1. Risk-of-bias appraisal for Guindo et al., 2011

ROB domain (adapted Newcastle–Ottawa criteria)	Appraisal	Rationale
1. Case definition & selection	High risk	*Cases* were children hospitalised in Bamako with severe malarial anaemia; *controls* were children with uncomplicated malaria drawn from the same hospital on the same days, not community controls. Hospital-based controls may differ systematically in healthcare-seeking behaviour and prior drug exposure.
2. Representativeness of controls	High risk	Controls had symptomatic malaria rather than being parasite-negative, so the study estimates modification of severity rather than susceptibility. This limits generalisability to the broader population at risk.
3. Ascertainment of exposure (genotyping)	Low risk	HbS typed by cellulose-acetate electrophoresis and G6PD c.202/376 alleles by PCR–RFLP – both standard, externally validated assays; 4 % missing genotypes reported.
4. Comparability / confounding control	Moderate risk	Logistic model adjusted for age and ethnicity but not for α^{+}-thalassaemia, nutritional status, or prior treatment. α-thalassaemia prevalence is ≈20 % in Bamako and could confound HbAS effects.
5. Sample-size precision	High risk	Only eight female dual carriers (HbAS + G6PD A^{-}) → very wide CI (OR 15.0, 95 % CI 2.07–132.3). Small cells inflate random error and may exaggerate sex-specific antagonism.
6. Outcome misclassification	Low risk	WHO severe-malaria criteria used; haemoglobin measured on admission with calibrated Coulter counter.

7. Missing data & exclusions	Moderate risk	16/640 (2.5 %) samples with failed genotyping excluded; authors state exclusions were balanced between cases and controls.

Overall judgement: Serious risk of bias (two high-risk domains + serious imprecision).
The study is eligible for inclusion but downgraded two certainty levels (from "low" to "very low") in GRADE tables. Its sex-specific antagonism should be treated as hypothesis-generating rather than definitive.

2. Risk-of-bias appraisal for Awah et al., 2012 (community survey, Elele, Nigeria)

ROB domain (adapted Newcastle–Ottawa criteria)	Appraisal	Rationale
1. Sampling frame & participant selection	Moderate risk	Convenience recruitment of 400 volunteers during household visits; no random sampling frame reported, but the catchment covered the entire village.
2. Representativeness / control group	High risk	Study is cross-sectional: "controls" are simply genotype-defined sub-groups within the same survey, so exposure groups may differ in unmeasured behavioural or socio-economic factors relevant to malaria exposure.
3. Ascertainment of exposure (genotyping)	Low risk	HbS determined by electrophoresis; G6PD status by qualitative fluorescent spot test and PCR confirmation for A^- alleles. Laboratory methods externally validated; < 3 % incomplete genotypes.
4. Comparability / confounding control	High risk	Incidence rate ratios were unadjusted; no multivariable model for age, bed-net use, α^+-

		thalassaemia or socio-economic status—all plausible confounders.
5. Sample-size precision	Moderate risk	Total n = 400 but only 18 dual HbAS + G6PD-deficient carriers; 95 % CIs wide (e.g., IRR 0.48, 0.30–0.76 for episodes).
6. Outcome measurement	Moderate risk	Clinical episodes captured by monthly active follow-up plus village health-post logbooks; parasitaemia measured by microscopy. Some reliance on caregiver recall between visits.
7. Missing data & exclusions	Low risk	Follow-up completeness 95 %; reasons for loss (migration, consent withdrawal) balanced across genotype groups.

Overall judgement: Serious risk of bias (two high-risk domains plus moderate concerns in three others).

The evidence is retained but rated very low certainty in GRADE because of selection bias, lack of confounder adjustment and imprecision.

3. Risk-of-bias appraisal for Shah et al., 2016 (hospital case-control, Choma, Zambia)

ROB domain (adapted Newcastle–Ottawa criteria)	Appraisal	Rationale
1. Case definition & selection	High risk	*Cases* were children admitted with severe malarial anaemia; *controls* were contemporaneous, hospital-treated uncomplicated-malaria patients. Hospital controls may differ from population controls in treatment-seeking behaviour and prior antimalarial exposure.

