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Host Determinants of Reinfection with Schistosomes in Humans: A Systematic Review and Meta-analysis

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Abstract

Background: Schistosomiasis is still a major public health burden in the tropics and subtropics. Although there is an effective chemotherapy (Praziquantel) for this disease, reinfection occurs rapidly after mass drug administration (MDA). Because the entire population do not get reinfected at the same rate, it is possible that host factors may play a dominant role in determining resistance or susceptibility to reinfection with schistosomes. Here, we systematically reviewed and meta-analyzed studies that reported associations between reinfection with the principal human-infecting species (*S. mansoni*, *S. japonicum* and *S. haematobium*) and host socio-demographic, epidemiological, immunological and genetic factors.

Methodology/Principal Findings: PubMed, Scopus, Google Scholar, Cochrane Review Library and African Journals Online public databases were searched in October 2013 to retrieve studies assessing association of host factors with reinfection with schistosomes. Meta-analysis was performed to generate pooled odds ratios and standardized mean differences as overall effect estimates for dichotomous and continuous variables, respectively. Quality assessment of included studies, heterogeneity between studies and publication bias were also assessed. Out of the initial 2739 records, 109 studies were included in the analyses, of which only 32 studies with 37 data sets were eligible for quantitative data synthesis. Among several host factors identified, strong positive association was found with age and pre-treatment intensity, and only slightly for gender. These factors are major determinants of exposure and disease transmission. Significant positive association was found with anti-SWA IgG4 level, and a negative overall effect for association with IgE levels. This reconfirmed the concept that IgE/IgG4 balance is a major determinant of protective immunity against schistosomiasis. Other identified determinants were reported by a small number of studies to enable interpretation.

Conclusions: Our data contribute to the understanding of host-parasite interaction as it affects reinfection, and is a potential tool to guide planning and tailoring of community interventions to target high-risk groups.

Introduction

Schistosomiasis is still an important helminthic infection in terms of severe morbidity that can result as a consequence of infection. Over 200 million people are infected and more than 700 million people are still at risk of getting infected with schistosomiasis [1]. Although the disease can be effectively treated with Praziquantel, reinfection occurs rapidly after mass drug administration (MDA). An effective vaccine used singly or in combination with chemotherapy is the optimum approach [2]. However, such a vaccine is presently not available, although some candidates are still in the development pipeline. Since chemotherapy by MDA is presently the only available intervention in endemic areas, there is need to identify the host factors that increase susceptibility to reinfection with schistosomes for targeted intervention in high-risk groups.

In addition to the demographic, socioeconomic and epidemiological variables that may predispose certain subset of the population to reinfection, several human studies in endemic areas have provided insight into the potential resistance inducing immune response phenotypes [2–8]. Many of these studies have found associations between reinfection with schistosomes and IgE/IgG4 balance. Schistosomiasis has previously been shown to be under the control of the cytokine genes cluster on chromosome 5q31-q33 region, called SM1 [7,9,10]. It is also possible that several other immunogenetic factors in addition to this cytokine genes cluster, including the genes controlling IgE levels, may be associated with reinfection with schistosomiasis [11–15]. However, it remains to be determined whether variations in these genes or which of the variations in these genes are potential determinants of reinfection.

We undertook this meta-analysis to identify and describe studies that had identified host determinants, including socio-demographic, epidemiological and immunogenetic factors that are associated with reinfection with schistosomes.

Author Summary

One of the major challenges of schistosomiasis control is that disease prevalence reverts to baseline levels after mass drug administration due to high rate of reinfection. Host factors play a major role in determining resistance or susceptibility to reinfection with schistosomiasis and other diseases. We systematically searched and analyzed studies that identified potential host determinants of reinfection with schistosomes. Among demographic variables, age but not gender was strongly associated with reinfection with schistosomes. Pretreatment infection intensity was also identified as a major determinant of reinfection. Positive association with IgG4 levels and negative association with IgE levels reconfirmed the notion that IgE/IgG4 balance is the major factor controlling protective immunity against schistosomiasis. Other factors were reported by few studies to allow correct inferences. These results contribute to our understanding of host-parasite relationship as it affects reinfection, and will be useful for planning and targeting the limited resources for intervention on high-risk groups.

Methods

Protocol registration

This study was performed in accordance with the recommendations of the PRISMA statement [16,17]. This statement summarized in the PRISMA 2009 checklist is supplied as supplementary information (Figure S1). The protocol for this study was determined prior to commencement of the study, and was registered in PROSPERO-International prospective register of systematic reviews with identification number CRD42013006582 available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID = CRD42013006582.

Eligibility criteria

Studies that assessed host factors of reinfection with schistosomes were included in this review. While all eligible studies on all identified host determinants were included in the qualitative systematic review, only factors reported by more than one study and whose data can be reliably extracted were included in the quantitative meta-analysis. All relevant studies were included irrespective of study type, study design, language and date. We limited included studies to studies performed on human subjects. Reports were excluded if the reported information was on a study performed on animals, if they were case studies, correspondence or reviews, and if the data could not be reliably retrieved. Decisions on eligibility were made by two independent reviewers and all discrepancies and disagreements as regards study and report eligibility were resolved by discussion or consensus with a third reviewer, when necessary.

Information source

Studies analyzed in this review were identified by searching electronic public databases including: PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Scopus (http://www.scopus.com/), Google Scholar (http://scholar.google.com/), Cochrane Review Library (http://www.cochrane.org/cochrane-reviews) and African Journals Online (AJOL) (http://www.ajol.info/index.php/index/search). The searches were performed in October 2013 with no limit set for the dates of publications. Reference lists of eligible articles were also checked to obtain supplementary information and records of potentially relevant studies and reports.

Efforts were made to contact authors for full texts, clarification on data and for supplementary data, when necessary. When responses with the necessary details were not received from authors during the duration of the study and after two reminders, such studies were excluded and classified as "full text not available".

Search strategy

Initial searches were performed on PubMed and Scopus databases using the broad search term: "((reinfection OR re-infection OR resistance OR resistant OR susceptibility OR susceptible OR haplotype OR allele OR SNP OR "single nucleotide polymorphism" OR variant OR polymorphism OR "genetic factors" OR HLA OR "human leucocyte antigen") AND (schistosom* OR bilharzi*))" to retrieve socio-demographic, epidemiological and immunogenetic factors. For Advanced Google Scholar, we filled in the term "schistosoma OR schistosomiasis OR schistosome OR bilharzia OR bilharziasis" in the field "with all of the word", and the words "reinfection OR re-infection OR resistance OR resistant OR susceptibility OR susceptible OR "host factors" OR "genetic factors" OR haplotype OR allele OR SNP OR "single nucleotide polymorphism" OR variant OR polymorphism, in the field "with at least one of the words" to search the titles of articles in Google scholar database. The Cochrane Library and African Journals Online databases were searched with the broad term "schistosoma OR schistosomiasis OR bilharzia OR bilharziasis".

Study selection

Two independent reviewers performed initial eligibility assessments on the retrieved titles and abstracts, for inclusion in the systematic review. Full texts of eligible articles were then retrieved and reviewed for inclusion in the systematic review, and further screened for inclusion in the meta-analysis using the inclusion criteria. In both steps of the screening, inclusion or exclusion of a study by both reviewers was considered conclusive, while inclusion or otherwise of studies judged eligible, controversial or ambiguous by either of the reviewers was resolved by discussion and consensus between the two reviewers. When necessary, disagreements and discrepancies were resolved by consensus with a third reviewer. Care was taken to identify more than one report describing a single study. When such was encountered, the overlap was identified and resolved, with contacts made to the authors when necessary. The study selection procedure was summarized in a systematic review flow chart.

Data collection process and data items

We adopted the methodology and data extraction template of The Review Manager (RevMan v5.2) from The Nordic Cochrane Centre, Cochrane Collaboration, 2012 for data extraction, in addition to other relevant data items as determined by the reviewers. As much as possible, the following pieces of information were obtained from eligible study reports by two independent reviewers, in a non-blinded manner: study ID (lead author name and year), study type, study period, study location, problem addressed (reinfection with schistosomes), species studied, host factors assessed, study aim, recruitment, inclusion, exclusion, informed consent, ethical approval, number of participants, study completion rate, statistical methods and funding. Given that the host factors we identified and reviewed were not set a priori, the factors were included on first observation. Thus, a study assessing several host factors was included respectively in the meta-analysis for each of the factors; while overlap from multiple reports referring to a single study was resolved to avoid duplication. When studies in different locations were separately reported in a single

article, the study was included twice for each of the areas differentiated using footnotes.

Quality assessment of included studies

To assess risk of bias within selected studies, we adopted the quality assessment tool in the Cochrane RevMan v5.2 program. Briefly, this method takes into account four factors: selection bias by evaluating the sampling and randomization procedure, performance bias by assessing the level of blinding of personnel, detection bias by evaluating the level of blinding of outcome assessment, and reporting bias by assessing the presence of selective reporting in data presentation. We created a quality scoring system based on these RevMan v5.2 quality assessment items, with levels of risk of bias scored as "−1" for high risk, "0" for unclear risk and "1" for low risk of bias. These parameters (n = 4), in addition to availability of descriptions of (n = 8): host determinants, outcomes definitions, inclusion criteria, exclusion criteria, method of diagnosis, mass chemotherapy, confirmation of cure prior to inclusion and follow-up period, were used to create a quality score based on 12 items on a scale of 100% for each included study (Table S1).

Assessment of risk of bias across studies

To assess the risk of bias across studies, Begg's funnel plots were generated to assess publication bias across the reviewed studies [18,19]. Funnel plots were created for each factor by plotting the effects measure (odds ratio) against the standard error of its logarithm. The symmetries of the funnel plots were first assessed visually. When potential publication bias was identified, the trim and fill method proposed by Duvall and Tweedie [20] was applied. No further test of bias or symmetry of the funnel was performed since no publication bias was apparent.

Definition of outcomes and risk factors

The outcomes definitions were pre-determined by the reviewers, and all included studies sufficiently satisfy these criteria. Briefly, "resistance to reinfection" was defined as absence of parasite egg in multiple parasitological examinations after treatment (and confirmation of cure), followed by a follow-up period (for uniformity, data on ~12 months follow-up were pooled) despite exposure to the parasite. Conversely, "susceptibility to reinfection" was defined as positive parasitological examination within 6 to 12 months after chemotherapy and cure. Among the risk factors identified, we adopted <10 years old as the definition for younger children. This was because all the included studies defined younger children as either <9 or <10 years old, apart from three studies that defined younger children as <13, < 14 and <15 years old, respectively (Table S1). High (moderate to high) pretreatment infection intensities was defined as >50 eggs/10 ml of urine for *S. haematobium*, and >100 eggs/g of feces for *S. mansoni* and *S. japonicum*. For antibody levels, only data from studies utilizing the predetermined established method (ELISA) were pooled. While slight variations exist in the methods adopted in each study for estimation of antibody levels, these were ignored since similar conditions apply for the comparator in each study. Only data from studies satisfying these definitions were pooled in data synthesis.

Quantitative data synthesis (meta-analyses)

Data from eligible studies were combined using meta-analysis performed on The Review Manager (RevMan v5.2) from The Nordic Cochrane Centre, Cochrane Collaboration, 2012 [21]. We meta-analyzed and interpreted all host factors reported in more

than one studies without setting any cut off for the minimum number of studies required for valid interpretation. For each identified host factor reported as dichotomous outcome, 2×2 contingency tables were generated and the odds ratio (OR) with the corresponding 95% confidence intervals (95% CI) were calculated. For studies reporting continuous outcomes (such as antibody levels), the input data was mean and standard deviation (SD) with the standardized mean difference (SMD) as the effect measure. When the standard deviation was not reported, it was computed with the calculator function in RevMan v5.2, using other supplied data (e.g. mean, SEM, p-value etc.). For each factor analyzed, a forest plot showing the respective odds ratios or standardized mean differences with their corresponding 95% confidence interval for each study and for the pooled data were generated. The test of overall effect was assessed using the Z-statistics on RevMan v5.2 with statistical significance set at $p<0.05$. Subgroup analysis based on species studied was performed when necessary, especially for host factors with sufficient number of included studies.

