Quantifying genetic effects on disease mediated by assayed gene expression levels

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Disease variants identified by genome-wide association studies (GWAS) tend to overlap with expression quantitative trait loci (eQTLs), but it remains unclear whether this overlap is driven by gene expression levels ‘mediating’ genetic effects on disease. Here, we introduce a new method, mediated expression score regression (MESC), to estimate disease heritability mediated by the cis genetic component of gene expression levels. We applied MESC to GWAS summary statistics for 42 traits (average \( N = 323,000 \)) and cis-eQTL summary statistics for 48 tissues from the Genotype-Tissue Expression (GTEx) consortium. Averaging across traits, only \( 1\pm 2\% \) of heritability was mediated by assayed gene expression levels. Expression-mediated heritability was enriched in genes with evidence of selective constraint and genes with disease-appropriate annotations. Our results demonstrate that assayed bulk tissue eQTLs, although disease relevant, cannot explain the majority of disease heritability.

In the past decade, GWAS studies have shown that most disease-associated variants lie in noncoding regions of the genome\(^6\), leading to the hypothesis that regulation of gene expression levels is the primary biological mechanism through which genetic variants affect complex traits, and motivating large-scale eQTL studies\(^5\). Many statistical methods have been developed to integrate eQTL data with GWAS data to gain functional insight into the genetic architecture of disease. These methods include colocalization tests, which have shown that many genes have eQTLs that colocalize with GWAS loci\(^2\); transcriptome-wide association studies, which have shown that many genes exhibit significant cis genetic correlations between their expression and disease\(^1\); and partitioning of disease heritability, which has shown that eQTLs as a whole are significantly enriched for disease heritability\(^3\).

Despite these findings, the extent to which eQTLs from available studies capture mechanistic effects of gene expression on disease remains unclear\(^2\). In particular, eQTLs from the largest available gene expression reference panels\(^3\) are measured in bulk tissues in steady-state cellular conditions, which may not reflect the specific cell types or cellular contexts in which gene expression is causal for disease\(^4\). In addition, several different causal scenarios can result in similar patterns of enrichment/overlap between GWAS loci and eQTLs, as summarized in Fig. 1a: (1) mediation, (2) pleiotropy and (3) linkage. Of these three scenarios, only scenario (1) is informative for the mechanism of action of SNPs on disease, but existing methods are unable to consistently distinguish scenarios (2) and (3) from scenario (1). Colocalization tests can sometimes rule out linkage as an explanation for overlap between eQTLs and disease SNPs, but cannot rule out pleiotropy\(^1\). Transcriptome-wide association studies cannot rule out either pleiotropy or linkage\(^2\). Among the methods that partition disease heritability, some aim to rule out linkage through fine mapping of eQTLs\(^5\), but none aim to rule out pleiotropy. Thus, it remains unclear whether enrichment/overlap between eQTLs and disease SNPs usually reflects mediation, or whether it more commonly reflects pleiotropy and/or linkage\(^6\). For example, in the case of autoimmune diseases, most instances of overlap between significant disease loci and immune cell eQTLs are driven by linkage\(^7\), suggesting that linkage may be more prevalent than mediation\(^8\).

In this study, we aim to quantify the proportion of disease heritability mediated in cis by assayed expression levels (scenario (1) from above). We first define expression-mediated heritability under a generative model featuring both mediated and nonmediated (including pleiotropic and linkage) effects of SNPs on the trait. This definition can accommodate ‘assayed’ gene expression levels measured in a tissue or cellular context that are not necessarily causal for the disease. We introduce a method, MESC, to estimate expression-mediated heritability from GWAS summary statistics, linkage disequilibrium (LD) scores and eQTL effect sizes obtained from external expression panels. Intuitively, MESC distinguishes mediated from nonmediated effects in a set of genes via the idea that mediation (unlike pleiotropy and linkage) induces a linear relationship between the magnitude of eQTL effect sizes and disease effect sizes. We applied MESC to GWAS summary statistics for 42 diseases and complex traits and cis-eQTL data for 48 tissues from the GTEx consortium\(^1\) to quantify the proportion of disease heritability mediated by the expression levels of all genes as a whole, as well as by various functional gene sets.

**Results**

**Definition of expression-mediated heritability.** We briefly define heritability mediated by the cis genetic component of gene expression levels (\( h^2_{\text{med}} \)). cis-eQTL effects multiplied by gene-trait effects form an expression-mediated component of each SNP effect on trait (Fig. 1b). This component is then squared and summed across all SNPs to obtain \( h^2_{\text{med}} \) (Fig. 1c,d). Our definition of \( h^2_{\text{med}} \) additionally has two forms: \( h^2_{\text{med,causal}} \), in which cis-eQTL effect sizes are hypothetically obtained in the causal cell types and contexts for the disease, and \( h^2_{\text{med,assayed}} \), in which cis-eQTL effect sizes are obtained from assayed expression levels.
sizes are obtained in a given set of assayed tissues, \( T \) (for example from GTEx), \( h^2_{med,assayed}(T) \) and \( h^2_{med,causal} \) are related by the formula
\[
h^2_{med,assayed}(T) = r^2_T h^2_{med,causal},
\]
where \( r^2_T \) is the average squared genetic correlation between expression in \( T \) and expression in the unobserved causal cell types/contexts for the disease. In practice, we only aim to estimate \( h^2_{med,assayed}(T) \), but it is useful to conceptualize this quantity in terms of \( h^2_{med,causal} \) since \( h^2_{med,causal} \) has a more direct mechanistic interpretation. For brevity, we refer to \( h^2_{med,assayed}(T) \) as simply \( h^2_{med} \), for the remainder of the manuscript, where the set of tissues, \( T \), is implicit.

We also define a quantity \( h^2_{med}(D) \) corresponding to the heritability mediated by the expression levels of gene category \( D \), where \( D \) can be arbitrarily defined over any set of genes (for example, genes in a specific molecular pathway). See Methods for a more detailed definition of \( h^2_{med} \) and \( h^2_{med}(D) \).

**Estimating expression-mediated heritability using MECS.** To estimate \( h^2_{med} \), we propose an approach that involves regressing squared GWAS summary statistics on squared cis-eQTL summary statistics summed across genes (Fig. 1d). Differences in LD between SNPs are captured by conditioning on LD scores (Fig. 1e). In addition, to avoid bias (see Methods), we stratify the regression across both gene categories \( D \) and SNP categories \( C \). The final regression equation used to estimate \( h^2_{med} \) is
\[
E[r^2_D] = N \sum_c \tau_c \epsilon_{k,c} + N \sum_d \pi_d \epsilon_{k,d} + 1
\]
where \( r^2_D \) is the GWAS \( r^2 \) statistic of SNP \( k \), \( N \) is the number of samples, \( \tau_c \) is the per-SNP contribution to nonmediated heritability of SNPs in SNP category \( C \), \( \epsilon_{k,c} \) is the LD score \(^2\) of SNP \( k \) with respect to SNP category \( C \) (defined as \( \epsilon_{k,c} = \sum_{j \in C} r^2_{jk} \)), \( \pi_d \) is the SNP category \( D \) (defined as \( \sum_{j \in D} \sum_{k \in LD} r^2_{jk} \)). Here, \( r_{jk} \) refers to the LD between SNPs \( j \) and \( k \), while \( \beta_k \) refers to the causal cis-eQTL effect size of SNP \( j \) on gene \( i \). \( \epsilon_{k,d} \) can be conceptualized as the total expression \( cis \) heritability of genes in \( D \) that is tagged by SNP \( k \).

