To bridge this divide, expression quantitative trait loci (eQTL) and transcriptome-wide association studies (TWAS) introduce molecular phenotypes as intermediate layers, extending inference from the variant level to the gene expression level. In this framework, eQTL analyses characterize the mapping from genotype to gene expression, while TWAS integrates GWAS summary statistics with expression prediction models to generate gene-level association signals and prioritize candidate genes (Wainberg et al., 2019; Xie et al., 2021; Zhao et al., 2022).

However, it is essential to emphasize that, TWAS remains an association-based projection under LD structure, not a causal estimand. Due to co-regulation among nearby genes, LD contamination, tissue mismatch, and genetic confounding, TWAS signals may reflect non-causal variants, leading to the prioritization of "bystander genes" (Liu et al., 2019; Zhao et al., 2022; Tambets et al., 2024). Thus, TWAS provides necessary but insufficient evidence for causality, and its statistical target remains a gene-level reparameterization of the association estimand.

Colocalization analysis provides a critical interface at this stage. Rather than constituting an independent association test, colocalization evaluates whether GWAS and molecular QTL signals share the same underlying causal variant by comparing their posterior distributions. In this sense, colocalization can be understood as an inference on shared causal configuration estimands derived from fine-mapping posterior distributions. This probabilistic framework enables the integration of association and functional evidence across data domains.

Building upon this structure, Mendelian randomization (MR) advances inference from causal probability to causal effect estimands. By using genetic variants as instrumental variables (IVs), MR estimates the causal effect of an exposure (e.g., gene expression or protein level) on an outcome trait, thereby mitigating confounding and reverse causation under three core assumptions: relevance, independence, and exclusion restriction (Hemani et al., 2018; Jiang et al., 2022).

In practice, however, MR is subject to several challenges, including horizontal pleiotropy, complex LD structure, and weak instruments, all of which can introduce bias and inflate false positives (Barfield et al., 2018; Tambets et al., 2024). Moreover, the limited availability of strong and cross-tissue stable cis-eQTL instruments constrains the applicability and reproducibility of MR in causal gene identification (Lu et al., 2024). Recent methodological developments-such as MR-Egger, weighted median estimators, and MR-PRESSO-provide partial robustness to these violations, but their validity critically depends on the structural evidence provided by upstream eQTL/TWAS and colocalization analyses (Hemani et al., 2018; Zhao et al., 2022).

Based on these considerations, we propose a unified causal inference layer in statistical genetics, in which different methods correspond to distinct estimands within a coherent inferential hierarchy:

-GWAS: association estimand

-Fine-mapping: causal probability estimand

-eQTL/TWAS: mediation mapping estimand

-Colocalization: shared causal configuration estimand

-MR: causal effect estimand

Within this framework, complex trait analysis can be formalized as a "functionally integrated causal chain": GWAS → fine-mapping → eQTL/TWAS → colocalization → MR. This pipeline represents a progressive refinement from statistical association to causal effect estimation, where each layer is defined by its estimand, assumptions, and sources of uncertainty. This estimand-driven perspective can also be extended to multi-trait genetics, where shared genetic architecture is distinguished from causal interpretation through structural, locus-level, and pattern-level inference (Fang, 2026).

In this study, we systematically develop this unified framework by clarifying the statistical foundations, assumptions, and limitations of each component, with particular emphasis on their interfaces and error