Test of heterogeneity between studies

Heterogeneity (inconsistency) between studies was evaluated using the Cochrane Q (Chi^2 test) and I^2 statistics in RevMan v5.2 [22]. The statistical significance for heterogeneity using the Chi^2 test was set as $p<0.10$. Estimates of degree of heterogeneity using I^2 were made by setting 25%, 50%, or 75% as limits for low, moderate or high heterogeneity, respectively [22]. The fixed-effects model with weighting of the studies was used when there was a lack of significant heterogeneity ($p>0.10$), while the random-effects model with weighting of the studies was used when there was heterogeneity between studies ($p<0.10$) and I^2 values of over 50%. A major drawback of the random-effects model is that it assigns relatively equal weight to studies. Therefore, fixed-effects model was preferred over random-effects, although random-effects model was still applied when significant heterogeneity was recorded between studies.

Sensitivity analysis

For sensitivity analysis, we adopted the methods recommended for Cochrane systematic reviews. Each meta-analysis of the association of reinfection with a host factor was reanalyzed with the exclusion of each individual study to examine the effect of a single study on the outcome of meta-analysis. In addition, to examine the effect of the largest and smaller studies on the outcome of the meta-analysis, cumulative meta-analysis was performed with studies ordered according to the sample size. Also, sensitivity testing to identify the effect of subgroups was performed by subgroup analysis. This was achieved by comparing the results of the meta-analysis after exclusion of each subgroup.

Results

Study selection

Using the broad search terms, initial screening of public databases yielded 2739 study reports. Out of these studies, 295 were included for full text reading based on initial title and abstract screening using the inclusion criteria. The two reviewers agreed with 284 decisions and 11 discrepancies were resolved by discussion and consensus. For some reasons that are outlined in Figure 1, further 186 study reports were excluded and a total of 109 studies identifying 39 host factors were included for the data synthesis. However, some of the identified host determinants were reported by only 1 study and were further excluded in the final meta-analysis. Finally, 32 study reports on 26 host determinants of

322
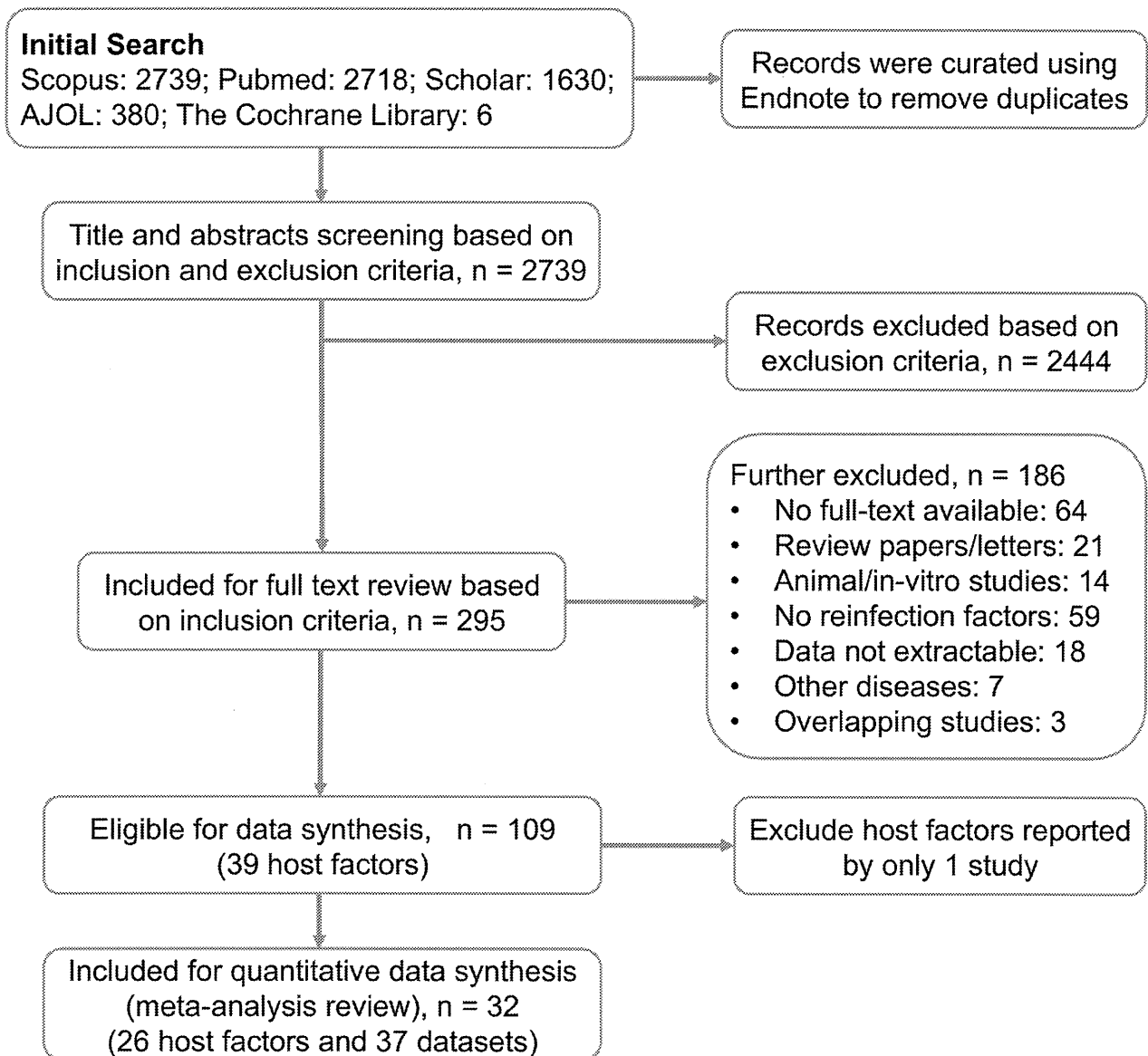
Figure 1. Flow diagram for the search and systematic review process.
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reinfection were included in the final quantitative data synthesis (meta-analysis). Five of these study reports were on two independent data sets [23–27], thus, a total of 37 datasets were included in the meta-analysis (Figure 1).

Characteristics of included studies

The characteristics of the 32 studies included in the meta-analysis were fully described in Table S1. This table outlined the study ID, study location, study period, sample size, gender ratio, age range and species studied. Out of the 32 included studies; 13 were on reinfection with *Schistosoma mansoni*, 7 were on *S. japonicum*, 10 studies assessed reinfection with *S. haematobium*, and 2 studies were on both *S. mansoni* and *S. haematobium*. Based on the population of subjects studied; 20 studies were on both children and adults, 11 studies were on children alone, while only 1 study was on adult subjects alone. All included studies were cohort studies (n = 32), with prospective data collection method (Table S1).

Quality assessment of included studies

Assessment of risk of bias within selected studies was performed using the quality assessment tool in Cochrane RevMan v5.2 program, modified as detailed in the Method section. The result of the quality assessment based on the 12 items on a scale of 100% showed that only 2 studies scored the maximum points (100%). The other included studies scored over 75% points in the study quality assessment, indicating their suitability for inclusion in the meta-analysis (Table S1).

Synthesis of results and meta-analysis

A total of 39 host factors comprising socio-demographic, epidemiological, immunological, genetic variants and other variables were identified from the included studies. However, 13 of these host determinants were reported in only one study and were subsequently excluded in the meta-analysis. The full list of 39 identified host factors with their corresponding statistics and effect

Table 1. Host determinants of reinfection with schistosomes identified in this study.

Host Factors	No Reps	Model	Heterogeneity		Association		Study References
			X^2 (p-val.)	I^2	OR/SMD (95% CI)	Z (p-val.)	
1. Demographic factors							
Age (<10)	19	Rand.	p<0.0001	74	1.91 [1.41, 2.60]	p<0.0001	[24,26,28–42]
Gender (M)	20	Rand.	p<0.0001	88	1.45 [1.02, 2.05]	p=0.04	[24–26,28–32,34–36,39,40,42–46]
2. Epidemiological factors							
PTI	7	Rand.	p=0.006	67	2.85 [1.97, 4.12]	p<0.0001	[26,27,30,36,40]
Exposure	4	Rand.	p<0.0001	91	2.34 [0.93, 5.85]	p=0.07	[36,40,42,44]
HTA	5	Rand.	p<0.0001	91	2.24 [0.63, 7.91]	p=0.21	[37,42,44,47]
3. Antibodies							
· IgE							
SWA	7	Rand.	p<0.0001	88	−0.06 [−0.59, 0.46]	p=0.82	[23,31,39,44,49,53]
SEA	8	Rand.	p=0.001	71	−0.03 [−0.38, 0.32]	p=0.88	[23,31,39,44,48–51]
· IgG4							
SWA	5	Fixed	p=0.38	5	0.47 [0.26, 0.68]	p<0.0001	[23,31,39,44,49]
SEA	9	Rand.	p<0.0001	89	0.41 [−0.13, 0.95]	p=0.14	[23,31,39,44,48–51]
· IgG1							
SWA	4	Rand.	p<0.0001	89	0.71 [0.06, 1.37]	p=0.03	[23,31,45,49]
SEA	5	Rand.	p=0.005	73	0.56 [0.10, 1.03]	p=0.02	[23,31,48,49,51]
· IgG2							
SWA	3	Rand.	p<0.0001	90	0.67 [−0.15, 1.49]	p=0.11	[23,31,49]
SEA	4	Rand.	p<0.0001	92	0.87 [0.02, 1.71]	p=0.04	[23,31,49,51]
· IgG3							
SWA	2	Rand.	p=0.04	77	−0.22 [−0.85, 0.42]	p=0.51	[31,49]
SEA	3	Fixed	p=0.70	0	0.04 [−0.21, 0.29]	p=0.77	[31,49,51]
· IgA							
SWA	3	Rand.	p<0.0001	95	0.50 [−0.67, 1.67]	p=0.40	[23,31,49]
SEA	5	Rand.	p<0.0001	93	0.54 [−0.42, 1.50]	p=0.27	[23,31,48,49,51]
· IgM							
SWA	3	Rand.	p<0.0001	98	1.84 [−1.11, 4.79]	p=0.22	[23,31,49]
SEA	3	Rand.	p<0.0001	96	1.19 [−0.50, 2.89]	p=0.17	[23,31,51]
4. Cytokines							
IFN-g	4	Fixed	p=0.36	1	−0.22 [−0.52, 0.08]	p=0.15	[6,44,45,48]
IL-10	4	Fixed	p=0.08	56	−0.15 [−0.44, 0.13]	p=0.29	[6,44,45,48]
TNF-a	2	Rand.	p=0.03	79	−0.27 [−0.77, 0.22]	p=0.28	[6,51]
IL-5	3	Rand.	p=0.004	82	−0.17 [−1.38, 1.04]	p=0.78	[6,44,48]

Table 1. Cont.

Host Factors	No Reps	Model	Heterogeneity χ^2 (p-val.)	I^2	Association OR/SMD (95% CI)	Z (p-val.)	Study References
5. Immune cell surface marker							
CD4	2	Fixed	p = 0.25	23	-0.62 [-1.05, -0.18]	p = 0.005	[6,81]
CD8	2	Rand.	p = 0.003	89	0.08 [-1.71, 1.86]	p = 0.93	[6,81]
CD19	2	Fixed	p = 0.40	0	0.38 [-0.04, 0.81]	p = 0.08	[6,81]

NB: No = Number of included studies; OR = odds ratio; SMD = standardized mean difference; p-val. = p-value; PTI = Pre-treatment intensity of infection; Exposure = Exposure rate; HTA = High transmission area; SWA = Schistosoma adult worm antigen; SEA = Schistosoma egg antigen.

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estimates were included as supplementary information (Table S2). The summary of the meta-analyses on 26 host factors, including: the number of pooled studies, data analyses model adopted, the tests of heterogeneity, association analyses and the study references were presented in Table 1. Several demographic, epidemiological and immunological variables were identified, with some of the variables showing strong association with reinfection with schistosomes. These are further described in subsequent sections.