**Equation (1)** allows us to estimate \( \pi_d \) and \( \tau_c \) via computationally efficient multiple regression of GWAS \( r^2 \) statistics against LD scores and expression scores. For the equation to provide unbiased estimates of \( \hat{h}^2_{med} \), two main effect-size independence assumptions must be satisfied, violations of which can be addressed via careful partitioning of SNPs and/or genes (Methods and Supplementary Note).

Throughout this study, we present estimates of three quantities that are a function of \( h^2_{med} \) and/or \( h^2_{med}(D) \): (1) the proportion of heritability mediated by expression (defined as \( \hat{h}^2_{med}/\hat{h}^2_{total} \)), (2) the proportion of expression-mediated heritability for gene category \( D \) (defined as \( \hat{h}^2_{med}(D)/\hat{h}^2_{med} \)) and (3) the enrichment of expression-mediated heritability for \( D \) (defined as the proportion of expression-mediated heritability in \( D \) divided by the proportion of genes in \( D \)). We estimate standard errors and \( P \) values for all quantities by jack-knifing over blocks of SNPs \(^2\) (Methods). We have released open source software implementing our method (https://github.com/douglasayao/mesc).
Simulations assessing calibration and bias. We performed simulations to assess the calibration and bias of MESC in estimating $h_{\text{med}}^2/h_s^2$ and its standard error from simulated complex trait and expression data under a variety of genetic architectures (Methods). We performed all simulations using real genotypes from UK Biobank\cite{38} ($N_{\text{GWAS}}=10,000$ GWAS samples; $N_{\text{EQT}}=100–1,000$ expression samples, $M=98,499$ SNPs from chromosome 1).

We evaluated the bias of MESC in estimating various values of $h_{\text{med}}^2/h_s^2$ in the following scenarios: (1) when varying expression panel sample size (Fig. 2a), (2) when varying the proportion of SNPs and genes with nonzero effects (Fig. 2b), (3) when simulating eQTL effect sizes in the gene expression panel that differ from those used to generate the complex trait phenotype, emulating the scenario in which assayed tissues differ from the causal tissue(s) for the disease (Fig. 2c), (4) when using different methods to estimate expression scores (5 in total) (Supplementary Fig. 1), (5) when varying total disease heritability (Supplementary Fig. 2) and (6) when including rare variants and inducing an inverse relationship between eQTL GWAS effect-size magnitude and minor allele frequency (Supplementary Fig. 3), consistent with negative selection acting on both gene expression\cite{39,40} and complex trait\cite{31,42}. We observed that MESC produced unbiased or nearly unbiased estimates of $h_{\text{med}}^2/h_s^2$ across all simulated genetic architectures with expression panel sample size greater than 500 when using the best-performing method to estimate expression scores, namely, LASSO with REML correction (Methods). We note that available expression panel sample sizes for individual tissues are typically smaller than 500, which necessitates meta-analysis across tissues to attain larger expression panel sample sizes (Methods). For scenario (3), we expect, in theory, that MESC will estimate the quantity $r_T^2(T)h_{\text{med(causal)}}^2$ when using expression scores from a noncausal tissue with average squared genetic correlation of expression $r_T^2(T)$ with the causal tissue. Our simulation results support this theoretical expectation.

Next, we assessed the bias of MESC in two biologically plausible scenarios corresponding to violations of the two main effect-size independence assumptions (Methods), and we assessed how well partitioning genes and SNPs ameliorated this bias. The assumptions can be summarized as: (1) gene–eQTL independence, where eQTL and gene effect-size magnitude are independent within each gene category, and (2) pleiotropy–eQTL effect-size independence, where
detected significant heritability enrichment of a SNP category (comprehensive functional SNP annotations) enabled us to obtain genetic component of assayed expression levels ($h_{\text{med}}^2/h_i^2$) for 42 diseases and complex traits from the UK Biobank and other publicly available datasets (average $N=339,000$). See Supplementary Note for the procedure behind selecting these ten traits and Extended Data Fig. 2 for estimates of $h_{\text{med}}^2/h_i^2$ for all 42 traits. Error bars represent jackknife standard errors. For each trait, we report the $h_{\text{med}}^2/h_i^2$ estimate for ‘all tissues’ (expression scores meta-analyzed across all 48 GTEx tissues) and ‘best tissue group’ (expression scores meta-analyzed within seven tissue groups). Here, ‘best’ refers to the tissue group resulting in the highest estimates of $h_{\text{med}}^2/h_i^2$ compared to all other tissue groups. $h_{\text{med}}^2/h_i^2$ estimates meta-analyzed across all 42 traits (average $N=323,000$). Error bars represent standard errors from random-effects meta-analysis. Here, ‘best tissue’ refers to the individual tissue resulting in the highest estimates of $h_{\text{med}}^2/h_i^2$ compared to all other tissues. BMI, body mass index; CNS, central nervous system.

Fig. 3 | Estimates of proportion of heritability mediated by expression from GTEx. a, Estimated proportion of heritability mediated by the cis genetic component of assayed gene expression levels ($h_{\text{med}}^2/h_i^2$) for ten genetically uncorrelated traits (average $N=339,000$). See Supplementary Table 1 for list of traits). In total, we produced three different types of expression scores: (1) expression scores for each individual GTEx tissue, (2) expression scores meta-analyzed within groups of GTEx tissues with common biological origin (Supplementary Table 2) and (3) expression scores meta-analyzed across all 48 GTEx tissues. Each type of expression score was used to estimate $h_{\text{med}}^2/h_i^2$ for each complex trait (Methods). To avoid biases, we partitioned genes by five expression cis-heritability bins and SNPs by the baselineLD model. We performed several analyses evaluating the robustness of these SNP and gene categories, finding that our estimates of $h_{\text{med}}^2/h_i^2$ were similar when using other reasonable choices of SNP and gene categories but very biased when not partitioning genes or SNPs at all (Supplementary Note).

Across all 42 traits, we observed an average $h_{\text{med}}^2/h_i^2$ of 0.11 (s.e. of 0.02) from the all-tissue meta-analyzed expression scores. We did not observe a relationship between $h_{\text{med}}^2/h_i^2$ and $h_{\text{med}}^2/h_i^2$ across traits ($R^2=0.004$) (Extended Data Fig. 1). Of the 42 traits, 26 had $h_{\text{med}}^2/h_i^2$ estimates greater than 0 at nominal significance ($P<0.05$), with 10 reaching Bonferroni significance ($P<0.05/42$). In Fig. 3a, we report $h_{\text{med}}^2/h_i^2$ estimates from all-tissue and tissue-group meta-analyzed expression scores for a representative set of ten genetically uncorrelated traits (full results in Extended Data Fig. 2 and Supplementary Tables 3 and 4). We observed consistently lower estimates of $h_{\text{med}}^2/h_i^2$ from individual-tissue expression scores than from meta-tissue expression scores, as well as a positive correlation between tissue sample size and magnitude of individual-tissue $h_{\text{med}}^2/h_i^2$ ($R^2=0.71$) (Extended Data Fig. 3), suggesting downward biases in individual-tissue $h_{\text{med}}^2/h_i^2$ estimates due to low sample size.

