In practice, these conditions are not directly testable and may be violated in subtle ways. For example, a variant may influence multiple biological processes, giving rise to additional pathways that complicate interpretation. For this reason, instrument selection is often informed by upstream analyses. Colocalization, in particular, can be used to identify regions where GWAS and molecular QTL signals are consistent with a shared underlying variant, thereby increasing confidence that the selected instruments reflect a common source of variation (Zuber et al., 2022).

5.2 Common estimation approaches

Among available methods, inverse-variance weighted (IVW) regression is most commonly used as the primary estimator. It combines ratio estimates from multiple instruments-defined as the effect of a variant on the outcome divided by its effect on the exposure-using inverse-variance weighting. When all instruments are valid, or when biases average out, IVW provides efficient estimates. In the case of a single instrument, the Wald ratio is typically used.

When heterogeneity is present across instruments, random-effects formulations can be applied to account for additional variance. A number of complementary approaches have been developed to improve robustness. MR-Egger regression introduces an intercept term to detect directional bias and, under certain conditions, provides a corrected estimate, although at the cost of reduced precision. The weighted median estimator remains consistent when a subset of instruments is invalid, while mode-based estimators offer further robustness under specific assumptions about the distribution of effects.

In settings where multiple exposures may contribute jointly-such as gene expression, splicing, or protein abundance-multivariable MR (MVMR) can be used to disentangle their contributions. Although this approach can provide more detailed insight into complex regulatory relationships, it also requires stronger assumptions and higher data quality.

5.3 Weak instruments and pleiotropy

The strength of the instruments plays a central role in determining the stability of MR estimates. Weak associations between instruments and exposure can lead to biased estimates that are closer to observational correlations, along with inflated uncertainty. The F statistic is commonly used as a diagnostic measure, with a value of around 10 often considered a practical benchmark in univariable analyses. In multivariable settings, instrument strength needs to be evaluated separately for each exposure.

When weak instruments are detected, several strategies may help improve performance, including applying stricter selection thresholds, restricting analyses to cis-regulatory variants, or using reference datasets that better match the target population. Issues such as sample overlap and winner's curse can further weaken instruments and should be considered during study design and interpretation.

Pleiotropy introduces an additional layer of complexity. Overall heterogeneity can be assessed using statistics such as Cochran's Q, while the MR-Egger intercept provides a test for directional bias. MR-PRESSO offers procedures for identifying and correcting outlier instruments, and radial MR provides a useful visualization framework for detecting influential points. Sensitivity analyses, including leave-one-out procedures and directionality checks such as the Steiger test, can further help evaluate the robustness of the results.

In reporting, it is generally advisable to consider multiple diagnostics together. When estimates from different methods (e.g., IVW, weighted median, MR-Egger) are broadly consistent and diagnostic tests do not indicate major violations, the findings are more likely to be reliable. When discrepancies arise, it is often necessary to revisit earlier steps, including instrument selection, data harmonization, and upstream evidence.

5.4 Position within integrative analyses

In a broader analytical workflow, MR is typically applied after an initial round of signal prioritization. Colocalization can be used to identify loci where signals from different data sources are consistent, thereby