Association of demographic factors with reinfection with schistosomes

1. Age (<10 years old). Age was identified as a major factor that may predispose certain subsets of the population to reinfection with schistosomes. In this review, we assessed the odds of reinfection among children less than 10 years old as compared to the rest of the population (Figure 2). Significant heterogeneity was observed among the included studies (p<0.0001, $I^2 = 74\%$), therefore, random effects model was applied for the meta-analysis. This heterogeneity was probably due to the variability of the studied populations. While some of the studies were on school-aged children (5–18 years range), others included the whole population (Table S1). Pooled odds ratio showed that younger age (<10 years old) was positively associated with reinfection with schistosomes (OR = 1.91, 95% CI = 1.41–2.60, Z = 4.15, p<0.0001) (Figure 2).

Sensitivity analysis by analysis of subgroups based on the species studied showed that while reinfection with S. mansoni [28–32] and S. haematobium [26,33–40] showed strong association with age, such association was not observed when only studies on S. japonicum [24,41,42] were considered (Figure 2). The positive association between reinfection and age (<10 years old) lost its statistical significance (p = 0.13) when all the studies on S. haematobium subgroup were excluded from the meta-analysis (Table S3). Also, sensitivity analysis by exclusion of a single study from the analysis (Table S3) or cumulative meta-analysis (Table S4) showed that the result of the meta-analysis was robust as the inclusion or exclusion of any single study did not affect the outcome of the odds ratio, Z-score and p-value.

2. Gender (male). We also assessed whether gender (being male) was a predisposing factor of reinfection with schistosomes. Because there was significant heterogeneity among the studies (p< 0.0001, $I^2 = 88\%$), random effects model was applied. Pooled odds ratio showed that although there was a positive association of male gender and reinfection with schistosomes, the association was only slightly statistically significant (OR = 1.45, 95% CI = 1.02–2.05, Z = 2.09, p = 0.04). Sensitivity analysis by subgroup analysis based on the species studied showed that statistically significant positive association was observed for association of male gender with reinfection with S. mansoni (p = 0.02) [25,28–32,43–45] and S. japonicum (p = 0.002) [24,42,46], while the association for reinfection with S. haematobium [26,34–36,39,40] was not statistically significant (p = 0.30) (Figure 3). The exclusion of S. mansoni or S. japonicum subgroups in the meta-analysis yielded effect measures which were not statistically significant (p = 0.22 and p = 0.23, respectively). Conversely, the exclusion of data from S. haematobium subgroup significantly affected the result of the meta-analysis (OR = 1.85, 95% CI = 1.34–2.55, Z = 3.76, p = 0.0002) (Table S3). Furthermore, sensitivity analyses by exclusion of single studies (Table S3) and cumulative meta-analysis (Table S4) showed that two large studies [26,34] significantly affected the result of the pooled effect estimate (Z-score and p-value).

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M–H, Random, 95% CI	Odds Ratio M–H, Random, 95% CI
1.1.1 S. mansoni							
Pinot 2010	7	67	8	96	4.3%	1.28 [0.44, 3.73]	
Tukahebwa 2013	61	127	80	249	7.4%	1.95 [1.26, 3.03]	
Caldas 2000	24	57	23	106	6.0%	2.62 [1.30, 5.28]	
Roberts 1993	44	80	1	9	1.7%	9.78 [1.17, 81.87]	
Pinot 2013	72	144	1	11	1.7%	10.00 [1.25, 80.16]	
Subtotal (95% CI)		475		471	21.1%	2.33 [1.44, 3.77]	
Total events	208		113				

Heterogeneity: Tau^2 = 0.08; Chi^2 = 5.54, df = 4 (P = 0.24); I^2 = 28%
Test for overall effect: Z = 3.44 (P = 0.0006)

1.1.2 S. japonicum							
Zhang 1997	11	48	49	123	5.7%	0.45 [0.21, 0.96]	
Wu 1993	42	179	147	561	7.7%	0.86 [0.58, 1.28]	
Acosta 2002 (1)	5	46	8	84	3.8%	1.16 [0.36, 3.77]	
Acosta 2002 (2)	6	52	8	87	4.0%	1.29 [0.42, 3.94]	
Subtotal (95% CI)		325		855	21.2%	0.81 [0.55, 1.17]	
Total events	64		212				

Heterogeneity: Tau^2 = 0.02; Chi^2 = 3.41, df = 3 (P = 0.33); I^2 = 12%
Test for overall effect: Z = 1.12 (P = 0.26)

1.1.3 S. haematobium							
Medhat 1998	12	43	19	58	5.2%	0.79 [0.34, 1.88]	
Saathoff 2004	78	134	365	662	7.7%	1.13 [0.78, 1.65]	
Hagan 1991	14	38	16	74	5.2%	2.11 [0.89, 5.00]	
Satayathum 2006	99	171	736	1918	8.0%	2.21 [1.61, 3.03]	
King 1988 (3)	72	120	200	500	7.6%	2.25 [1.50, 3.38]	
Ofoezie 2000	22	32	26	55	4.9%	2.45 [0.98, 6.13]	
King 1988 (4)	64	101	212	520	7.4%	2.51 [1.62, 3.91]	
Etard 1995	25	54	61	414	6.6%	4.99 [2.74, 9.09]	
Mutapi 1999	27	31	61	112	4.1%	5.64 [1.85, 17.19]	
Grogan 1997	33	33	11	24	1.0%	78.65 [4.32, 1430.96]	
Subtotal (95% CI)		757		4337	57.7%	2.32 [1.61, 3.35]	
Total events	446		1707				

Heterogeneity: Tau^2 = 0.21; Chi^2 = 33.21, df = 9 (P = 0.0001); I^2 = 73%
Test for overall effect: Z = 4.50 (P < 0.00001)

Total (95% CI)		1557		5663	100.0%	1.91 [1.41, 2.60]	
Total events	718		2032				

Heterogeneity: Tau^2 = 0.28; Chi^2 = 70.09, df = 18 (P < 0.00001); I^2 = 74%
Test for overall effect: Z = 4.15 (P < 0.0001)
Test for subgroup differences: Chi^2 = 19.09, df = 2 (P < 0.0001), I^2 = 89.5%
(1) 1994
(2) 1995
(3) Metrifonate
(4) Praziquantel

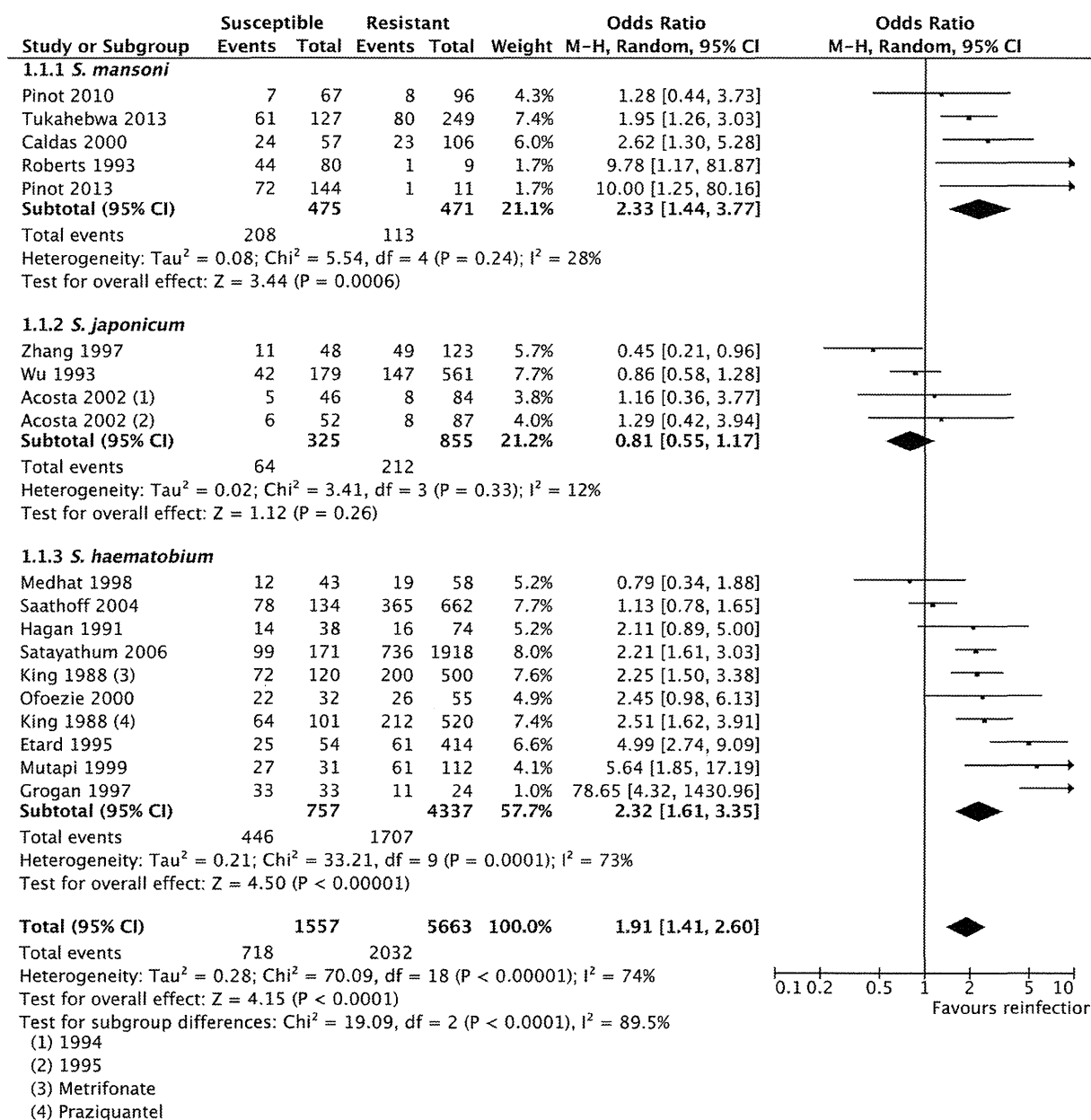0.1 0.2   0.5   1   2   5   10
Favours reinfection

Figure 2. Association of younger age (<10 years old) with reinfection with schistosomes. Presented here is the meta-analysis forest plot showing the pooled odds ratio and the corresponding 95% CI, subgroup analysis by species, and assessment of heterogeneity among studies. There was a strong statistically significant positive association between younger age (<10 years old) and reinfection with schistosomes. doi:10.1371/journal.pntd.0003164.g002

Association of epidemiological factors with reinfection with schistosomes

Three major epidemiological factors of reinfection were identified, including: pre-treatment infection intensity [26,27,30,36,40], rate of exposure [36,40,42,44] and levels of transmission in the studied area [37,42,44,47]. There was strong positive association between high (moderate to high) pretreatment infection intensities (>50 eggs/10 ml of urine for S. haematobium, and >100 eggs/g of feces for S. mansoni and S. japonicum) and reinfection with schistosomes (OR = 2.85, 95% CI = 1.97–4.12, Z = 5.57, $p<0.0001$). (Figure 4A). The positive association between high rates of exposure (we defined high rate of exposure as "above average" exposure rate for a specific population as determined by authors) and reinfection with schistosomes was not statistically significant (OR = 2.34, 95% CI: 0.93–5.85, Z = 1.81, $p = 0.07$) (Figure 4B). Although there was also a positive correlation between residence in high transmission area and reinfection, the association was not statistically significant (OR = 2.24, 95% CI = 0.63–7.91, Z = 1.25, $p = 0.21$) (Figure 4C).