For independent validation, we used cis-eQTL summary statistics from eQTLGen ($N_{\text{eQTL}}=31,684$ in blood only) to estimate $h_{\text{med}}^2/h_i^2$ for the same 42 traits. We obtained very similar $h_{\text{med}}^2/h_i^2$ estimates for GTEx all-tissue expression for blood/immune traits and lower $h_{\text{med}}^2/h_i^2$ for nonblood/immune traits (Extended Data Fig. 4 and Supplementary Table 5), consistent with the fact that eQTLGen only captures expression levels in blood, while GTEx all-tissue meta-analysis captures expression levels across diverse tissues.

Estimation of $h_{\text{med}}^2$ for 42 diseases and complex traits. We applied MESC to estimate the proportion of heritability mediated by the cis genetic component of assayed expression levels ($h_{\text{med}}^2/h_i^2$) for 42 independent diseases and complex traits from the UK Biobank and other publicly available datasets (average $N=323,000$; see Supplementary Table 1 for list of traits). In total, we produced three different types of expression scores: (1) expression scores for each individual GTEx tissue, (2) expression scores meta-analyzed within groups of GTEx tissues with common biological origin (Supplementary Table 2) and (3) expression scores meta-analyzed across all 48 GTEx tissues. Each type of expression score was used to estimate $h_{\text{med}}^2/h_i^2$ for each complex trait (Methods). To avoid biases, we partitioned genes by five expression cis-heritability bins and SNPs by the baselineLD model. We performed several analyses evaluating the robustness of these SNP and gene categories, finding that our estimates of $h_{\text{med}}^2/h_i^2$ were similar when using other reasonable choices of SNP and gene categories but very biased when not partitioning genes or SNPs at all (Supplementary Note).

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Genes with low expression heritability explain more \( h_{med}^2 \). To investigate the relationship between expression \( cis \) heritability (\( h_{cis}^2 \)) and amount of complex trait heritability mediated by those genes, we looked at the proportion of \( h_{med}^2 \) (defined as \( h_{med}^2(D)/h_{med}^2 \) for gene category \( D \)) mediated by genes stratified into ten equally sized bins by their \( h_{cis}^2 \). Across 26 traits with \( h_{med}^2 \) significantly greater than 0, we observed an inverse relationship between meta-tissue \( h_{cis}^2 \) and proportion of \( h_{med}^2 \) across gene bins (Fig. 4 and Supplementary Table 6), with 32% of \( h_{med}^2 \) explained by the lowest two bins (mean meta-tissue \( h_{cis}^2 = 0.014 \)) and only 3% of \( h_{med}^2 \) explained by the highest two bins (mean meta-tissue \( h_{cis}^2 = 0.30 \)). This result implies that genes with less heritable expression (that is, weaker/fewer eQTLs) have substantially larger causal effect sizes on the complex trait.

We considered several reasons why genes with less heritable expression might have larger causal effects on the complex trait. One explanation is that negative selection purifies out strong eQTLs for genes with large effect on complex traits\(^{5,45} \). Alternatively, genes with weaker/fewer eQTLs) may have substantially larger causal effect sizes on the complex trait.

\( h_{med}^2 \) enrichment in functional gene sets. To gain insight into the distribution of expression-mediated effect sizes across various functional gene sets, we estimated \( h_{med}^2 \) enrichment, defined as (proportion of \( h_{med}^2 \))/(proportion of genes), for these gene sets. We analyzed 827 gene sets from three main sources: (1) 10 gene sets reflecting various broad metrics of gene essentiality; (2) 780 gene sets reflecting specific biological pathways, including gene sets from the KEGG\(^{48} \), Reactome\(^{49} \) and Gene Ontology (GO)\(^{50} \) pathway databases; and (3) 37 gene sets comprising genes specifically expressed in 37 different GTEx tissues\(^9 \) (Methods; see Supplementary Table 7 for list of gene sets). We restricted our analyses to large gene sets with at least 200 genes, since we observed large standard errors in \( h_{med}^2 \) enrichment estimates for gene sets with 200 or fewer genes (Supplementary Fig. 4).

Out of 21,502 gene set–complex trait pairs (827 gene sets×26 complex traits), we observed 226 gene set–complex trait pairs (comprising 117 unique gene sets) with false discovery rate (FDR)-significant \( h_{med}^2 \) enrichment (\( q < 0.05 \) accounting for 21,502 hypotheses tested). Significant \( h_{med}^2 \) enrichment estimates ranged from 1.5× to 51× across gene set–complex trait pairs. The full list of \( h_{med}^2 \) enrichment estimates for all 21,502 gene set–complex trait pairs is reported in Supplementary Table 8.

In Fig. 5a, we show \( h_{med}^2 \) enrichment estimates for all ten broadly essential gene sets meta-analyzed across 26 complex traits (individual trait results in Extended Data Fig. 6). We observed Bonferroni-significant meta-trait \( h_{med}^2 \) enrichment (\( P < 0.05/10 \)) for eight gene sets, including ExAC loss-of-function intolerant genes\(^56 \) (3.9× enrichment; \( P = 2.3 \times 10^{-11} \)), FDA-approved drug targets\(^52 \) (5.2× enrichment; \( P = 2.0 \times 10^{-4} \)), genes essential in mice\(^13,35 \) (4.0× enrichment; \( P = 1.1 \times 10^{-2} \)) and genes nearest to GWAS peaks\(^9 \) (3.9× enrichment; \( P = 5.0 \times 10^{-5} \)).

Of the 780 pathway gene sets, we observed that 97 had a significant \( h_{med}^2 \) enrichment (\( q < 0.05 \)) in at least one of the 26 complex traits. In Fig. 5b, we show the \( h_{med}^2 \) enrichment estimates of a representative set of 140 gene set–complex trait pairs (full results in Extended Data Fig. 7). Most gene sets exhibited highly trait-specific patterns of enrichment that were consistent with the known biology of the trait, including fragile X mental retardation protein (FMRP)-interacting genes for schizophrenia\(^57,58 \), Wnt signaling for bone density\(^9 \) and hemostasis for platelet count\(^6 \).

Finally, we investigated whether genes specifically expressed in 37 different GTEx tissues\(^9 \) were enriched for \( h_{med}^2 \). We found significant \( h_{med}^2 \) enrichment (\( q < 0.05 \)) of genes specifically expressed in brain tissues for brain-related traits (schizophrenia and years of education) (Fig. 5c), demonstrating that the complex trait heritability of SNPs near genes specifically expressed in causal tissues (at least for the two traits here) is in part mediated by the expression of those genes.

Given that MESC can be used to prioritize disease-relevant gene sets on the basis of the magnitude of their \( h_{med}^2 \) enrichment, it falls alongside a large class of methods that aim to perform gene-set enrichment analysis from GWAS data\(^{48–52} \). We compared results from MESC to two other popular gene-set enrichment methods applied to the same GWAS summary statistics that we analyzed, MAGMA\(^{48} \) and DEPICK\(^{41} \). We observed that MESC highlighted both broadly concordant and unique gene sets compared with these other methods (Supplementary Note, Extended Data Fig. 8 and Supplementary Table 9).

