Strongyloides*, in which a sexual free-living stage alternates with a parthenogenic parasitic female, is another example where the availability of genetically identical, free-living larvae makes this system uniquely amenable to transgenesis [10].