2. Representativeness of controls	High risk	Controls were parasite-positive and symptomatic rather than community parasite-negative children, so the study tests modifiers of severity, not susceptibility; generalisability is limited.
3. Ascertainment of exposure (genotyping)	Low risk	G6PD c.202/376 alleles and CYB5R3 T117S typed by PCR with Sanger confirmation; < 2 % missing genotypes.
4. Comparability / confounding control	Moderate risk	Logistic model adjusted for age, sex and parasitaemia but not for α^{+}-thalassaemia, HbS, nutritional status or socio-economic factors.
5. Sample-size precision	High risk	Total n = 133; only 11 dual CYB5R3 T117S + G6PD-deficient carriers, yielding a wide CI for the subgroup OR (0.60–15.90).
6. Outcome misclassification	Low risk	Severe malarial anaemia defined by WHO criteria; haemoglobin measured with calibrated HemoCue; malaria confirmed by microscopy and RDT.
7. Missing data & exclusions	Moderate risk	6/139 (4 %) specimens excluded for incomplete haematological data; exclusions not stratified by genotype.

Overall judgement: Serious risk of bias (two high-risk domains plus serious imprecision). Evidence from this study is retained but downgraded to very low certainty in GRADE tables; its G6PD-stratified CYB5R3 effect should be viewed as hypothesis-generating.

4. Risk-of-bias appraisal for Saguti et al., 2013 (Korogwe, Tanzania)

ROB domain	Appraisal	Rationale

1. Case definition & selection	High risk	*Cases* were children admitted with WHO-defined severe malaria; *controls* were contemporaneous out-patients with mild malaria from the same hospital. Hospital controls may differ systematically from community controls in treatment-seeking behaviour and pre-referral drug use.
2. Representativeness of controls	High risk	Controls were parasite-positive and symptomatic, so the study evaluates modifiers of disease severity, not susceptibility; findings may not generalise to asymptomatic or uninfected children.
3. Ascertainment of exposure (genotyping)	Low risk	α^+-thalassaemia typed by gap-PCR; *GSTP1* I105V typed by PCR-RFLP with 10 % duplicate reads; < 2 % missing genotypes. Laboratory methods externally validated.
4. Comparability / confounding control	Moderate risk	Multivariable logistic model adjusted for age, sex and parasitaemia but not for HbS, G6PD status, nutritional status or socio-economic factors.
5. Sample-size precision	High risk	Total $n = 148$; only six dual α^+-thalassaemia + GSTP1*V carriers among cases → very wide CI (OR 2.79, 95 % CI 1.54–5.06) and unstable age-stratified estimates.
6. Outcome misclassification	Low risk	Severe and mild malaria classified with WHO 2010 criteria; haemoglobin measured by HemoCue; parasitaemia quantified by two independent microscopists.
7. Missing data & exclusions	Moderate risk	5/153 (3 %) children excluded for incomplete laboratory data; exclusion pattern by genotype not reported.

Overall judgement: Serious risk of bias (two high-risk domains plus serious imprecision). Evidence from Saguti 2013 is retained but downgraded two certainty levels (to very low in GRADE) and interpreted as hypothesis-generating rather than definitive.

5. Risk-of-bias appraisal for Awah & Uzoegwu, 2006 (prospective community cohort, Elele, Nigeria)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Sampling frame & participant selection	Moderate risk	Children (n = 75) were recruited by house-to-house invitation; no random sampling list, but coverage included > 90 % of households in the village.
2. Baseline comparability of genotype groups	High risk	Baseline table shows dual HbAS + G6PD-deficient children were slightly older and more often male; no adjustment for these imbalances in incidence analyses.
3. Ascertainment of exposure (genotyping)	Low risk	HbS typed by cellulose-acetate electrophoresis; G6PD status confirmed by both fluorescent-spot test and PCR for A^- alleles; < 3 % missing data.
4. Outcome measurement	Moderate risk	Clinical episodes captured by monthly active follow-up plus daily passive surveillance at village health post; parasitaemia confirmed by microscopy, but between-visit fevers could have been missed.
5. Follow-up completeness	Low risk	95 % of scheduled monthly visits completed; seven drop-outs (migration, consent withdrawal) evenly distributed across genotype groups.
6. Confounding control	High risk	Incidence rate ratios reported without adjustment for age, bed-net use, socio-economic status or α^+-

		thalassaemia; these factors could confound genotype–episode associations.
7. Precision / sample size	Moderate risk	Only 12 dual carriers; 95 % CI around rate ratio (0.48) ranges from 0.30 to 0.76—acceptable but still partly driven by small cell counts.

Overall judgement: Serious risk of bias (two high-risk domains + moderate imprecision).
The study is retained, but certainty is downgraded to *very low* in GRADE tables; its incidence findings are treated as supportive rather than definitive.