Discussion

We have developed a new method, MESC, to estimate complex trait heritability mediated by the \( cis \) genetic component of assayed expression levels (\( h_{med}^2 \)) from GWAS summary statistics and eQTL effect sizes estimated from an external expression panel. Our method is distinct from existing methods that identify and quantify overlap between eQTLs and GWAS hits (including colocalization tests\(^{26–28} \), transcriptome-wide association studies\(^{11,14,16} \) and heritability partitioning by eQTL status\(^{13,29} \)) in that it specifically aims to distinguish directional mediated effects from nondirectional pleiotropic and linkage effects. Moreover, our polygenic approach does not require individual eQTLs or GWAS loci to be significant and is not impacted by the sparsity of eQTL effect sizes, so, unlike other approaches\(^{3,13,27} \), we do not exclude genes or SNPs from our analyses on the basis of any significance thresholds. We applied our method to summary statistics for 42 traits and eQTL effect sizes estimated from 48 GTEx tissues. We show that across traits, a significant but modest proportion of complex trait heritability (0.11 ± 0.02) is mediated by the \( cis \) genetic component of assayed expression levels.
Although many previous approaches have hypothesized that SNPs impact complex traits by directly modulating gene expression levels, our results provide concrete genome-wide evidence for this hypothesis. However, the fact that our \( h^2_{med}/h^2_g \) estimates are low for most traits suggests that eQTLs estimated from steady-state expression in bulk postmortem tissues from GTEx do not capture most of the mediated effect of complex trait heritability, motivating additional assays to better identify molecular mechanisms impacted by regulatory GWAS variants.

There are two possible explanations for our low \( h^2_{med}/h^2_g \) estimates:

1. The proportion of complex trait heritability mediated by the cis genetic component of gene expression levels is, in fact, high in causal cell types/contexts for the trait, but eQTL data from bulk assayed tissues from GTEx are a poor proxy for eQTL data in causal cell types/contexts, causing \( h^2_{med}/h^2_g \) to be low. In other words, \( h^2_{med/cis} \) is high, while \( r^2_g(T) \) is low. Low \( r^2_g(T) \) may be addressed by larger assays measuring context-specific expression\(^{33,34}\) and/or single-cell expression\(^{34}\).

2. The proportion of complex trait heritability mediated by the cis genetic component of gene expression levels is low even in causal cell types/contexts for the trait. In particular, complex trait heritability may be mediated in ways other than through gene expression levels in cis, including through protein-coding changes, splicing or expression levels in trans. In these scenarios, additional assays, such as splicing\(^{35}\), histone mark\(^{34}\), chromosome conformation\(^{36}\) and trans-eQTL\(^{37}\) assays can potentially be informative for probing other molecular mechanisms impacted by GWAS variants. We note that much larger gene expression assays than are currently available are necessary to estimate heritability mediated by gene expression levels in trans using MESC (Supplementary Note). We anticipate that MESC can be used to estimate the proportion of disease heritability mediated by future QTL studies beyond cis-eQTLs.

We considered several other explanations for our low \( h^2_{med}/h^2_g \) estimates and justify that they do not apply to our analysis. Our low \( h^2_{med}/h^2_g \) estimates are not related to the fact that expression cis heritability \( h^2_{cis} \) is also low, since the level of environmental/stochastic noise in gene expression measurements does not affect our \( h^2_{med}/h^2_g \) estimates (Supplementary Note). Moreover, our \( h^2_{med}/h^2_g \) estimates are not biased by rare variant effects on gene expression\(^{38,39}\), since we only aim to estimate the proportion of common disease heritability mediated by gene expression levels (Supplementary Note).

We observed that expression scores meta-analyzed across tissues gave us higher estimates of \( h^2_{med}/h^2_g \) than individual-tissue expression scores. This result is consistent with previous studies that reported...
higher heritability enrichment of cis-eQTLs meta-analyzed across all GTEx tissues compared to individual tissues\(^7,9\), higher prediction accuracy for imputed expression using joint prediction from multiple tissues compared to individual tissues\(^9\) and high cis genetic correlations of expression between tissues overall\(^1,7,7^2\).

We observed a strong inverse relationship between proportion of \(h^2_{med}\) and expression cis heritability across genes, suggesting that genes with low expression cis heritability have large effects on complex traits. This result suggests that integrative association tests that prioritize genes on the basis of probability of colocalization between eQTLs and GWAS hits\(^6,9,9\) and/or significance of genetic correlation between expression and trait\(^1^1,1^2\) may not detect the most mechanistically important genes, since these methods have lower power for genes with weaker eQTLs. Instead, our result suggests that genes with weaker eQTLs should be prioritized, and it motivates the implementation of larger eQTL studies and/or cell-type-specific assays to more accurately detect these weak eQTLs.

There are several limitations to our method. First, our method makes the assumption that the magnitude of eQTL effect sizes is uncorrelated with the magnitude of both gene-trait effect sizes and nonmediated effect sizes within each SNP and gene category included in the model. Although we have evaluated the robustness of our choice of SNP and gene categories in both simulations and real data, these assumptions may still be violated. Second, our method relies on the accurate estimation of expression scores from external expression panel samples. For our method to be well powered, it requires large expression panel sample sizes, which can only be obtained through meta-analysis across individual tissues at current sample sizes. Third, the quantity that our method estimates in practice (that is, heritability mediated by assayed gene expression levels) can potentially be much smaller than the theoretical quantity of heritability mediated by expression levels in causal cell types/contexts if assayed gene expression levels do not adequately capture expression levels in causal cell types/contexts. Fourth, our method can only provide reliable \(h^2_{med}\) enrichment estimates for large gene sets of the order of 200 or more genes, so smaller gene pathways or individual genes cannot be prioritized using our method. Fifth, our method does not capture nonadditive effects of SNPs on gene expression or gene expression on trait.

Despite these limitations, our method provides a framework to distinguish mediated effects from pleiotropic and linkage effects and will be useful for quantifying the improvement of new molecular QTL studies over existing assays in capturing regulatory disease mechanisms. Moreover, partitioning mediated heritability can provide insight into regulatory effects mediated by specific gene sets or pathways.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at [https://doi.org/10.1038/s41588-020-0625-2](https://doi.org/10.1038/s41588-020-0625-2).

