

¹ Transcripts with high distal heritability mediate genetic effects on
² complex traits

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⁴ Anna L Tyler, J Matthew Mahoney, Mark P Keller, Candice N. Baker, Margaret Gaca, Anuj Srivastava,
⁵ Isabela Gerdes Gyuricza, Madeleine Braun, Nadia A Rosenthal, Alan D Attie, Gary A Churchill and Gregory
⁶ W Carter

⁷ **Abstract**

⁸ Gene expression is an important mediator of genetic effects on phenotype. Although many genes are subject
⁹ to simple, local regulation, recent evidence suggests that complex distal regulation may be more important
¹⁰ in mediating trait variability. To investigate this possibility, we combined two large, data sets modeling
¹¹ diet-induced obesity and metabolic disease in genetically diverse mice. Using a novel high-dimensional
¹² mediation analysis, we identified a heritable composite transcript that explained 30% of the variation across
¹³ all metabolic traits. The composite transcript was interpretable in terms of enriched biological processes
¹⁴ and predicted obesity status in an independent mouse cohort as well as in human cohorts with measured
¹⁵ gene expression. Transcripts contributing most strongly to this composite mediator tended to have complex,
¹⁶ distal regulation distributed throughout the genome. These results suggest that trait-relevant variation in
¹⁷ transcription is largely distally regulated, but is nonetheless identifiable, interpretable, and translatable across
¹⁸ species.

¹⁹ **Introduction**

²⁰ In the quest to understand the genetic architecture of complex traits, gene expression is an important mediator
²¹ between genotype and phenotype. There is ample evidence from genome-wide association studies (GWAS)
²² that regulation of gene expression accounts for the bulk of the genetic effect on complex traits, as most
²³ trait-associated variants lie in gene regulatory regions^{1–7}. It is widely assumed that these variants influence
²⁴ local transcription, and methods such as transcriptome-wide association studies (TWAS)^{8–11}, summary
²⁵ data-based Mendelian randomization (SMR)¹⁰, and others capitalize on this idea to identify genes associated

26 with multiple disease traits^{12–15}

27 Despite the great promise of these methods, explaining trait effects with local gene regulation has been more
28 difficult than initially assumed^{16;17}. Although trait-associated variants tend to lie in non-coding, regulatory
29 regions, they often do not have detectable effects on gene expression¹⁶ and tend not to co-localize with
30 expression quantitative trait loci (eQTLs)^{17;18}.

31 One possible explanation for these observations is that gene expression is not being measured in the appropriate
32 cell types and thus local eQTLs influencing traits cannot be detected¹⁶. An alternative explanation that has
33 been discussed in recent years is that effects of these variants are mediated not through local regulation of
34 gene expression, but through distal regulation^{18–20;15}.

35 In this model, a gene's expression is influenced by many variants throughout the genome through their
36 cumulative effects on a broader regulatory network. In other words, the heritable component of the
37 transcriptome is an emergent state arising from the myriad molecular interactions defining and constraining
38 gene expression.

39 To assess the role of wide-spread distal gene regulation on complex traits, we investigated diet-induced obesity
40 and metabolic disease as an archetypal example. Diet-induced obesity and metabolic disease are genetically
41 complex with hundreds of variants mapped through GWAS [REFS]. These variants are known to act through
42 multiple tissues that interact dynamically with each other [REFS], including adipose tissue, pancreatic
43 islets, liver, and skeletal muscle. The multi-system etiology of metabolic disease complicates mechanistic
44 dissection of the genetic architecture, requiring large, dedicated data sets that include high-dimensional,
45 clinically relevant phenotyping, dense genotyping in a highly recombined population, and transcriptome-wide
46 measurements of gene expression in multiple tissues.

47 Measuring gene expression in multiple tissues is critical to adequately assess the extent to which local gene
48 regulation varies across the tissues and whether such variability might account for previous failed attempts to
49 identify trait-relevant local eQTL. Such data sets are extremely difficult to obtain in human populations,
50 particularly in the large numbers of subjects required for adequate statistical power. Thus, to further
51 investigate the role of local and distal gene regulation on complex traits, we generated two complementary
52 data sets: A discovery data set in a large population of diversity outbred (DO) mice²¹, and an independent
53 validation data set derived by crossing inbred strains from the Collaborative Cross (CC) mice²² to form CC
54 F1 mice (CC-RIX). Both populations modeled diet-induced obesity and metabolic disease¹²