Association of immunological factors with reinfection with schistosomes

Among the immunological factors identified in this review, most studies reported association between humoral responses and probability of reinfection with schistosomes. An interesting finding was a negative standardized mean difference observed in the
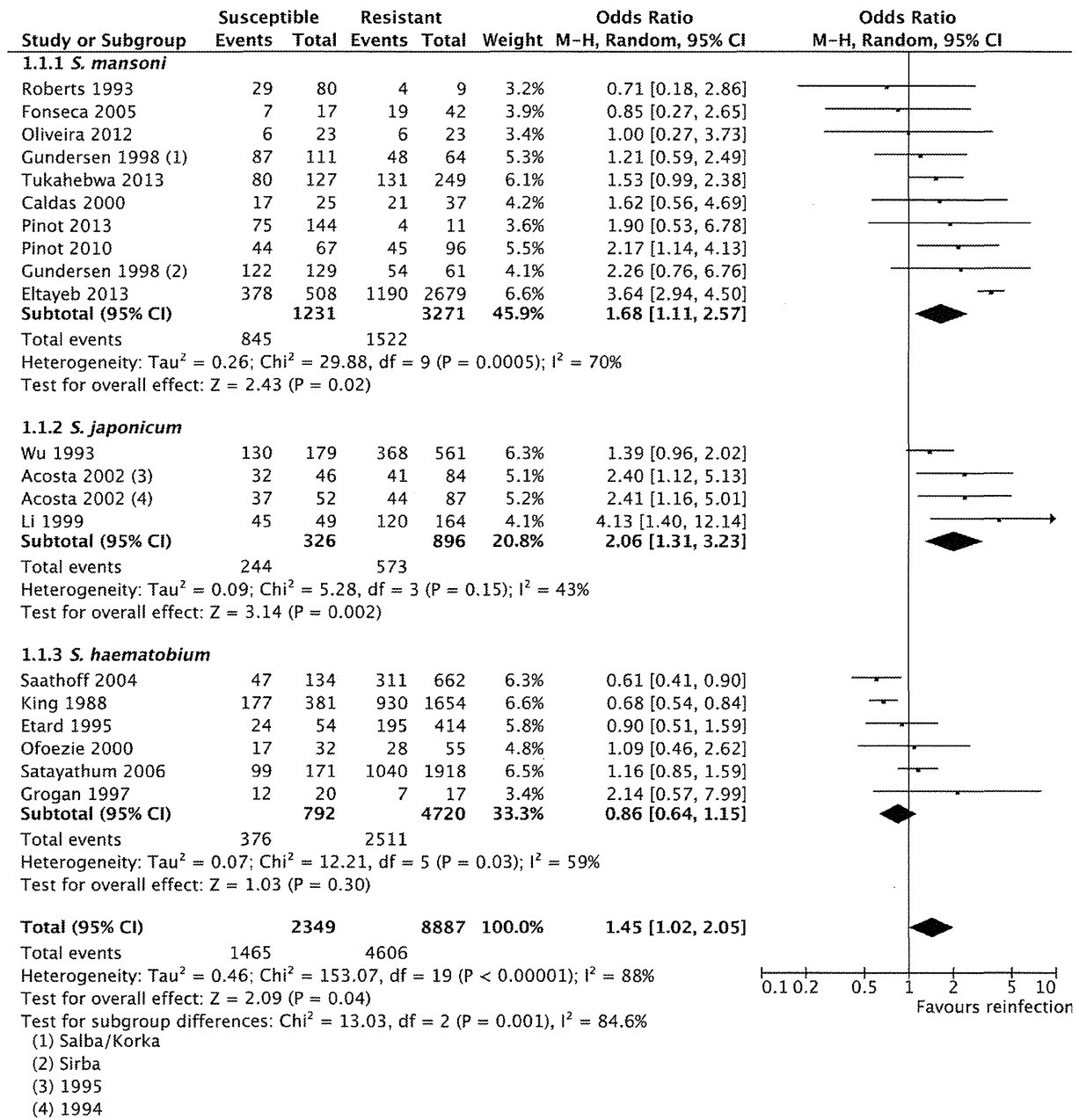
326

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M–H, Random, 95% CI	Odds Ratio M–H, Random, 95% CI
1.1.1 S. mansoni							
Roberts 1993	29	80	4	9	3.2%	0.71 [0.18, 2.86]	
Fonseca 2005	7	17	19	42	3.9%	0.85 [0.27, 2.65]	
Oliveira 2012	6	23	6	23	3.4%	1.00 [0.27, 3.73]	
Gundersen 1998 (1)	87	111	48	64	5.3%	1.21 [0.59, 2.49]	
Tukahebwa 2013	80	127	131	249	6.1%	1.53 [0.99, 2.38]	
Caldas 2000	17	25	21	37	4.2%	1.62 [0.56, 4.69]	
Pinot 2013	75	144	4	11	3.6%	1.90 [0.53, 6.78]	
Pinot 2010	44	67	45	96	5.5%	2.17 [1.14, 4.13]	
Gundersen 1998 (2)	122	129	54	61	4.1%	2.26 [0.76, 6.76]	
Eltayeb 2013	378	508	1190	2679	6.6%	3.64 [2.94, 4.50]	
Subtotal (95% CI)		1231		3271	45.9%	1.68 [1.11, 2.57]	
Total events	845		1522				

Heterogeneity: Tau² = 0.26; Chi² = 29.88, df = 9 (P = 0.0005); I² = 70%
Test for overall effect: Z = 2.43 (P = 0.02)

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M–H, Random, 95% CI	Odds Ratio M–H, Random, 95% CI
1.1.2 S. japonicum							
Wu 1993	130	179	368	561	6.3%	1.39 [0.96, 2.02]	
Acosta 2002 (3)	32	46	41	84	5.1%	2.40 [1.12, 5.13]	
Acosta 2002 (4)	37	52	44	87	5.2%	2.41 [1.16, 5.01]	
Li 1999	45	49	120	164	4.1%	4.13 [1.40, 12.14]	
Subtotal (95% CI)		326		896	20.8%	2.06 [1.31, 3.23]	
Total events	244		573				

Heterogeneity: Tau² = 0.09; Chi² = 5.28, df = 3 (P = 0.15); I² = 43%
Test for overall effect: Z = 3.14 (P = 0.002)

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M–H, Random, 95% CI	Odds Ratio M–H, Random, 95% CI
1.1.3 S. haematobium							
Saathoff 2004	47	134	311	662	6.3%	0.61 [0.41, 0.90]	
King 1988	177	381	930	1654	6.6%	0.68 [0.54, 0.84]	
Etard 1995	24	54	195	414	5.8%	0.90 [0.51, 1.59]	
Ofoezie 2000	17	32	28	55	4.8%	1.09 [0.46, 2.62]	
Satayathum 2006	99	171	1040	1918	6.5%	1.16 [0.85, 1.59]	
Grogan 1997	12	20	7	17	3.4%	2.14 [0.57, 7.99]	
Subtotal (95% CI)		792		4720	33.3%	0.86 [0.64, 1.15]	
Total events	376		2511				

Heterogeneity: Tau² = 0.07; Chi² = 12.21, df = 5 (P = 0.03); I² = 59%
Test for overall effect: Z = 1.03 (P = 0.30)

Total (95% CI)		2349		8887	100.0%	1.45 [1.02, 2.05]	
Total events	1465		4606				

Heterogeneity: Tau² = 0.46; Chi² = 153.07, df = 19 (P < 0.00001); I² = 88%
Test for overall effect: Z = 2.09 (P = 0.04)
Test for subgroup differences: Chi² = 13.03, df = 2 (P = 0.001), I² = 84.6%
(1) Salba/Korka
(2) Sirba
(3) 1995
(4) 1994

0.1 0.2   0.5   1   2   5   10
Favours reinfection

Figure 3. Association of gender (male) with reinfection with schistosomes. Presented here is the meta-analysis forest plot showing the pooled odds ratio and the corresponding 95% CI, subgroup analysis by species, and assessment of heterogeneity among studies. The observed positive association between reinfection and gender was only slightly significant.
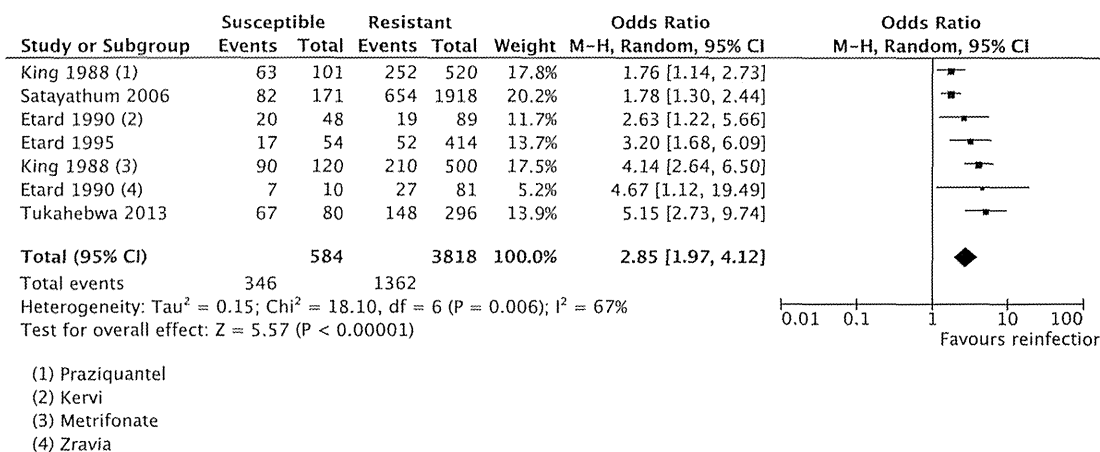doi:10.1371/journal.pntd.0003164.g003

association between IgE levels and reinfection with schistosomes (Figure 5 and Figure S2) inferred from meta-analysis on 8 studies [23,31,39,44,48–51]. However, subgroup analyses of these associations with IgE levels against adult worm antigen (SWA) (Figure 5A) and egg antigen (SEA) (Figure 5B) were not statistically significant (For anti-SWA IgE, SMD = −0.06, 95% CI = −0.59–0.46, Z = 0.23, p = 0.82; for anti-SEA IgE, SMD = −0.03, 95% CI = −0.38–0.32, Z = 0.15, p = 0.88). Sensitivity analysis by exclusion of individual studies showed that the exclusion of any of the included studies in this meta-analysis did not affect the pooled effect estimates (Table S3).

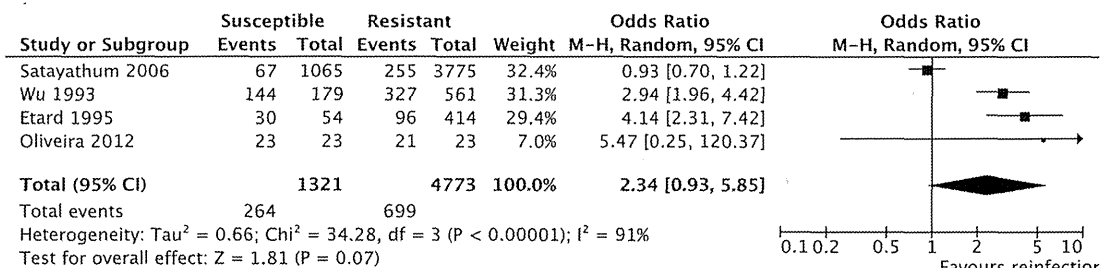Conversely, strong positive association was observed between IgG4 levels and reinfection with schistosomes (Figure 6 and Figure S2). However, while the association between reinfection and anti-SWA IgG4 levels was statistically significant (SMD = 0.47, 95% CI = 0.26–0.68, Z = 4.35, p<0.0001) (Figure 6A); the association between reinfection and anti-SEA IgG4 levels was not statistically significant (SMD = 0.41, 95% CI = −0.13–0.95, Z = 1.48, p = 0.14) (Figure 6B). Sensitivity analysis showed that while the exclusion of any single study did not affect the result of the association between reinfection with schistosomes and anti-SWA IgG4 levels, the exclusion of one study [39] resulted in positive association with statistical significance for the association of anti-SEA IgG4 with reinfection with schistosomes (Table S3).