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Methods

Definition of $h^2_{\text{med}}$. We model trait $y$ for $N$ individuals as follows:

$$y = X\gamma + X\alpha + \varepsilon$$

(2)

where $y$ is an $N$ vector of phenotypes (standardized to mean 0 and variance 1), $X$ is an $N \times M$ genotype matrix for $M$ SNPs (standardized to mean 0 and variance 1), $\gamma$ is an $M$ vector of nominated SNP effect sizes on the trait (including pleiotropic, linkage and trans-eQTL-mediated effects), $B$ is an $M \times M$ matrix of cis-eQTL effect sizes in the causal cell types/contexts for genes, $\alpha$ is a $G$ vector of causal gene expression effect sizes on the trait and $\varepsilon$ is an $N$ vector of environmental effects. We treat all variables as random. We define $h^2_{\text{med}}$ as follows:

$$h^2_{\text{med}} = \text{Var}[X\alpha]$$

Under the assumption that $\gamma$ and $\beta$ are independent of each other, we can rewrite this as follows:

$$h^2_{\text{med};\text{cis}} = \frac{\text{Var}[X\alpha]}{\text{Var} [E[X\beta | B, \alpha]]} = \frac{\text{Var} [E[X\beta | B, \alpha]]}{\text{Var} [E[X\beta | B, \alpha]]}$$

$$= \text{Var} [E[X\beta | B, \alpha]]$$

where $E[X\beta]$ is the average squared per-gene effect of expression on trait and $E[h^2_{\text{med}}]$ is the average cis heritability of expression across all genes. The second line above follows the first because $E[X\beta | B, \alpha] = 0$. We define $h^2_{\text{nomed}}$ in a similar fashion:

$$h^2_{\text{nomed}} = \text{Var}[X\gamma]$$

where $E[X\gamma]$ is the average squared per- SNP effect on trait that is not mediated by gene expression. We consider additional expression causality scenarios, such as reverse mediation, cis-by-trans mediation and mediation by unobserved intermediaries (Supplementary Fig. 8), and we justify that these scenarios do not compromise our definition of $h^2_{\text{nomed}}$ (Supplementary Note).

In practice, expression levels in causal cell types/contexts for the complex trait are probably not assayed. Given a set of assayed tissues $T$ (which may or may not be causal for the complex trait), we define $h^2_{\text{med}}(T)$ as follows:

$$h^2_{\text{med}}(T) = \frac{\text{Var}[X\alpha]}{\text{Var} [E[X\beta | B, \alpha]]}$$

while we define $h^2_{\text{nomed}}(T)$ as $h^2_{\text{nomed}} + (1 - r^2(T)) h^2_{\text{med}}$. Here, $r^2(T) = \frac{\sum_{i=1}^{M} \text{Cov}[X\beta_i, \text{Var}[X\beta]]}{\text{Var}[X\beta]}$ and denotes the average squared genetic correlation between expression in assayed tissues $T$ versus in causal cell types/contexts, where $\beta$ represents cis-eQTL effect sizes on gene $i$ in $T$. Note that $\beta$ can refer to either single tissue or meta-tissue cis-eQTL effect sizes, depending on whether $T$ contains one or multiple tissues.

Unstratified MESC. For illustrative purposes, we walk through a derivation for MESC in the idealized scenario that we know, (1) the true eQTL effect sizes, $\beta$, of each SNP on each gene and (2) the true phenotypic effect sizes, $\alpha$, of each SNP on $y$.

Under our generative model for the trait (equation (2)), the total effect of SNP $k$ on the complex trait is

$$\alpha_k = \sum_{i}^{G} \beta_{ki} \alpha_i + \epsilon_k$$

Given the conditional independence of $\alpha$ and $y$ given $\beta$, upon squaring $\alpha_k$, we have

$$E[\alpha_k^2 | \beta_{ik} \ldots \beta_{ik}] = \sum_{i}^{G} E[\alpha_i^2 | \beta_{ik} \ldots \beta_{ik}] + E[\beta_{ik}^2 | \beta_{ik} \ldots \beta_{ik}]$$

Assuming unconditional independence of $\alpha$ and $y$ which requires that we make additional effect-size independence assumptions involving $\beta$ see Model assumptions), this simplifies to

$$E[\alpha_k^2 | \beta_{ik} \ldots \beta_{ik}] = E[\alpha_i^2 | \beta_{ik} \ldots \beta_{ik}] + E[\beta_{ik}^2]$$

(3)

We use equation (3) to estimate $E[\alpha_k^2]$ by regressing $\alpha_k^2$ for all SNPs on $\sum_i^G \beta_{ik}^2$ and taking the slope, while we estimate $E[\beta_{ik}^2]$ by taking the intercept. See Fig. 1d for a plot illustrating this approach. $E[\alpha_k^2]$ can be multiplied by $\text{GE}[\text{med}]$ to obtain $h^2_{\text{med}}$ while $E[\beta_{ik}^2]$ can be multiplied by $\text{GE}[\text{med}]$ to obtain $h^2_{\text{med}}$.

When we perform this regression using eQTL effect sizes obtained from non-cancerous tissues $T$ with squared genetic correlation $r^2(T)$ with the causal tissues($\delta$), we obtain an estimate of the quantity $h^2_{\text{med}}(T)$ rather than $h^2_{\text{med}}$ (Supplementary Note). Moreover, in practice we perform this regression using GWAS and eQTL summary statistics, in which case we account for differences in LD between SNPs with an LD score covariate (see Supplementary Note for derivation and regression equation).

Model assumptions. The two main effect-size independence assumptions that are needed to derive equation (3) are:

1. Across all genes, the magnitude of gene effect sizes is uncorrelated with the magnitude of eQTL effect sizes (that is, $\text{Corr}(\alpha, \beta^2) = 0$). We refer to this assumption as gene–eQTL effect-size independence.
2. Across all SNPs, the magnitude of non-mediated effect sizes is uncorrelated with the magnitude of eQTL effect sizes (that is, $\text{Corr}(\beta^2, \beta^2) = 0$). We refer to this assumption as pleiotropy–eQTL effect-size independence.

Violations of either of these two assumptions will result in biased estimates of $h^2$, where the direction of bias is the same as the direction of correlation between eQTL effect-size magnitude and gene or non-mediated effect-size magnitude. See Supplementary Note for a discussion of realistic scenarios in which these assumptions might be violated, as well as an illustration of how conditioning on SNP- and gene-level annotations can ameliorate any resulting bias.

Stratified MESC. In this section, we extend unstratified MESC to estimate $h^2_{\text{med}}$ partitioned over groups of genes. Note that stratified MESC can be viewed as a special form of stratified LD score regression (Supplementary Note). Given $D$ potentially overlapping gene categories $D_1, \ldots, D_D$, we define $h^2_{\text{med}}$ partitioned over gene categories as follows:

$$h^2_{\text{med}}(D_k) = \frac{\sum_{i \in D_k} \alpha_i^2 \beta_i^2}{\text{Var} [E[X\beta | B, \alpha]]}$$

where $h^2_{\text{med}}(D_k)$ is the heritability mediated in cis through the expression of genes in category $D_k$, $\text{Var} [E[X\beta | B, \alpha]]$ is the average squared causal effect of expression on trait for genes in $D_k$ and $E[h^2_{\text{med}}(i | \in D_k)]$ is the average cis heritability of expression of genes in $D_k$. Similar to our definition of $h^2_{\text{med}}$, the second line above relies on an independence assumption between $\alpha$ and $\beta$, namely that $\alpha_i \perp \beta_i | i \in D_k$.

For gene $i$, we model the variance of gene effect size $\alpha_i$ as

$$\text{Var}(\alpha_i) = \sum_{d \in D_k} x_{id}$$

If gene categories $D_k$ form a disjoint partition of the set of all genes, we have

$$x_{id} = \frac{\text{Var}(\alpha_i) | \alpha_i \in D_k}{\text{Var}(\alpha_i | \alpha_i \in D_k)}$$

On the other hand, if gene categories are overlapping, then $x_{id}$ can be conceptualized as the contribution of annotation $D_k$ to $h^2_{\text{med}}$ conditional on contributions from all other gene categories included in the model.