55 The DO population and CC recombinant inbred lines were derived from the same eight inbred founder mouse
56 strains, five classical lab strains, and three strains more recently derived from wild mice²¹. They represent

57 three subspecies of mouse *Mus musculus domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*,
58 and capture 90% of the known variation in laboratory mice²³. The DO mice are maintained with a breeding
59 scheme that ensures equal contributions from each founder across the genome thus rendering almost the
60 whole genome visible to genetic inquiry²¹. The CC mice were initially outcrossed to recombine the genomes
61 from all eight founders, and then inbred for at least 20 generations to generate multiple inbred lines. Because
62 these two populations have common ancestral haplotypes we could directly and unambiguously compare
63 the local genetic effects on gene expression at the whole-transcriptome level while varying the population
64 structure driving distal regulation.

65 In the DO population, we paired clinically relevant metabolic traits from 500 mice [REF], including body
66 weight, plasma levels of insulin and glucose and plasma lipids, with transcriptome-wide gene expression in
67 four tissues related to metabolic disease: adipose tissue, pancreatic islets, liver, and skeletal muscle. We
68 measured similar metabolic traits in the CC-RIX and gene expression from three of the four tissues used
69 in the DO: adipose tissue, liver, and skeletal muscle. Because the CC-RIX carry the same founder alleles
70 as the DO, local gene regulation is expected to match between the populations, but because the alleles are
71 recombined through the genome, distal effects are expected to vary from those in the DO, allowing us to
72 directly assess the role of local gene regulation in driving trait-associated transcript variation. Together, these
73 data enable a comprehensive view into the genetic architecture of metabolic disease.

74 Results

75 To comprehensively assess the genetic control of gene expression in metabolic disease in mice, we assayed
76 metabolic traits and multi-tissue gene expression in DO mice.

77 Genetic variation contributed to wide phenotypic variation

78 Although the environment was consistent across all animals, the genetic diversity present in the DO population
79 resulted in widely varying distributions across physiological measurements (Fig. 1). For example, body
80 weights of adult individuals varied from less than the average adult B6 body weight to several times the body
81 weight of a B6 adult in both sexes (Males: 18.5 - 69.1g, Females: 16.0 - 54.8g) (Fig. 1A). Fasting blood
82 glucose (FBG) also varied considerably (Fig. 1B), although few of the animals had FBG levels that would
83 indicate pre-diabetes (19 animals, 3.8%), or diabetes (7 animals, 1.4%) according to previously developed
84 cutoffs (pre-diabetes: FBG \geq 250 mg/dL, diabetes: FBG \geq 300, mg/dL)²⁴. Males had higher FBG than
85 females on average (Fig. 1C) as has been observed before suggesting either that males were more susceptible
86 to metabolic disease on the high-fat diet, or that males and females may require different thresholds for

87 pre-diabetes and diabetes.

88 Body weight was strongly positively correlated with food consumption (Fig. 1D $R^2 = 0.51$, $p < 2.2 \times 10^{-16}$)
 89 and fasting blood glucose (FBG) (Fig. 1E, $R^2 = 0.21$, $p < 2.2 \times 10^{-16}$) suggesting a link between behavioral
 90 factors and metabolic disease. However, the heritability of this trait and others (Fig. 1F) indicates that
 91 background genetics contribute substantially to correlates of metabolic disease in this population.