Equally, positive associations were observed for association of reinfection with schistosomes with levels of IgG1, IgG2, IgA and
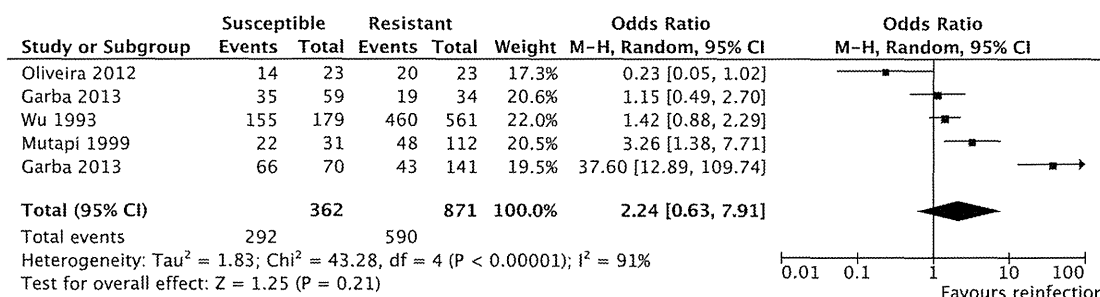
A

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
King 1988 (1)	63	101	252	520	17.8%	1.76 [1.14, 2.73]	
Satayathum 2006	82	171	654	1918	20.2%	1.78 [1.30, 2.44]	
Etard 1990 (2)	20	48	19	89	11.7%	2.63 [1.22, 5.66]	
Etard 1995	17	54	52	414	13.7%	3.20 [1.68, 6.09]	
King 1988 (3)	90	120	210	500	17.5%	4.14 [2.64, 6.50]	
Etard 1990 (4)	7	10	27	81	5.2%	4.67 [1.12, 19.49]	
Tukahebwa 2013	67	80	148	296	13.9%	5.15 [2.73, 9.74]	
Total (95% CI)		584		3818	100.0%	2.85 [1.97, 4.12]	
Total events	346		1362				

Heterogeneity: Tau^2 = 0.15; Chi^2 = 18.10, df = 6 (P = 0.006); I^2 = 67%
Test for overall effect: Z = 5.57 (P < 0.00001)

0.01 0.1 1 10 100
Favours reinfection

(1) Praziquantel
(2) Kervi
(3) Metrifonate
(4) Zravia

B

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
Satayathum 2006	67	1065	255	3775	32.4%	0.93 [0.70, 1.22]	
Wu 1993	144	179	327	561	31.3%	2.94 [1.96, 4.42]	
Etard 1995	30	54	96	414	29.4%	4.14 [2.31, 7.42]	
Oliveira 2012	23	23	21	23	7.0%	5.47 [0.25, 120.37]	
Total (95% CI)		1321		4773	100.0%	2.34 [0.93, 5.85]	
Total events	264		699				

Heterogeneity: Tau^2 = 0.66; Chi^2 = 34.28, df = 3 (P < 0.00001); I^2 = 91%
Test for overall effect: Z = 1.81 (P = 0.07)

0.1 0.2 0.5 1 2 5 10
Favours reinfection

C

Study or Subgroup	Susceptible Events	Total	Resistant Events	Total	Weight	Odds Ratio M-H, Random, 95% CI	Odds Ratio M-H, Random, 95% CI
Oliveira 2012	14	23	20	23	17.3%	0.23 [0.05, 1.02]	
Garba 2013	35	59	19	34	20.6%	1.15 [0.49, 2.70]	
Wu 1993	155	179	460	561	22.0%	1.42 [0.88, 2.29]	
Mutapi 1999	22	31	48	112	20.5%	3.26 [1.38, 7.71]	
Garba 2013	66	70	43	141	19.5%	37.60 [12.89, 109.74]	
Total (95% CI)		362		871	100.0%	2.24 [0.63, 7.91]	
Total events	292		590				

Heterogeneity: Tau^2 = 1.83; Chi^2 = 43.28, df = 4 (P < 0.00001); I^2 = 91%
Test for overall effect: Z = 1.25 (P = 0.21)

0.01 0.1 1 10 100
Favours reinfection

Figure 4. Association of epidemiological factors with reinfection with schistosomes. (A) Meta-analysis forest plot for the association of reinfection with high pretreatment intensity showing positive association with reinfection. (B) Forest plot for the association of reinfection with high rate of exposure showing association with reinfection without statistical significance. (C) Meta-analysis forest plot for the association of reinfection with residence in high transmission area did not show statistically significant association with reinfection.
doi:10.1371/journal.pntd.0003164.g004

IgM against SWA (Figure S3A, B, D and E) and SEA (Figure S4A, B, D and E); while negative association was observed between reinfection and levels of IgG3 (Figure S3C and Figure S4C). However, there were limited number of studies reporting these factors and the associations were not statistically significant except for IgG1.

Some cellular immune response factors were also identified. However, there were consistently small number of studies reporting these factors, and the associations were not statistically significant (Table 1 and Table S2). We observed negative standardized mean differences from the associations of reinfection with levels of IFN-γ, IL-5, IL-10, IL-13, TNF-α and CD4$^+$ T-helper cells; and positive effect estimates from the association of reinfection with proportions of CD8, CD19 and CD16 positive cells. Other factors including the HLA gene polymorphisms, levels of total protein, albumin, total cholesterol, low-density lipoproteins and very low-density lipoproteins were also identified. However, these factors were all reported by only one study (Table S2).

Risk of bias across studies

To assess outcome reporting bias and publication bias across studies, we generated funnel plots for two representative host
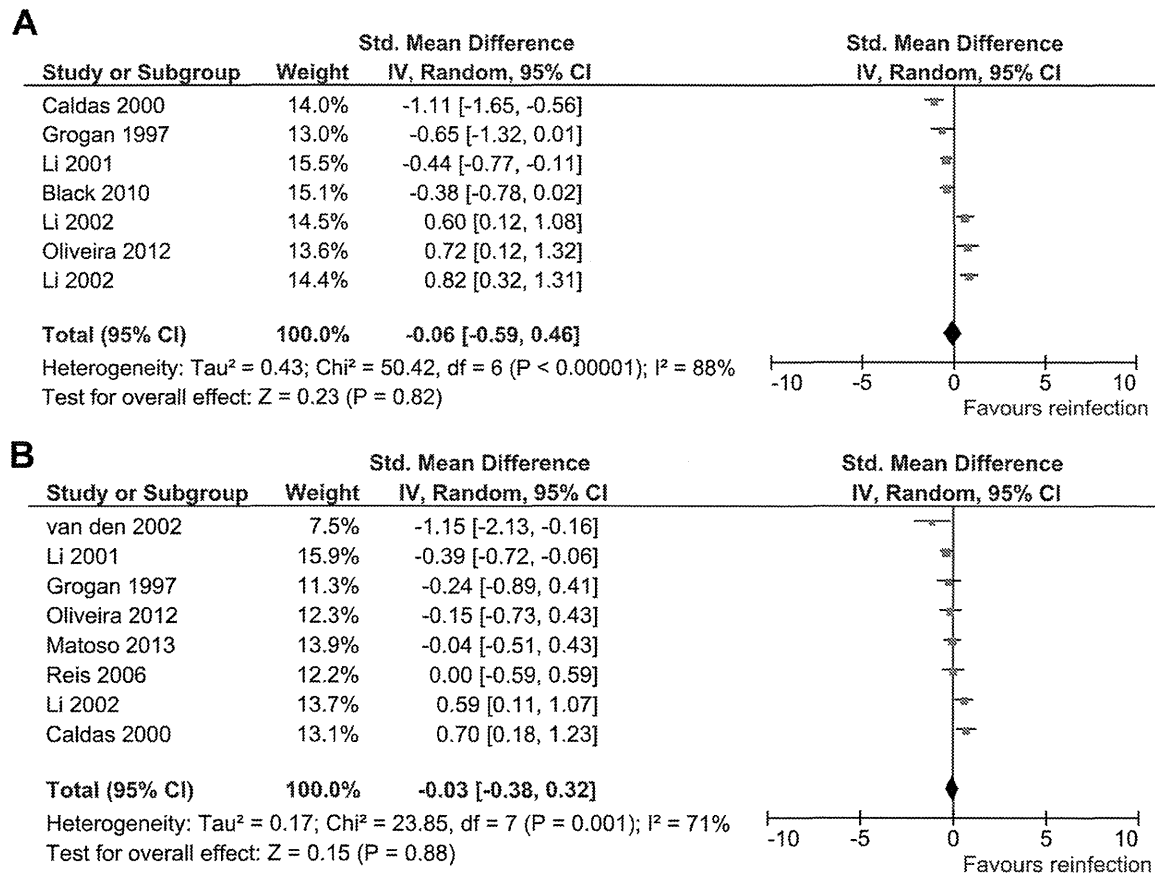
A

Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Caldas 2000	14.0%	-1.11 [-1.65, -0.56]
Grogan 1997	13.0%	-0.65 [-1.32, 0.01]
Li 2001	15.5%	-0.44 [-0.77, -0.11]
Black 2010	15.1%	-0.38 [-0.78, 0.02]
Li 2002	14.5%	0.60 [0.12, 1.08]
Oliveira 2012	13.6%	0.72 [0.12, 1.32]
Li 2002	14.4%	0.82 [0.32, 1.31]
Total (95% CI)	100.0%	-0.06 [-0.59, 0.46]

Heterogeneity: Tau^2 = 0.43; Chi^2 = 50.42, df = 6 (P < 0.00001); I^2 = 88%
Test for overall effect: Z = 0.23 (P = 0.82)

Std. Mean Difference IV, Random, 95% CI

-10   -5   0   5   10
Favours reinfection

B

Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
van den 2002	7.5%	-1.15 [-2.13, -0.16]
Li 2001	15.9%	-0.39 [-0.72, -0.06]
Grogan 1997	11.3%	-0.24 [-0.89, 0.41]
Oliveira 2012	12.3%	-0.15 [-0.73, 0.43]
Matoso 2013	13.9%	-0.04 [-0.51, 0.43]
Reis 2006	12.2%	0.00 [-0.59, 0.59]
Li 2002	13.7%	0.59 [0.11, 1.07]
Caldas 2000	13.1%	0.70 [0.18, 1.23]
Total (95% CI)	100.0%	-0.03 [-0.38, 0.32]

Heterogeneity: Tau^2 = 0.17; Chi^2 = 23.85, df = 7 (P = 0.001); I^2 = 71%
Test for overall effect: Z = 0.15 (P = 0.88)

Std. Mean Difference IV, Random, 95% CI

-10   -5   0   5   10
Favours reinfection

Figure 5. Association of IgE levels with reinfection with schistosomes. (A) Meta-analysis forest plot for the association of reinfection with anti-SWA IgE levels showing the pooled standardized mean difference and the corresponding 95% CI and assessment of heterogeneity among studies. The observed negative overall effect was not statistically significant. (B) Meta-analysis forest plot for the association of reinfection with anti-SEA IgE levels showing the pooled standardized mean difference and the corresponding 95% CI and assessment of heterogeneity among studies. The observed negative overall effect was not statistically significant.
doi:10.1371/journal.pntd.0003164.g005

factors with sufficient number of included studies (age and gender) by plotting the odds ratio (OR) on the x-axis, and the standard error of the log of odds ratio (SE(log[OR]) on the y-axis (Figure 7). The funnel plot showed the typical cone appearance with good symmetry, with the studies apparently distributed on either side of the pooled outcome effect estimate (Figure 7). Two outlier studies [29,32] observed from the association of reinfection with age were of significantly small size (Figure 7A). To exclude potential small study effect, these studies were excluded and their exclusion did not affect the result of the pooled effect size. Equally, we also applied the trim and fill method proposed by Duvall and Tweedie [20] by adding studies equivalent to these outliers, which appear to be missing. Again, this did not affect the symmetry and the result of the pooled effect estimate. Also, subgroup analysis showed that all studies on S. japonicum were on the left side of the cone unlike the other species. However, exclusion of this subgroup (Table S3), or trim and fill method did not affect the results of the combined effect estimates. These indicate that there is minimal publication bias in these studies and no further test of bias was carried out.