Given $C$ potentially overlapping SNP categories $C_1, \ldots, C_C$, we define $h^2_{\text{med}}$ partitioned over $C$ categories as follows:

$$h^2_{\text{med}}(C_k) = \frac{\sum_{j \in C_k} \alpha_j^2}{\text{Var} [E[X\beta | B, \alpha]]}$$

where $h^2_{\text{med}}(C_k)$ is the heritability mediated by SNPs in category $C_k$, $C_k$ is the number of SNPs in $C_k$, and $E[h^2_{\text{med}} | j \in C_k]$ is the average squared non-mediated effect size of SNPs in $C_k$.

For SNP $j$, we model the variance of non-mediated effect size $\alpha_j$ as follows:

$$\text{Var}(\alpha_j) = \sum_{c \in C_j} x_{jc}$$

If SNP categories $C_k$ form a disjoint partition of the set of all SNPs, we have

$$x_{jc} = \frac{\text{Var}(\alpha_j) | \alpha_j \in C_k}{\text{Var}(\alpha_j | \alpha_j \in C_k)}$$

On the other hand, if SNP categories are overlapping, then $x_{jc}$ can be conceptualized as the contribution of annotation $C_k$ to $h^2_{\text{med}}$ conditional on contributions from all other SNP categories included in the model.

The equation for stratified MESC is

$$E[Z^2] = N \sum_{c} \ell_N + N \sum_{d} \ell_{D, c} + 1$$

(4)

where $Z^2$ is the GWAS $\chi^2$ statistic of SNP $k$, $N$ is the number of samples, $\ell_N$ is the LD score of SNP $k$ with respect to SNP category $C_k$ (defined as $\ell_N = \sum_{c \in C_k} \ell_{c}$ and $L_{c, d}$ is the expression score of SNP $k$ with respect to gene category $D_d$ (defined as $L_{c, d} = \sum_{i \in c} \sum_{j \in d} \ell_{c, ij}^2$). Here, $\ell_{c}$ refers to the LD between
SNPs $j$ and $k$. See Supplementary Note for a derivation of this equation. Analogous to unstratified MESC, when we perform this regression using expression scores in assayed tissues $T$ rather than expression scores in causal cell types/contexts, we will estimate $\hat{\beta}_{cis}^{D^2} = \sum_{D} \sum_{D} \phi_{cis}(D^2) \tilde{D}_{cis}^{D^2}(D^2) \tilde{D}_{cis}^{D^2}(D^2)$, where $\phi_{cis}(D^2)$ is the average squared genetic correlation of expression between $T$ and causal cell types/contexts for genes in $D^2$.

**Estimation of expression scores.** To carry out the regression described in equation (3), we first estimate expression scores $\hat{L}_{cis}^{D^2}$ from an external expression panel. We estimate $\hat{L}_{cis}^{D^2}$ from either eQTL summary statistics or individual-level genotypes and expression measurements, where the latter provides less noisy estimates of $\hat{L}_{cis}^{D^2}$ where it is available. In our case, we use the first procedure to estimate expression scores from eQTLGen data (since only eQTL summary statistics are provided), whereas we used the second procedure for GTEx data.

**eQTL summary statistics.** We can estimate $\hat{L}_{cis}^{D^2}$ from eQTL summary statistics using the following formula: $\hat{L}_{cis}^{D^2} = \sum_{D} \sum_{D} \phi_{cis}(D^2) \tilde{D}_{cis}^{D^2}(D^2) \tilde{D}_{cis}^{D^2}(D^2)$ from the right-hand side of the formula is in expectation equal to $\hat{L}_{cis}^{D^2}$ (Supplementary Note).

**Individual-level genotypes and expression data.** We estimate $\hat{L}_{cis}^{D^2}$ by first using LASSO to obtain regularized estimates of causal eQTL effect sizes ($\hat{h}_{cis}^2$), then multiply $\hat{h}_{cis}^2$ by the element-wise squared LD matrix $R^2$ as follows: $\hat{L}_{cis}^{D^2} = \sum_{D} \sum_{D} \rho_{cis}(\hat{h}_{cis}^2)$, where $\rho_{cis}$ is a scaling factor we apply to $\hat{L}_{cis}^{D^2}$ so that $\rho_{cis} \in [0, 1]$ for each gene and $\hat{L}_{cis}^{D^2}$ is the restricted maximum likelihood (REML) estimate of expression cis-heritability for gene $i$. We observed that scaling our estimates in this manner reduces noise and bias compared to unscaled estimates (Supplementary Fig. 9). We obtain approximately unbiased estimates of the squared LD between two SNPs using the formula $\rho_{cis}^2 = \rho^2 - \frac{\rho^2}{\rho^2}$, where $\rho^2$ denotes the standard unbiased estimator of $\rho^2$. We refer to this overall procedure as ‘LASSO with REMEL correction’ and show that it provides the best performance in simulations compared to other methods (Supplementary Note).

**Meta-analysis of expression scores.** Given our method of computing expression scores from individual-level genotypes and expression data outlined above (‘Estimation of expression scores’), we meta-analyze expression scores across tissues as follows. We first obtain meta-tissue expression cis-heritability ($\hat{h}_{cis}^2$) estimates for each gene by averaging individual-tissue $\hat{h}_{cis}^2$ estimates across tissues. We scale individual-tissue LASSO-predicted causal eQTL effect sizes to the meta-tissue $\hat{h}_{cis}^2$, then average the scaled causal eQTL effect sizes across tissues. Finally, we multiply the averaged causal eQTL effect sizes by the element-wise squared LD matrix to obtain expression scores. In simulations, we show that this method of meta-analyzing expression scores produces nearly unbiased estimates of $\rho_{cis}^2$ at five tissues 200 samples per tissue (Supplementary Fig. 10), which is comparable to the number expression panel samples in a given tissue group (Supplementary Table 2).

**Simulations.** All simulations were conducted using genotypes from UK Biobank for a random subset of Assay ID 3 SNPs on chromosome 1 ($N = 98,499$ SNPs). All simulations followed the same overall procedure outlined below in chronological order. See Supplementary Note for specific parameters used in each simulation.

1. Simulation of expression data. We simulated 1–5 $Q$ eQTLs each for $G = 1,000$ genes, with effect sizes drawn from a normal distribution and locations randomly selected in a 1 megabase (Mb) window around the gene. Total $\hat{h}_{cis}^2$ was fixed at 0.05 for all simulations. We then simulated expression phenotypes for 100–1,000 expression panel samples (genotypes randomly selected from UK Biobank) using an additive generative model with normally distributed environmental noise added, representing an expression panel.
2. Simulation of GWAS data. We simulated nonmediated SNP effect sizes and gene–trait effect sizes from normal or point-normal distributions for all SNPs and genes corresponding to various levels of $\hat{h}_{cis}^2$. Total $\hat{h}_{cis}^2$ was fixed at 0.5 for all simulations (other than for Supplementary Fig. 2, in which we varied $\hat{h}_{cis}^2$). Together with the eQTL effect sizes simulated in the previous step, we used these effect sizes to simulate trait phenotypes using an additive generative model with normally distributed environmental noise added for 10,000 GWAS samples (genotypes randomly selected from UK Biobank and distinct from the expression panel samples). We then produced GWAS summary statistics from this simulated dataset using customary least squares.
3. Estimation of expression scores. We estimated expression scores from the expression panel samples using LASSO with REMEL correction (see Estimation of expression scores). For computational ease, we did not actually use REMEL to predict expression cis-heritability for each gene in each simulation; instead, we took the true expression cis-heritability of the gene and added noise drawn from $N(0,0.017)$ to simulate REMEL prediction error, which is consistent with empirical standard error estimates produced by GCTA (Supplementary Fig. 11).
4. Estimation of $\hat{h}_{cis}^2$. We estimated $\hat{h}_{cis}^2$ using MESC with the previously estimated expression scores, in-sample LD scores (computed from the 10,000 GWAS samples) and GWAS summary statistics.