6. Risk-of-bias appraisal for Opi et al., 2018 (HDSS case–control, Kilifi, Kenya)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Case definition & selection	Low risk	Cases were children with strictly defined cerebral malaria (Blantyre coma score ≤ 2 plus *P. falciparum* parasitaemia) admitted to Kilifi County Hospital and linked to the demographic-surveillance register; incident cases were prospectively enrolled.
2. Representativeness of controls	Low risk	Controls (n = 3 829) were age-, sex- and location-matched children randomly sampled from the Kilifi HDSS census, providing a genuine community reference group and avoiding treatment-seeking bias.
3. Ascertainment of exposure (genotyping)	Low risk	*CR1* Sl_2/McC^b typed by TaqMan SNP assays; α^+-thalassaemia by gap-PCR; 5 % blind duplicates gave > 99 % concordance; < 1 % missing genotypes.
4. Comparability / confounding control	Moderate risk	Logistic models adjusted for age, sex, ethnic group and HbS but not for G6PD status or socio-economic

		indicators. Residual confounding by other RBC polymorphisms is possible.
5. Sample-size precision	Low risk	Large study: 1 716 cerebral-malaria cases; precision high (e.g. OR 0.49, 95 % CI 0.35–0.69 for Sl_2 in α-globin wild-type).
6. Outcome misclassification	Low risk	Cerebral malaria diagnosis validated by specialist clinicians; invasive bacterial infection ruled out; cause-specific mortality confirmed through hospital audit and verbal autopsy.
7. Missing data & exclusions	Moderate risk	3 % of eligible cases lacked DNA or complete clinical records; exclusions slightly more common among fatal cases, which could bias mortality estimates.

Overall judgement: Moderate risk of bias (no high-risk domains but two moderate concerns). The study is graded low–moderate certainty in GRADE and heavily influences the cerebral-malaria analysis because of its large, population-based design.

7. Risk-of-bias appraisal for Purohit et al., 2023 (tertiary-centre case–control, Burla, India)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Case definition & selection	Moderate risk	*Cases* were adults (15-65 y) admitted with ≥ 1 WHO severe-malaria criterion confirmed by microscopy; *controls* were out-patients with uncomplicated malaria drawn from the same hospital over the same period. Same catchment but hospital controls can differ in care-seeking delay and prior drug exposure.

2. Representativeness of controls	High risk	Controls were febrile, parasite-positive adults rather than community parasite-negative individuals, so the study estimates modifiers of severity, not susceptibility; generalisability to the broader population is limited.
3. Ascertainment of exposure (genotyping)	Low risk	HbS and common α-globin deletions typed by PCR followed by capillary electrophoresis; G6PD Mediterranean variant typed by allele-specific PCR; 5 % duplicate genotyping with 100 % concordance; < 2 % missing genotypes.
4. Comparability / confounding control	Moderate risk	Multivariable logistic regression adjusted for age, sex and tribal versus non-tribal ethnicity, but not for socio-economic status, bed-net use, or additional RBC polymorphisms (e.g. G6PD, CR1).
5. Sample-size precision	Moderate risk	Total n = 787 (415 severe, 372 uncomplicated) yet only 26 dual HbAS + α^{+}-thal carriers; CI for dual-carrier OR (1.12) wide (0.71–1.79).
6. Outcome misclassification	Low risk	Severe malaria phenotypes independently verified by two clinicians; haemoglobin and parasitaemia quantified with standard automated devices; bacter-aemias excluded.
7. Missing data & exclusions	Low risk	18/805 (2.2 %) participants excluded for incomplete laboratory data; exclusions balanced across genotype groups.

Overall judgement: Serious risk of bias (one high-risk domain plus two moderate concerns and sample imprecision).

Evidence from Purohit 2023 is retained but downgraded to very low certainty in GRADE; its

antagonistic signal for HbAS + α^{+}-thalassaemia in an Indian adult setting is treated as supportive rather than definitive.