Discussion

Identification of host factors that predispose certain subsets of the population to reinfection with schistosomes, and indeed any other disease, is a major strategy that will guide planning and

tailoring of community interventions to target high-risk groups. It is also important for targeting health education and limited resources for disease prevention. Our meta-analysis has identified some of the host determinants of reinfection with schistosomes. The outcomes showed strong positive association with age, and pretreatment intensity of infection, and only slight association with gender. Also, the IgE/IgG4 balance, which is well recognized as a major determinant of reinfection [2–5], was again reconfirmed by our meta-analysis. Our results showed that younger age and pretreatment intensity of infection, which are connected with behavioral differences in population subsets and disease transmission, play predominant role in determining the probability of reinfection with schistosomes. This is due to differences in rate of exposure, but not necessarily absence of protective immunity [52]. Conversely, the immunological parameters related with protective immunity, which may itself be associated with age and accumulated experience [40,52–56]; also play major role in determining protection from infection but not exposure to the pathogen. It can therefore be inferred that exposure and age-related factors play a predominant role in disease transmission, while immunological factors control protective immunity against reinfection.

Younger age was positively associated with the rate of reinfection. Schistosomes are transmitted through skin penetration by the infective cercariae during water contact activities. Given that younger children are mainly involved in such high-risk water
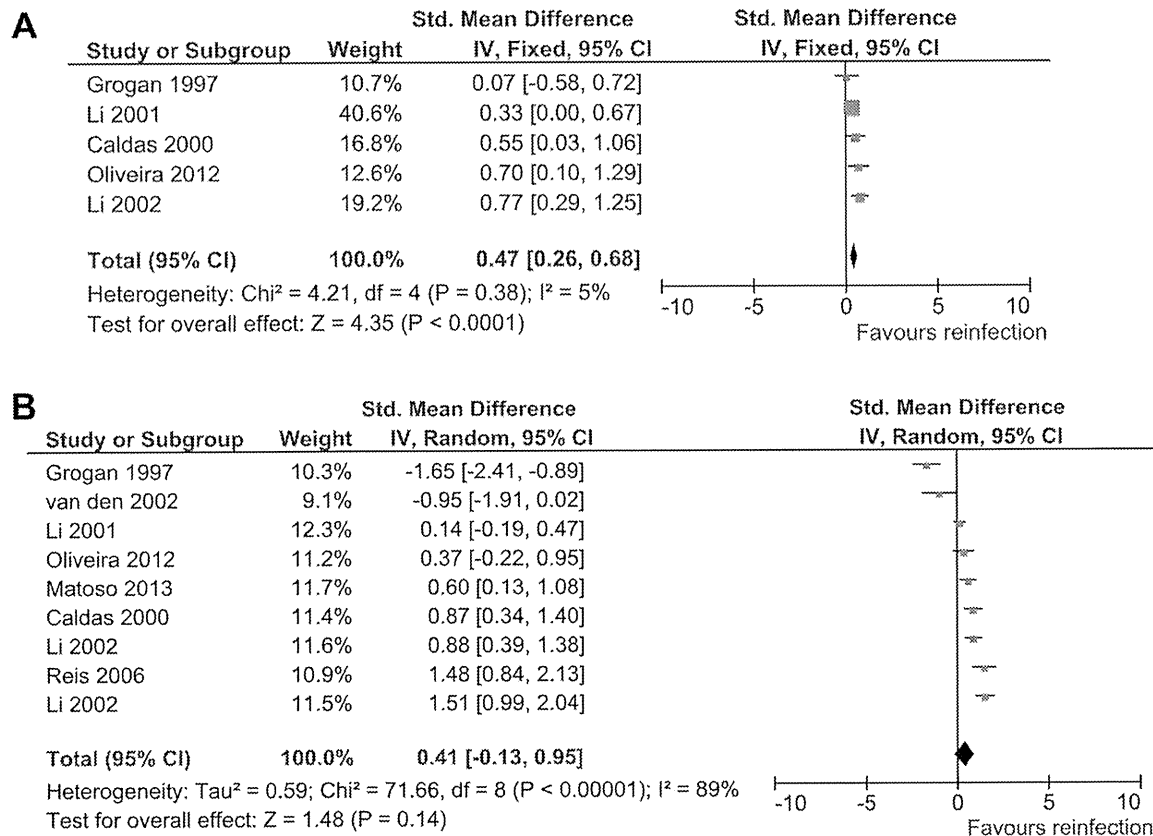
A

Study or Subgroup	Weight	Std. Mean Difference IV, Fixed, 95% CI
Grogan 1997	10.7%	0.07 [-0.58, 0.72]
Li 2001	40.6%	0.33 [0.00, 0.67]
Caldas 2000	16.8%	0.55 [0.03, 1.06]
Oliveira 2012	12.6%	0.70 [0.10, 1.29]
Li 2002	19.2%	0.77 [0.29, 1.25]
Total (95% CI)	**100.0%**	**0.47 [0.26, 0.68]**

Heterogeneity: Chi² = 4.21, df = 4 (P = 0.38); I² = 5%
Test for overall effect: Z = 4.35 (P < 0.0001)

Std. Mean Difference IV, Fixed, 95% CI
-10 -5 0 5 10
Favours reinfection

B

Study or Subgroup	Weight	Std. Mean Difference IV, Random, 95% CI
Grogan 1997	10.3%	-1.65 [-2.41, -0.89]
van den 2002	9.1%	-0.95 [-1.91, 0.02]
Li 2001	12.3%	0.14 [-0.19, 0.47]
Oliveira 2012	11.2%	0.37 [-0.22, 0.95]
Matoso 2013	11.7%	0.60 [0.13, 1.08]
Caldas 2000	11.4%	0.87 [0.34, 1.40]
Li 2002	11.6%	0.88 [0.39, 1.38]
Reis 2006	10.9%	1.48 [0.84, 2.13]
Li 2002	11.5%	1.51 [0.99, 2.04]
Total (95% CI)	**100.0%**	**0.41 [-0.13, 0.95]**

Heterogeneity: Tau² = 0.59; Chi² = 71.66, df = 8 (P < 0.00001); I² = 89%
Test for overall effect: Z = 1.48 (P = 0.14)

Std. Mean Difference IV, Random, 95% CI
-10 -5 0 5 10
Favours reinfection

Figure 6. Association of IgG4 levels with reinfection with schistosomes. (A) Meta-analysis forest plot for the association of reinfection with anti-SWA IgG4 levels showing the pooled standardized mean difference and the corresponding 95% CI and assessment of heterogeneity among studies. IgG4 level was highly significantly associated with reinfection with schistosomes. (B) Meta-analysis forest plot for the association of reinfection with anti-SEA IgG4 levels showing the pooled standardized mean difference and the corresponding 95% CI and assessment of heterogeneity among studies. The observed positive overall effect was not statistically significant.
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contact activities like domestic chores and recreation, this result is expected and is consistent with the results of other big intervention studies [26,30,34,36]. However, species based subgroup analysis did not show positive association between age and reinfection with *S. japonicum*. It is not clear whether this is related to differences in the study cultural settings since unlike *S. haematobium* and *S. mansoni*; the distribution of *S. japonicum* is limited to South East Asia. However, limited number of studies assessed reinfection with *S. japonicum*, and unlike studies on the other species which sometimes involved only children, all studies on *S. japonicum* involved both adults and children; a major source of heterogeneity among the included studies. Also, *S. japonicum* has some peculiarities that may also contribute to the observed heterogeneity, including: its zoonotic nature, the generally much lower prevalence (especially in recent decades) and the fact that exposure is often mainly during occupational activities instead of domestic and recreational ones.

Our analyses showed only slight association between gender and reinfection with schistosomes. Although boys and girls may have major behavioral but not biological differences that can affect rate of reinfection [28], this distinction is very minimal among the younger age group. Even among the older children, there are only changes in the kind of water contact activities, which may not necessarily translate to major change in rate of exposure to the disease. Surprisingly, subgroup analysis showed strong positive association between gender and reinfection with *S. japonicum*.

Although this may be due to differences in gender role in various cultural settings, the observed association may not be very reliable since the analysis was based on only four studies on *S. japonicum*. Also on cultural differences, Fulford *et al.* (1996) and other workers identified that patterns of water contact vary dramatically between even culturally rather similar communities [57,58]. Therefore, absence of strong association with gender even with apparent behavioral differences between genders remains inconclusive. This implies that gender difference in reinfection pattern varies in difference cultural settings [58].

Apart from the demographic factors, three major epidemiological factors were positively correlated with reinfection: high pretreatment intensity, high rate of exposure and residence in high transmission area. High pretreatment intensity is related to possibility of failed or incomplete treatment [36], especially when studies did not include a follow up study dedicated to confirming cure in the treated population. As would be expected, there was also a positive correlation between reinfection and high exposure rate as inferred from the four studies assessing this factor, but the association was not statistically significant. Although some studies distinguished between high transmission areas and low transmission areas based on relative availability of potable water and sanitation [36], there was no association between residence in either of these areas, and rate of reinfection with schistosomes. This could be because water contact activities do not depend exclusively on lack of domestic water or sanitation,
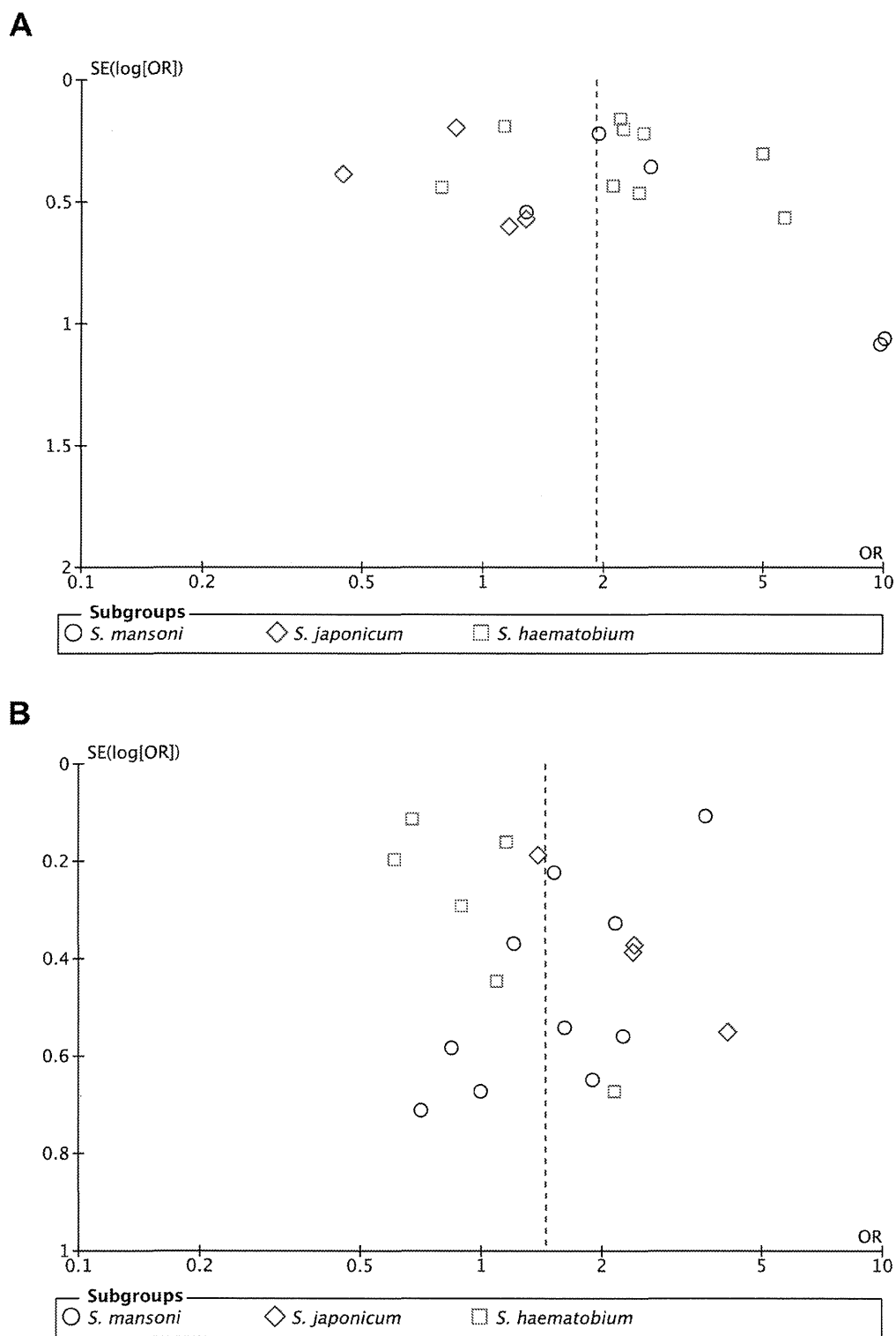
330

A

B

Figure 7. Funnel plots for assessment of publication bias. (A) Funnel plots for assessment of publication bias for studies assessing association of reinfection with age showing lack of significant publication bias in the included studies. (B) Funnel plots for assessment of publication bias for gender.
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but is a function of several interrelated factors, including: perception, distinct cultural practices and the need for recreation. These render residence in either high or low transmission areas less important as a risk factor of reinfection with schistosomes.