**Data and quality control.** Genotypes. For MESC, we used European samples in 1000 Genomes as reference SNPs to compute LD scores. Regression samples were obtained from HapMap 3 (ref. 3). Notably, by restricting regression SNPs to HapMap 3 SNPs, we estimate ‘common’ disease heritability mediated by gene expression levels (see Supplementary Note for discussion of rare versus common variant $\hat{h}_{cis}^2$), SNPs with GWAS $P$-values $>\max(0.001, N)$ (where $N$ is the number of GWAS samples) and in the major histocompatibility complex region were excluded. See the Supplementary Note of ref. 4 for justification of these procedures.

For computing expression scores, we downloaded genotypes derived from sequencing data for GTEx v.7 from the GTEx portal (see Data availability) as described in ref. 1. We retained SNPs that were from HapMap 3 (ref. 3).

**Expression data.** We obtained processed and quantile-normalized gene expression data for GTEx v.7 from the GTEx portal (see Data availability) as described in ref. 1. For each tissue, the following covariates were included in all analyses: three genetic principal components, sex, platform and 14–35 expression factors as selected by the main GTEx analysis.

**Estimation of expression scores from GTEx data.** We used REMEL as implemented in GCTA to estimate the expression cis-heritability for each gene in each individual GTEx tissue. We then used LASSO as implemented in PLINK (with the LASSO tuning parameter set as the estimated expression cis-heritability of the gene) to estimate eQTL effect sizes for each gene in each individual GTEx tissue. In all procedures, we excluded gene–tissue pairs for which LASSO did not converge when predicting effect sizes. For Fig. 3 and Extended Data Fig. 2, we obtained causal eQTL effect-size estimates in three different ways.

**Meta-analysis across all tissues.** For each gene, we averaged the expression cis-heritability estimates across all 48 tissues. Within each tissue, we scaled the LASSO-predicted eQTL effect sizes to the averaged cis-heritability value. We then averaged the scaled eQTL effect sizes for each gene across all tissues. Genes were retained if they had a LASSO-converged eQTL effect size in at least one tissue.

**Meta-analysis in tissue groups.** Of the 48 tissues, we grouped together 37 of them into seven broad tissue groups: adipose, blood/immune, cardiovascular, central nervous system, digestive, endocrine and skin (Supplementary Table 2). Within each tissue group, we averaged the expression cis-heritability estimates for each gene and scaled the LASSO-predicted eQTL effect sizes to the averaged cis-heritability value. We then averaged the scaled eQTL effect sizes for each gene across the tissues for each tissue group. Genes were retained in each tissue group if they had a LASSO-converged eQTL effect size in at least one tissue within that tissue group.

**Individual tissues.** For each individual tissue, we scaled the LASSO-predicted eQTL effect sizes to the within-tissue group averaged cis-heritability estimates.

The final eQTL effect sizes were then multiplied by the element-wise squared LD matrix (estimated from 1000 Genomes) to order to obtain expression scores (see Estimation of expression scores).

**Set of 42 independent traits.** Analogous to previous studies, we initially considered a set of 34 traits from publicly available sources and 55 traits from UK Biobank for which GWAS summary statistics had been computed using BOLT-LMM v.2.3 (refs. 7,8) (see Data availability). We restricted our analysis to 47 traits with $z$ scores of total SNP heritability above 6 (computed using stratified LD score regression). The 47 traits included five traits that were duplicated across two datasets (genetic correlation of at least 0.9). For duplicated traits, we retained the dataset with the larger sample size, leaving us with a total of 42 independent traits. When meta-analyzing estimates across traits, we performed random-effects meta-analysis using the R package metareg.

**BaselineLD categories.** In all our analyses, we stratified SNPs by 72 functional categories specified by the baselineLD model v.2.0 (refs. 14,5) (see Data availability). These annotations include coding, conserved, regulatory (for example, promoter, enhancer, histone marks, transcription factor binding sites) and LD-related annotations. The original baselineLD model v.2.0 contains 76 total categories; we retained four categories corresponding to QTL MaxCPP annotations because the information contained in these annotations is redundant with the eQTL effect-size information contained in expression scores.

**Gene-set analyses.** To obtain unbiased estimates of $\hat{h}_{cis}^2$ enrichment for the genes in our analysis, we must ensure that the gene–eQTL effect-size independence assumption holds within each gene set (see Model assumption). To this end, we captured potential correlations between the magnitude of eQTL effect sizes and gene–trait effect sizes within gene sets, we partitioned each gene set into three equally sized bins on the basis of the magnitude of their expression cis-heritability relative to other genes.
in the gene set. We then estimated $\hat{h}^2_{\text{reg}}(D)$ for each individual bin and aggregated these values together to estimate the overall $\hat{h}^2_{\text{reg}}$ enrichment of the gene set.

**Broad gene sets.** We obtained gene sets corresponding to all coding genes, genes near significant GWAS hits in the NHGRI GWAS catalog, genes essential in mice, genes essential in cultured cell lines, genes with any disease association in ClinVar and genes that are FDA-approved drug targets, from the Macarthur laboratory GitHub page (see Data availability). We obtained an additional gene set for genes essential in cell lines, genes depleted for protein-truncating mutations, and genes depleted for missense mutations from the supplementary data of the respective papers.

**Pathway gene sets.** We initially considered a set of 7,246 gene sets from the `canonical pathways' and 'GO gene sets' collections from the Molecular Signatures Database (see Data availability), consisting of gene sets from BioCarta, Reactome, KEGG, GO, PID and other sources. We restricted our analysis to 780 gene sets for which the number of genes with LASSO estimates of eQTL effect sizes that converged in individual GTEx tissues was at least 100, when averaged across all individual tissues. Note that this roughly corresponds to gene sets with greater than 200 total genes; see Supplementary Table 7.

**Tissue-specific expression gene sets.** We initially considered the full set of 48 GTEx tissues. We restricted our analysis to 37 gene sets for which the focal tissue belonged to one of the seven main tissue groups we defined in our previous analyses (Supplementary Table 2). From ref. 27, we obtained the set of 10% most specifically expressed genes in each of the 37 tissues.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
GWAS summary statistics for 42 diseases and complex traits can be found at https://data.broadinstitute.org/alkesgroup/sumstats_formatted/. Genotypes for 1000 Genomes Phase 3 data can be found at ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502. GTEx v.7 data can be found at https://www.gtexportal.org/home/datasets, although to access genotypes one is required to have an approved application. eQTLGen data can be found at https://www.eqtlgen.org/cx-eqtl. html. BaselineLD v2.0 annotations can be found at https://data.broadinstitute.org/alkesgroup/LDSCORE/. Gene sets can be found from the Macarthur laboratory, https://github.com/macarthur-lab/gene_lists, and Molecular Signatures Database, http://software.broadinstitute.org/gsea/msigdb/collections.jsp. S-LDSC software can be found at https://github.com/bulk/idsc, BOLT-LMM software can be found at https://data.broadinstitute.org/alkesgroup/BOLT-LMM/downloads/.