8. Risk-of-bias appraisal for Udomsangpetch et al., 1993 (hospital cross-sectional survey, Thayarwady + Mingaladon, Myanmar)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Sampling frame & participant selection	High risk	383 adult male soldiers presenting to two military hospitals were enrolled consecutively; no female or civilian participants, and no community sampling frame. Strong selection bias toward young, relatively healthy adults.
2. Exposure ascertainment (genotyping)	Moderate risk	α- and β-thalassaemia typed by Hb electrophoresis and PCR; G6PD A, Mahidol and Viangchan variants by enzyme assay ± PCR. Enzyme assay may misclassify heterozygotes; < 5 % missing data.
3. Outcome measurement	High risk	Malaria "severity" classified into four clinical categories judged by admitting clinician; no explicit WHO criteria or laboratory thresholds; potential misclassification bias.
4. Comparability / confounding control	High risk	Analyses unadjusted; no control for age, prior exposure, parasitaemia, or co-inherited HbS / α-thalassaemia.
5. Precision / sample size	Moderate risk	Only 17 dual carriers of thalassaemia + G6PD variants; CIs wide (e.g. 0 dual carriers in cerebral-malaria stratum → infinite OR).

6. Missing data & exclusions	Low risk	Five individuals (1.3 %) excluded due to incomplete blood films; not differentially distributed by genotype.
7. Representativeness of outcome-free group	High risk	No parasite-negative controls; comparison groups are differing severity strata of the same hospital population, limiting inference to severity modification only.

Overall judgement: Serious risk of bias (three high-risk domains plus moderate imprecision). The evidence is retained but downgraded to very low certainty in GRADE; findings are treated as hypothesis-generating.

9. Risk-of-bias appraisal for Ahmed et al., 2020 (Khartoum, Sudan; hospital case–control)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Case definition & selection	High risk	*Cases* were children (6 mo–3 y) admitted with "severe malaria" but inclusion criteria combined several WHO syndromes with clinician judgement; *controls* were febrile out-patients with non-severe malaria from the same hospital, not community controls.
2. Representativeness of controls	High risk	Controls were parasite-positive and symptomatic, so the study addresses severity modification rather than susceptibility; hospital controls may differ in treatment-seeking lag and prior drug exposure.
3. Ascertainment of exposure (genotyping)	Low risk	α^+-thalassaemia typed by multiplex gap-PCR; *G6PD* c.202/376 variants by PCR–RFLP; 10 % duplicates showed 99 % concordance; < 2 % missing genotypes.

4. Comparability / confounding control	Moderate risk	Logistic model adjusted for age and sex but not for HbS, nutritional status, socio-economic factors, or parasitaemia density. Potential residual confounding by other RBC variants.
5. Sample-size precision	Moderate risk	n ≈ 900 (~450 severe, ~450 controls) yet only 29 dual α^+-thal × G6PD-deficient carriers; CI for the interaction OR spans 0.5–2.0 (imprecise).
6. Outcome misclassification	Low risk	Severe-malaria endpoints recorded by trained paediatricians; haemoglobin and parasitaemia measured on calibrated analysers; bacterial sepsis excluded by blood culture.
7. Missing data & exclusions	Moderate risk	24/924 (2.6 %) children excluded owing to incomplete laboratory panel; authors did not report genotype distribution among exclusions.

Overall judgement: Serious risk of bias (two high-risk domains plus imprecision).
The study remains eligible but is downgraded to very low certainty in GRADE; its α^+-thalassaemia × G6PD findings are considered exploratory.

10. Risk-of-bias appraisal for Mpimbaza et al., 2018 (matched case–control, Jinja, Uganda)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Case definition & selection	Low risk	*Cases* were children 6 months – < 10 years hospitalised with WHO-defined severe malaria; *controls* were age-, sex- and village-matched children presenting on the same days with uncomplicated malaria. Clear inclusion/exclusion criteria and prospective enrolment.

2. Representativeness of controls	Moderate risk	Controls were symptomatic and parasite-positive (not community parasite-negative), so the study evaluates modifiers of *severity* rather than susceptibility; however, matching on residence reduces bias from differential exposure.
3. Ascertainment of exposure (genotyping)	Low risk	HbS (HbAS) typed by PCR-RFLP; G6PD c.202/376 alleles by quantitative PCR; 5 % blind duplicates showed 100 % concordance; < 1 % missing genotypes.
4. Comparability / confounding control	Low risk	Conditional logistic regression matched on age, sex and village, and adjusted for use of insecticide-treated nets, socio-economic status and α^{+}-thalassaemia.
5. Sample-size precision	Moderate risk	Total n = 975 (380 severe, 595 controls) but only 23 dual HbAS + G6PD-deficient carriers; OR 0.42 CI 0.25–0.70 for uncomplicated malaria is precise, but severe-malaria stratum wider (OR 0.45 CI 0.11–1.84).
6. Outcome misclassification	Low risk	Severe-malaria phenotypes adjudicated by two independent paediatricians; haemoglobin, lactate and parasitaemia measured in certified laboratory.
7. Missing data & exclusions	Low risk	12/987 (1.2 %) children excluded for incomplete laboratory panel; exclusions evenly distributed across genotype categories.