Among the immunological factors identified, most studies reported association between different antibody isotype levels and probability of reinfection with schistosomes. Interestingly, a negative association was recorded from the association between IgE levels and reinfection with schistosomes. On the other hand, strong positive association was observed between IgG4 levels and reinfection with schistosomes. These observations are consistent with the consensus perspective that IgE/IgG4 balance plays central role in controlling protective immunity against infection or reinfection with schistosomes [2–5,8,31,38,39,41,44,56,59–63]. While increased IgE levels are protective against infection and reinfection, elevated IgG4 levels increase predisposition to infection and reinfection. Recent studies have found strong association between IgE levels and certain loci in the human genome, including: the cytokine gene cluster on chromosome 5q31-q33 (SM1), which also controls infection with schistosomiasis [7,64–67]; FCER1A on chromosome 1q23, which is the gene encoding the alpha chain of the high affinity receptor for IgE [11,14]; STAT4 on chromosome 2q32 [12,64] which controls Th1 development; STAT6 on chromosome 12q13 [12,13,15,64] and GATA3 on chromosome 10p15 [12,64] which control Th2 development; and the Th2 cytokine receptor cluster in 16p12 region of the human genome [7,64]. We had expected to identify studies assessing association between reinfection and several immunogenetic factors including variations in these loci; however, there were few or no studies on the host immunogenetic factors of reinfection with schistosomes, an important theme for further research. We are presently proposing a study that will identify association between major single nucleotide polymorphisms (SNPs) in these loci, and reinfection with schistosomes and other helminthic infections.

Our analyses on cellular immune response factors showed negative effect estimates for the associations of reinfection with levels of IFN-γ, IL-5, IL-10, TNF-α and CD4$^+$ T-helper cells; and positive effect estimates for the association of reinfection with the proportions of CD8$^+$, CD19$^+$ and CD16$^+$ cells. However, these inferences are not very reliable since there was consistently limited number of studies reporting these host factors, and the associations were not statistically significant (Table 1 and Table S2). An interesting observation though is the negative effect estimate recorded from the association of reinfection with schistosomes with levels of IFN-γ. Studies in both human and animal models have shown that protective immunity against schistosomiasis is mainly Th1 dependent [2,4,6,68–75]. The egg antigen drives a dominant Th2 response. Thus, induction and sustenance of a Th1 environment at the acute phase of infection thru onset of egg deposition is required for sterile and anti-pathology protection [68,69,75–78]. Negative effect estimates were also recorded from the association of reinfection with levels of IL-5 and IL-13. This is consistent with the notion that these cytokines control release and survival of eosinophil [31], which has been shown to induce antibody dependent protective immunity against schistosomiasis [79,80]. Other factors including the HLA gene polymorphisms, levels of total protein, albumin, total cholesterol, low-density lipoproteins and very low-density lipoproteins were also identified. However, these factors were all reported by only one study (Table S2).

In conclusions, this study has identified the major host determinants of resistance or susceptibility to reinfection with schistosomes; although we had anticipated studies on immunogenetic factors in

addition to the identified socio-demographic, epidemiological and immunological factors. Therefore, there is need to explore the association between reinfection with schistosomes and host immunogenetic factors, especially the variations in the genes controlling immune response against schistosomiasis. This will be an interesting subject for further studies. Strong association with age and water contact related factors has reaffirmed that these factors play dominant role in determining exposure to pathogen and disease transmission. We also reconfirmed the major role played by IgE/IgG4 balance in controlling protective immunity against infection and reinfection with schistosomes.

Supporting Information

Figure S1 The PRISMA 2009 checklist. This study followed the guidelines of the PRISMA statement for conduct and reporting systematic reviews and meta-analyses.
(DOC)

Figure S2 Association of IgE and IgG4 levels with reinfection with schistosomes (detailed). This is the same data as shown in Figure 5 and Figure 6, except that the details of calculation of continuous variables are shown.
(PDF)

Figure S3 Association of levels of anti-SWA antibody isotypes with reinfection with schistosomes. Forest plots for the association of reinfection with IgG1 (**A**), IgG2 (**B**), IgG3 (**C**), IgA (**D**), and IgM (**E**).
(TIF)

Figure S4 Association of levels of anti-SEA antibody isotypes with reinfection with schistosomes. Forest plot for the association of reinfection with IgG1 (**A**), IgG2 (**B**), IgG3 (**C**), IgA (**D**), and IgM (**E**).
(TIF)

Table S1 Characteristics of studies included in the meta-analysis of host factors of reinfection with schistosomes.
(XLSX)

Table S2 Summary of overall effect measures for host determinants of reinfection identified in this study. This Table is similar to Table 1, but other host determinants reported by only one study are listed as well.
(DOC)

Table S3 Sensitivity analysis by exclusion of individual studies or subgroups from the meta-analysis.
(XLS)

Table S4 Sensitivity analysis by sample size ordered cumulative meta-analysis for the association of age and gender with reinfection with schistosomes.
(XLS)

Acknowledgments

Author Contributions

Conceived and designed the experiments: ECM NTH. Performed the experiments: ECM NTH AAW. Analyzed the data: ECM NTH AAW CIE ON KH. Contributed reagents/materials/analysis tools: ECM NTH CIE ON KH. Wrote the paper: ECM NTH. Revised the manuscript for important intellectual content: CIE ON KH.

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333

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334

Molecular basis for the reverse reaction of African human trypanosomes glycerol kinase

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Summary

The glycerol kinase (GK) of African human trypanosomes is compartmentalized in their glycosomes. Unlike the host GK, which under physiological conditions catalyzes only the forward reaction (ATP-dependent glycerol phosphorylation), trypanosome GK can additionally catalyze the reverse reaction. In fact, owing to this unique reverse catalysis, GK is potentially essential for the parasites survival in the human host, hence a promising drug target. The mechanism of its reverse catalysis was unknown; therefore, it was not clear if this ability was purely due to its localization in the organelles or whether structure-based catalytic differences also contribute. To investigate this lack of information, the X-ray crystal structure of this protein was determined up to 1.90 Å resolution, in its unligated form and in complex with three natural ligands. These data, in conjunction with results from structure-guided mutagenesis suggests that the trypanosome GK is possibly a transiently autophosphorylating threonine kinase, with the catalytic site formed by non-conserved residues. Our results provide a series of structural peculiarities of this enzyme, and gives unexpected insight into the reverse catalysis mechanism. Together, they provide an encouraging molecular framework for the development of trypanosome GK-specific inhibitors, which may lead to the design of new and safer trypanocidal drug(s).

Introduction

Trypanosoma brucei gambiense (Tbg) and *Trypanosoma brucei rhodesiense* (Tbr) are hemo-parasitic unicellular eukaryotes that cause human African trypanosomiasis (HAT), a disease that is still of great public health concern due to lack of a vaccine and satisfactory treatment (Migchelsen *et al.*, 2011). They are transmitted to humans through the bite of tsetse flies, hence have a complicated life cycle that involves alternating between insect-adapted forms such as the procyclic forms (PCFs) in the midgut of the vector and bloodstream forms (BSFs) in the human host (Simpson *et al.*, 2006). These organisms are classified as one of the most successful parasitic organisms because they have evolved with sophisticated and well-orchestrated strategies to permit their development. Notable among these are antigenic variation and their energy metabolism strategies (Hannaert *et al.*, 2003; Stijlemans *et al.*, 2011). A closer look at their intricate energy metabolism has revealed a number of distinct structural and biochemical features in the human stage of the parasites, and portrayed them as an Achilles' heel that might be exploitable for the purpose of developing a new chemotherapy. Importantly, some of these features are either absent or different from the type of metabolism operating in the host (Verlinde *et al.*, 2001).

The BSFs, unlike the PCFs and the host cells, contain a largely repressed mitochondrion that lacks the full complement of Krebs cycle and oxidative phosphorylation enzymes (Le Febvre and Hill, 1986; Nolan and Voorheis, 2000), hence, all their energy needs are derived from the glycolysis (Hannaert *et al.*, 2003). The glycolytic pathway of these parasites is compartmentalized within peroxisome-like organelles called glycosomes. These organelles sequester the first seven glycolytic enzymes, as well as two enzymes of glycerol metabolism including the NAD-dependent glycerol-3-phosphate dehydrogenase and glycerol kinase (GK) (Opperdoes and Borst, 1977; Guerra-Giraldez *et al.*, 2002). In BSFs, a net of two ATP molecules per glucose catabolized are produced from glycolysis, which is tightly coupled to the activity of the mitochondrially localized trypanosome alternative oxidase (TAO) (Fig. S1). However, during anaerobiosis or when TAO is inhibited, GK participates in glycolysis and aids in furnishing the BSFs with a net of one ATP per molecule of glucose consumed. Hence, GK becomes essential to BSFs (Haanstra *et al.*, 2008; Ohashi-Suzuki *et al.*, 2011; Gualdron-Lopez *et al.*, 2013; Saimoto *et al.*, 2013).

GK belongs to the sugar kinase/heat shock protein 70/actin superfamily [proteins capable of phospho transfer or hydrolysis from ATP (Hurley *et al.*, 1993)], and is present in all domains of life, where it generally functions as the connection between carbohydrate metabolism and lipid synthesis. GK catalyzes phospho group transfer from ATP to glycerol (forwards reaction) forming ADP and glycerol 3-phosphate (G3P) (Dipple *et al.*, 2001; Rahib *et al.*, 2007). In *T. brucei*, five identical tandemly arranged genes on chromosome 9 encode GK (Colasante *et al.*, 2006). In contrast to the GK of other organisms where under physiological conditions only the forward reaction is feasible, trypanosome GK is able to additionally catalyze the reverse reaction, i.e. phospho transfer from G3P to ADP to produce ATP and glycerol (Kralova *et al.*, 2000; Bringaud *et al.*, 2006). In fact, GK of BSFs utilizes this reverse reaction for participation in anaerobic glycolysis to rescue them from death when TAO is inhibited (Minagawa *et al.*, 1997; Ohashi-Suzuki *et al.*, 2011). Furthermore, it has been described long ago that under aerobic condition, trypanosomes can utilize both glucose and glycerol to the same extent, and glycerol can become sole energy source when glucose is absent (Marshall, 1948). Therefore, targeting trypanosome GK together with glucose metabolism would also be relevant to drug discovery.

The present study was conceived for two reasons. First, other organisms do not perform reverse catalysis by GK (Kralova *et al.*, 2000; Verlinde *et al.*, 2001). Owing to the lack of knowledge about the mechanism, it was unclear whether the ability of the trypanosomal GK to catalyze the reverse reaction is solely due to its compartmentalization in glycosomes, or if structure-based catalytic differences also contribute to facilitate it. Second, although GK in conjunction with TAO is a promising target for chemotherapy, an effective and selective parasite GK inhibitor has not yet become available partly due to unavailability of information on its structure and reverse reaction mechanism. So far, prokaryotic GKs are the most widely studied. Of the eukaryotes, only structural information of *Plasmodium falciparum* GK is available, which in addition to being functionally different from trypanosome GK, is not essential for growth of the asexual blood stages of this organism (Schnick *et al.*, 2009).