**Code availability**
Software implementing MESC can be found at https://github.com/douglasyao/mesc.

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**Author contributions**
D.W.Y., L.J.O., A.L.P. and A.G. conceived the project. D.W.Y. and A.G. designed experiments. D.W.Y. performed the experiments and analyzed the data. D.W.Y. and A.G. wrote the manuscript with input from L.J.O. and A.L.P.

**Competing interests**
The authors declare no competing interests.

**Additional information**
Extended data is available for this paper at https://doi.org/10.1038/s41588-020-0625-2.
Supplementary information is available for this paper at https://doi.org/10.1038/s41588-020-0625-2.
Correspondence and requests for materials should be addressed to D.W.Y. or A.G.
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Extended Data Fig. 1 | Relationship between \( h^2_{\text{med}} \) and \( h^2_g \). \( h^2_{\text{med}} \) estimates were obtained using all-tissue meta-analyzed expression scores. \( h^2_g \) estimates were obtained using stratified LD-score regression. Error bars represent jackknife standard errors.
Extended Data Fig. 2 | $h^2_{\text{med}}/h^2_g$ estimates for all diseases and expression scores. Same as Fig. 3a, but containing $h^2_{\text{med}}/h^2_g$ estimates for all 42 traits from all three types of expression scores: ‘All tissues’ (expression scores meta-analyzed across all 48 GTEx tissues), ‘Best tissue group’ (expression scores meta-analyzed within 7 tissue groups), and ‘Best tissue’ (expression scores computed within individual tissues). Here, ‘best’ refers to the tissue/tissue group resulting in the highest estimates of $h^2_{\text{med}}/h^2_g$ compared to all other tissues/tissue groups. Error bars represent jackknife standard errors.
Extended Data Fig. 3 | Relationship between individual tissue sample size and magnitude of $h^2_{med}/h^2_g$, $h^2_{med}/h^2_g$ estimates from expression scores estimated in each of 48 individual GTEx tissues were meta-analyzed across 42 complex traits, then plotted against the number of samples in each tissue. We use the following abbreviations: adipose visceral, adipose visceral omentum; brain ACC, brain anterior cingulate cortex BA24; brain CBG, brain caudate basal ganglia; brain CH, brain cerebellar hemisphere; brain FC, brain frontal cortex BA9; brain nABG, brain nucleus accumbens basal ganglia; brain PBG brain putamen basal ganglia; cells CETL, cells EBV-transformed lymphocytes; cells TF, cells transformed fibroblasts; esophagus GJ, esophagus gastroesophageal junction; heart AA, heart atrial appendage; heart LV, heart left ventricle; skin NSES, skin not sun exposed suprapubic; skin SELL, skin sun exposed lower leg; small intestine, small intestine terminal ileum.
Extended Data Fig. 4 | $h_{med}^2/h_g^2$ estimates for 42 diseases and complex traits using data from eQTGen. We estimated expression scores for all SNPs using cis-eQTL summary statistics from eQTGen ($N = 31,684$ blood samples), then estimated $h_{med}^2/h_g^2$ using GWAS summary statistics for the same 42 traits analyzed in the main text. Expression cis-heritability estimates for eQTGen data were obtained using LD-score regression. For sake of comparison, we also display $h_{med}^2/h_g^2$ estimates obtained from expression scores from GTEx all-tissue meta-analysis and GTEx whole blood only. (a) $h_{med}^2/h_g^2$ estimates for 42 individual traits, organized into blood/immune and non-blood/immune traits. Error bars represent jackknife standard errors. (b) Results from a meta-analyzed across traits. Error bars represent standard errors from random-effects meta-analysis. Note that low estimates of $h_{med}^2/h_g^2$ for GTEx whole blood expression scores are caused by the small sample size of the GTEx whole blood data set ($N = 369$).
Extended Data Fig. 5 | Relationship between expression cis-heritability and metrics of gene essentiality. For each gene, pLI (probability of loss-of-function intolerance) was obtained from Lek et al. 2016 Nature and $s_{\text{hel}}$ (selection against protein-truncating variants) was obtained from Cassa et al. 2017 Nature Genetics.
Extended Data Fig. 6 | $h^2_{med}$ enrichment estimates for all 10 broadly essential gene sets across all 26 complex traits. Same as Fig. 5a, but showing $h^2_{med}$ enrichment estimates for individual traits rather than meta-analyzed estimates.
Extended Data Fig. 7 | $h^2_{med}$ enrichment estimates for 97 pathway-specific gene sets across all 26 complex traits. Same as Fig. 5b, but plotting all pathway-specific gene sets (out of 780 total) with FDR-significant $h^2_{med}$ enrichment in at least one of the 26 complex traits. For ease of display, we grouped together related traits and gene sets.
Extended Data Fig. 8 | Comparison between gene set enrichment estimates from MESC, MAGMA, and DEPICT. See Supplementary Note for details on these analyses. (a) Venn diagram showing the overlap between significantly enriched trait-gene set pairs (FDR < 0.05) identified by the three methods. (b) Scatterplots of -log10 enrichment p-values from MESC vs. MAGMA (left), MESC vs. DEPICT (middle), and MAGMA vs. DEPICT (right). Each point represents a trait-gene set pair. (c) List of all 32 gene sets–complex traits pairs detected as significant by MESC (FDR q-value < 0.05) that are not detected as significant by MAGMA or DEPICT. See Supplementary Table 9 for enrichment estimates for all gene set–complex traits pairs.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection We analyzed existing datasets. Thus, no software was used to collect data.

Data analysis S-LDSC v1.0.1 software was used: https://github.com/bulik/Ldsc. BOLT-LMM v2.3.4 software was used: https://data.broadinstitute.org/alkesgroup/BOLT-LMM/downloads/. Custom MESC software is available at https://github.com/douglasyao/mesc.

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## Life sciences study design

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| Sample size       | Our work is based on existing summary statistics. We restricted our analyses to data sets for which the z-score of SNP heritability is larger than 6. |
|-------------------|----------------------------------------------------------------------------------------------------------|
| Data exclusions   | Our study was restricted to data sets of European ancestry. No data was excluded from European individuals. |
| Replication       | We analyzed existing data sets. No replication was performed.                                            |
| Randomization     | We analyzed existing data sets. Thus, no randomization was performed.                                      |
| Blinding          | We analyzed existing data sets. Thus, blinding was irrelevant to our work.                                |

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| Population characteristics | Our study involved publicly available data sets (e.g. UK Biobank and existing summary statistics). We restricted our data to individuals of European ancestry. |
|----------------------------|-----------------------------------------------------------------------------------------------------------------|
| Recruitment                | We analyzed existing data sets. Thus, no recruitment was performed.                                                |
| Ethics oversight           | We analyzed existing data sets. Thus, ethics oversight was irrelevant to our work.                                 |

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