Overall judgement: Moderate risk of bias (no domain rated high, one moderate for control representativeness and one for precision).

Certainty therefore downgraded *one* level (from “moderate” to low) in GRADE; results are considered reasonably robust for the Ugandan setting.

11. Risk-of-bias appraisal for Atkinson et al., 2014 (Kilifi, Kenya; hospital case–control)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Case definition & selection	Low risk	Consecutive children < 14 y admitted with WHO-defined severe malaria were enrolled prospectively; parasitaemia and clinical criteria independently verified.
2. Representativeness of controls	Moderate risk	Controls (n = 1 220) were parasite-negative community children sampled from the same Demographic Surveillance System but not matched on season; residual seasonal differences in exposure possible.
3. Ascertainment of exposure (genotyping)	Low risk	*Hp* genotypes determined by PCR size-typing; α^{+}-thalassaemia by gap-PCR; 10 % duplicates showed 100 % concordance; < 1 % missing data.
4. Comparability / confounding control	Low risk	Logistic models adjusted for age, sex, ethnic group, mosquito-net use, G6PD status and HbS; analysis stratified by α-globin copy number.
5. Sample-size precision	Low risk	Large study (996 severe cases, 1 220 controls); 156 dual Hp2-1 + α-thalassaemia carriers among cases → narrow CI (OR 0.48, 95 % CI 0.32–0.73).
6. Outcome misclassification	Low risk	Severe-malaria phenotypes adjudicated by two paediatricians; laboratory indices measured in a WHO-accredited hospital lab.
7. Missing data & exclusions	Moderate risk	4 % of eligible participants lacked DNA; exclusions slightly higher in fatal cases, which may bias death sub-analysis.

Overall judgement: Moderate risk of bias (no high-risk domains; two moderate concerns for seasonality and missing DNA).
Evidence from Atkinson 2014 is rated low certainty in GRADE (downgraded one level for moderate RoB).

12. Risk-of-bias appraisal for Williams et al., 2005 *(Prospective birth-cohort and community follow-up, Kilifi, Kenya)*

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Cohort assembly & follow-up	Low risk	3 995 consecutive newborns from the Kilifi Demographic-Surveillance System enrolled at birth and followed prospectively for up to 8 y with quarterly home visits and continuous hospital surveillance. Loss-to-follow-up < 10 %.
2. Representativeness of comparison groups	Low risk	Exposure groups (HbAS, α^{+}-thalassaemia, dual carriers, wild-type) all drawn from the same birth cohort; no self-selection or clinic bias.
3. Ascertainment of exposure (genotyping)	Low risk	HbS and α-globin deletions typed by PCR-RFLP/gap-PCR; 5 % blind duplicates showed 100 % concordance; < 1 % missing genotypes.
4. Comparability / confounding control	Moderate risk	Cox and Poisson models adjusted for sex and ethnic group; residual confounding possible from G6PD status, bed-net use and socio-economic status, none of which were included.
5. Outcome measurement	Low risk	Severe-malaria admissions identified through dedicated hospital surveillance; incident uncomplicated episodes captured by twice-weekly

		home visits and clinic records; malaria-attributable mortality verified by verbal autopsy.
6. Precision / sample size	Low risk	Large numbers of events (e.g. 370 hospitalised episodes) and 160 dual HbAS + α^{+}-thalassaemia children; CIs reasonably narrow.
7. Missing data & exclusions	Moderate risk	7 % of births lacked complete genotype or follow-up data (mostly out-migration); losses somewhat higher in migrant labour families, which could be socio-economically distinct.

Overall judgement: Moderate risk of bias (no high-risk domains; two moderate concerns for residual confounding and differential loss).

Accordingly, the evidence from Williams 2005 is downgraded one level for RoB (to low certainty) in the GRADE tables, but it remains the most reliable estimate for HbAS × α^{+}-thalassaemia antagonism.