This study represents the first successful attempt to provide molecular insights into the structure of trypanosome GK. Using X-ray crystallography and structure-guided mutagenesis, we identified how it binds ADP and G3P in the active site groove and orient them for phospho transfer using residues that are not conserved in other organisms. The solved structure for the ligand-free form as well as in complex with each of three natural ligands (glycerol, G3P, and ADP) revealed a number of striking features such as (i) the participation of a beta-hairpin in the regulation of active cleft area upon glycerol or G3P binding, (ii) coordination of ADP and G3P in the active site by trypanosome-specific residues, and (iii) an additional ADP binding site far away from the active site cleft, and a new binding pattern for the ADP in the form of an adduct of two ADPs intercalated by a Mg^{2+} at this novel site. Together, these observations provide an encouraging molecular framework for the development of trypanosome GK-specific inhibitors that may lead to the design of a new and safer anti-trypanosomal drugs.

Results

Structure determination

Full-length TbgGK (512 residues) was overexpressed as an N-terminal hexahistidine-tagged fusion protein and purified as described in *Experimental procedures*. The ligand-free TbgGK obtained after extensive washing of the protein-glycerol (TbgGK in purification buffer containing 10% glycerol) sample in 10 mM MOPS buffer, pH 6.8, containing 10 mM $MgSO_4$ to remove glycerol, was crystallized by the vapor diffusion method using a reservoir solution composed of 0.1 M HEPES pH7.0, 12% (w/v) isopropanol and 4% (w/v) sorbitol, whereas crystals of the glycerol-bound TbgGK were obtained under a different condition [0.1 M HEPES buffer, pH 7.0, 30% (w/v) PEG400 and 11% (w/v) 1,6-hexanediol]. Crystals of TbgGK with either G3P or ADP were prepared by soaking crystals of the glycerol-bound form in the reservoir solution supplemented with each compound. Other substrates

Table 1. Data collection and refinement statistics of TbgGK crystals.

Ligand	None	Glycerol	ADP	G3P
Data collection				
Space group	$P2_12_12_1$	$P2_1$	$P2_1$	$P2_1$
Cell parameters				
$a/b/c$ (Å)	68.77/131.97/148.61	62.49/122.14/154.53	62.16/153.84/120.10	64.17/120.90/153.55
$\alpha/\beta/\gamma$ (°)	90/90/90	90/90.76/90	90/89.95/90	90/90.02/90
Wavelength (Å)	0.98	0.98	1.00	0.98
Resolution (Å)	50.0–2.9 (2.95–2.90)	50.0–2.40 (2.45–2.40)	50–1.9 (1.93–1.90)	50–2.7 (2.75–2.70)
Total reflections	158 585	237 428	554 224	237 052
Unique reflections	30 848	90 635	176 540	64 589
Completeness (%)	99.4 (99.6)	95.8 (96.11)	92.3 (80.9)	99.8 (99.7)
R_{merge} / (%)	5.0 (67.5)	4.3 (47.4)	3.8 (47.0)	4.6 (45.1)
I/σ	19.4 (1.71)	14.6 (1.7)	14.0 (3.1)	17.1 (3.3)
Refinement				
Resolution range (Å)	30–2.90	30–2.40	30–1.90	30–2.70
Number of non-hydrogen atoms in:				
Protein	7 897	15 833	15 814	15 826
Ligand	–	42	135	30
Water molecules	9	204	1 092	151
Magnesium atoms	–	–	2	–
R_{work}/R_{free}	0.201/0.282	0.190/0.251	0.211/0.251	0.205/0.290
R.m.s. deviation from ideal values				
Bond length (Å)	0.013	0.015	0.006	0.009
Bond angle (°)	1.58	1.65	0.94	1.24
Temperature factor (Å²)				
Protein	66.81	51.9	32.7	66.7
Magnesium	–	–	35.3	–
Ligand	–	55.8	35.3	57.7
Water	48.1	52.2	41.2	53.4
Ramachandran plot				
Most favored and allowed regions (%)	100	99.7	99.6	99.9
Disallowed region (%)	0.0	0.3	0.4	0.1
PDB codes	3WXI	3WXK	3WXL	3WXJ

Values in parentheses are for the highest resolution shell.

were unable to bind the ligand-free form even when soaked for days and at concentrations of up to 20 mM ADP or G3P binding was achieved only when the glycerol-bound crystals were used for soaking. X-ray diffraction data were collected using synchrotron radiation (Table 1), and the structure of the glycerol-bound form was solved by molecular replacement using the structure of *P. falciparum* GK (PDB code 2W40; Schnick *et al.*, 2009) as a search model. The refined structure was then used as a template of molecular replacement for the structure determination of the ligand-free TbgGK and TbgGK–G3P and –ADP complexes.

Overall structure of ligand-free TbgGK

The crystal structure of the ligand-free TbgGK (pdb code: 3WXI) represents the first structure of a eukaryotic, potentially essential GK. In accord with the result of size-exclusion chromatography, the enzyme exists as a homodimer with an approximate dimension of 40 × 60 × 120 Å in the asymmetric unit (Fig. 1A). The monomer is

composed of 18 β-strands (β1–β18) and 18 α-helices (α1–α18). In the dimer structure, monomers (referred to as chains A and B) are related by a non-crystallographic twofold axis to each other and are predominantly associated through an intermolecular anti-parallel β-sheet (β14^A–β15^A–β15^B–β14^B) and extensive hydrogen bonds between residues from each of three pairs of α-helices (α12^A–α14^B, α14^A–α12^B and α18^A–α18^B; Fig. 1B and Table S1). The contact surface area of each monomer is approximately 5700 Å², representing about 30% of the monomer's total surface area.

There is no significant difference between monomer structures as indicated by root-mean-square (rms) deviation calculated for superimposed 512 Cα positions (0.87 Å), and each is folded into two functional domains I (residues 1–261) and II (residues 268–511) (Fig. 2A and Fig. S3). This architecture is similar to the patterns observed in the GKs of *Escherichia coli* (Hurley *et al.*, 1993), *Enterococcus casseliflavus* (Yeh *et al.*, 2004), *Staphylococcus aureus* (Minasov *et al.*, 2009), *P. falciparum* (Schnick *et al.*, 2009), *Thermococcus kodakaraensis*
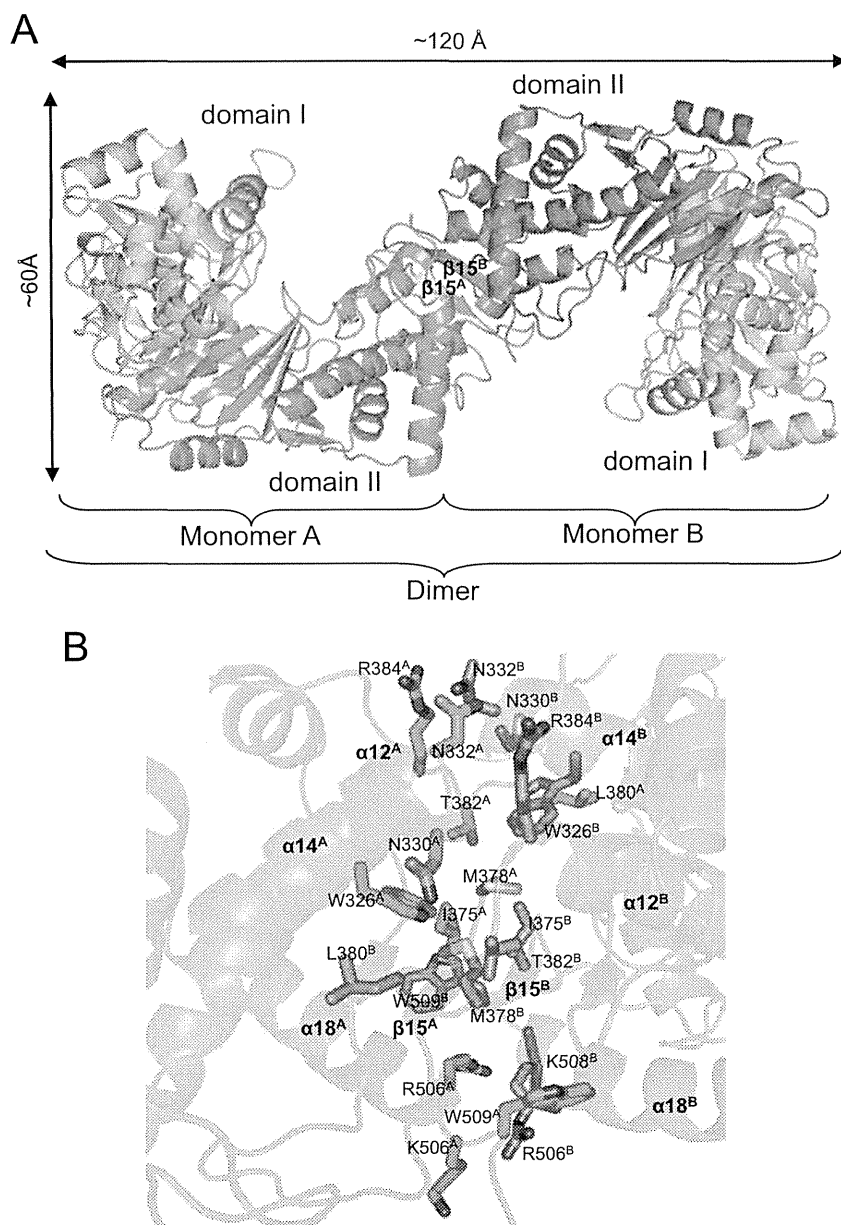
A

Fig. 1. Structure of ligand-free TbgGK. (A) Cartoon diagram of the TbgGK dimer and (B) zoomed-in view of the inter-monomer joint showing amino acid residues involved in the dimer formation. Superscript identifies the monomer chains A or B. Cyan and green represent domains I and II of monomer A, while the other cyan and magenta are domains I and II of the monomer B respectively. $\beta15^A$ and $\beta15^B$ from monomers A and B, respectively, form an inter-subunit antiparallel β-sheet and contribute to the dimer formation. Color codes of the stick model are; green and magenta: carbon atom of monomer A and B respectively; blue: nitrogen atom; red: oxygen atom.

B

Fig. 2. Monomer structures of ligand-free, glycerol-, and G3P-bound TbgGK.

A. Cartoon diagram showing the unligated enzyme having a $\beta10/\beta11$ loop encroaching the V-shaped active site cleft. The loop is maintained by a disulfide linkage (S–S; green stick) between the sulfo groups of C278 and C319.

B. Structure of the TbgGK-glycerol complex, revealing the binding sites of catalytic (cg) and non-catalytic glycerol (ncg) molecules (yellow sticks) at different domains in the active site cleft of TbgGK. In the glycerol-bound TbgGK, the $\beta10/\beta11$ loop is out of the active site cleft, and assumes an open conformation.

C. Residues around cg and ncg. The cg is tightly bound at the catalytic site by well-conserved residues. While the aromatic residues Trp104 and Phe279 form hydrophobic interactions with the cg, it is bound via hydrogen bonds to Arg84, Glu85 and Asp254. The ncg is weakly bound to the non-catalytic site near the $\beta10/\beta11$ loop via a single hydrogen bond with the main chain nitrogen of Thr276.

D. G3P-bound TbgGK. In this form, a hydrogen bond between T276 (shown as yellow stick) from the $\beta10/\beta11$ loop and the bound G3P maintains the loop in closed conformation.

E. Residues around G3P. The G3P is depicted as a yellow stick with its $2F_o$–F_c map contoured at 3.0 σ. Dotted lines represents hydrogen bonds.