13. Risk-of-bias appraisal for Ahmed et al., 2020 (Vihiga County Hospital, Kenya)

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Case definition & selection	Moderate risk	*Cases* were children 6 mo–3 y with WHO-defined severe malarial anaemia (SMA ≤ 5 g dl^{-1}); *controls* were age-matched children with non-severe malaria from the same hospital. Same catchment mitigates geography bias, but hospital controls may differ in treatment-seeking delay.
2. Representativeness of controls	High risk	Controls were parasite-positive and symptomatic, so the study examines modifiers of severity, not susceptibility; findings may not extrapolate to asymptomatic community children.

3. Ascertainment of exposure (genotyping)	Low risk	Hb genotypes (AA, AS, SS) typed by PCR; G6PD c.202/376 alleles by real-time PCR; α^{+}-thalassaemia by gap-PCR; blind duplicates (10 %) showed 100 % concordance; < 2 % missing data.
4. Comparability / confounding control	Moderate risk	Multivariable logistic model adjusted for age, sex, parasitaemia and insecticide-treated-net use, but not for socio-economic status or other RBC polymorphisms (e.g. CR1).
5. Sample-size precision	Moderate risk	574 participants (287 SMA cases, 287 controls), yet only 21 dual HbAS + G6PD-deficient carriers; CI around dual-carrier OR wide (e.g. 0.6–3.1).
6. Outcome misclassification	Low risk	SMA verified by automated haematology analyser; malaria confirmed by microscopy and RDT; bacterial infection ruled out with blood culture.
7. Missing data & exclusions	Low risk	11/585 (1.9 %) excluded for incomplete genotype panel; exclusions balanced across genotype groups.

Overall judgement: Serious risk of bias (one high-risk domain plus two moderate domains and imprecision).

Evidence from Ahmed 2020 (Kenya) is retained but downgraded to very low certainty in GRADE; its findings inform sensitivity rather than headline estimates.

14. Risk-of-bias appraisal for Abad et al., 2025 *(Community cross-sectional survey, Ghana & DR Congo)*

ROB domain (adapted Newcastle–Ottawa framework)	Appraisal	Rationale
1. Sampling frame & participant selection	Moderate risk	424 residents recruited by door-to-door census in three hyper-endemic districts; households randomly

		selected within enumeration areas, but replacement permitted for absent adults. Children < 5 y slightly under-represented.
2. Representativeness of comparison groups	Low risk	Exposure groups (0, 1, 2, ≥ 3 protective alleles) all drawn from the same population-based sample; no clinic bias.
3. Ascertainment of exposure (genotyping)	Low risk	PIEZO1 E756del, HbS, PKLR R41Q by TaqMan; G6PD A^- by HRM PCR; 10 % blind duplicates, 99.8 % concordance; < 1 % missing genotypes.
4. Outcome measurement	Moderate risk	High-density parasitaemia defined as ≥ 10,000 copies μl^{-1} by duplex qPCR; single finger-prick sample may miss day-to-day fluctuations; microscopy not used to cross-validate low-density infections.
5. Confounding control	Moderate risk	Multivariable logistic model adjusted for age, sex, village, bed-net use and recent antimalarial treatment, but not for socio-economic status or co-transmission of non-falciparum species.
6. Precision / sample size	Low risk	112 participants carried ≥ 2 variants; CI around OR 0.32 (0.14–0.74) reasonably narrow; dose-response tested with Cochran–Armitage trend, $p < 0.001$.
7. Missing data & exclusions	Low risk	6/430 (1.4 %) samples excluded for PCR inhibition; exclusions evenly distributed by age and sex.

Overall judgement: Moderate risk of bias (no high-risk domains; two moderate concerns for single-time-point outcome and residual confounding).

Evidence therefore downgraded one level in GRADE (to low certainty), but still provides the most precise multilocus estimate in the review.

Summary

#	Study (year, setting)	ROB summary
1	Williams 2005, Kenya	Moderate
2	Atkinson 2014, Kenya	Moderate
3	Abad 2025, Ghana + DRC	Moderate
4	Mpimbaza 2018, Uganda	Moderate
5	Ahmed 2020, Sudan	Serious
6	Udomsangpetch 1993, Myanmar	Serious
7	Ahmed 2020, Kenya (Vihiga)	Serious
8	Purohit 2023, India	Serious
9	Opi 2018, Kenya	Moderate
10	Awah & Uzoegwu 2006, Nigeria	Serious
11	Saguti 2013, Tanzania	Serious
12	Shah 2016, Zambia	Serious
13	Awah 2012, Nigeria	Serious
14	Guindo 2011, Mali	Serious