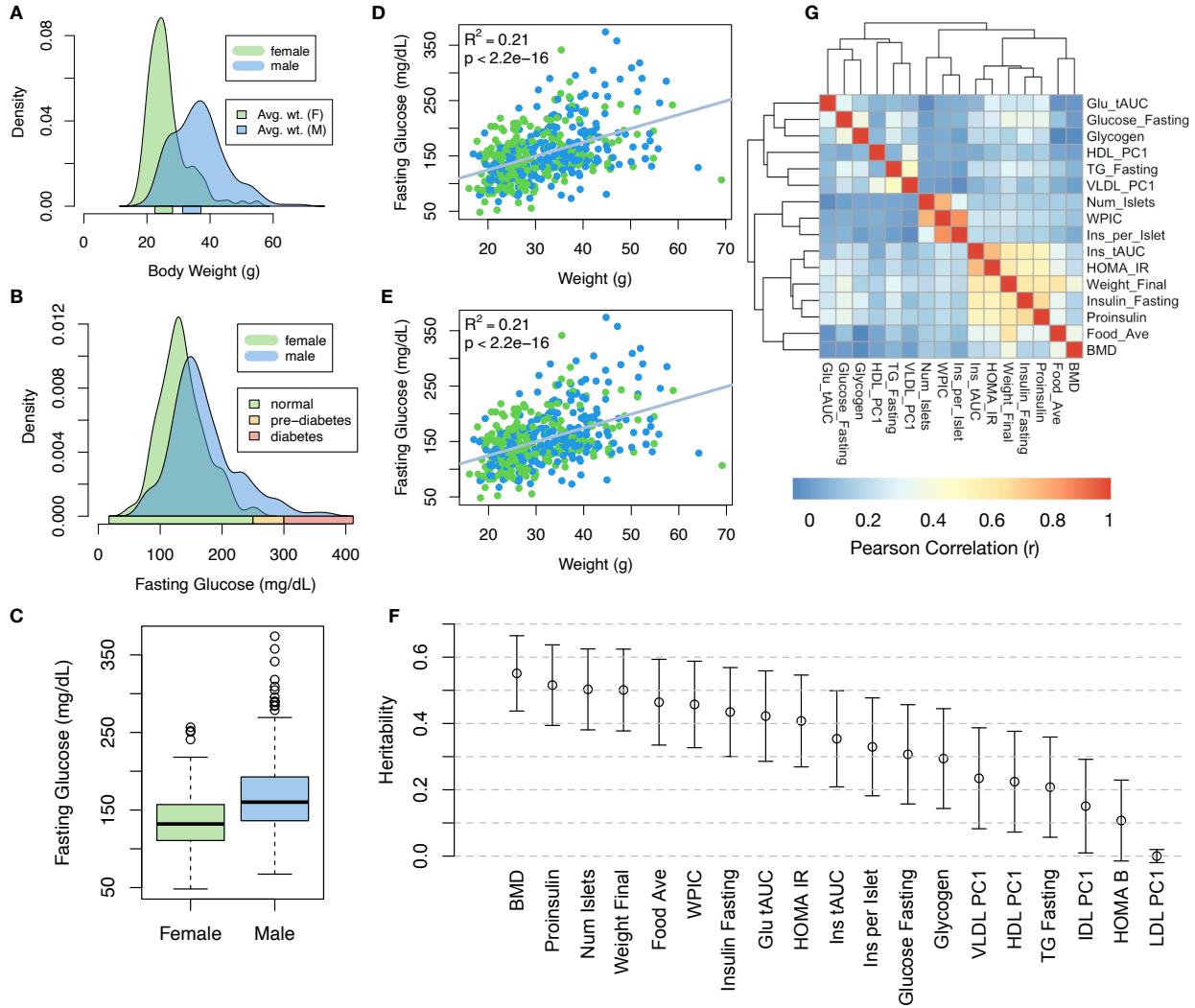


Figure 1: Clinical overview. **A.** Distributions of final body weight in the diversity outbred mice. Sex is indicated by color. The average B6 male and female adult weights at 24 weeks of age are indicated by blue and green bars on the x-axis. **B.** The distribution of final fasting glucose across the population split by sex. Normal, pre-diabetic, and diabetic fasting glucose levels for mice are shown by colored bars along the x-axis. **C.** Males had higher fasting blood glucose on average than females. **D.** The relationship between food consumption and body weight for both sexes. **E.** Relationship between body weight and fasting glucose for both sexes. **F.** Heritability estimates for each physiological trait. Bars show standard error of the estimate. **G.** Correlation structure between physiological traits.

92 The trait correlations (Fig. 1G) showed that most of the metabolic trait pairs were only modestly correlated

93 indicating complex relationships among the measured traits. This low level of redundancy suggests a broad
94 sampling of multiple heritable aspects of metabolic disease including overall body weight, glucose homeostasis,
95 pancreatic composition and liver function.

96 **Distal Heritability Correlated with Phenotype Relevance**

97 To comprehensively assess the genetic control of gene expression in metabolic disease we assayed adipose, islet,
98 liver, and skeletal muscle gene expression in the DO cohort. We performed eQTL analysis using R/qtl2²⁵
99 (Methods) and identified both local and distal eQTLs for transcripts in each of the four tissues (Supp. Fig
100 1). Significant local eQTLs far outnumbered distal eQTLs (Supp. Fig. 1F) and tended to be shared across
101 tissues (Supp. Fig. 1G) whereas the few significant distal eQTLs we identified tended to be tissue-specific
102 (Supp. Fig. 1H)

103 We calculated the heritability of each transcript in terms of local and distal genetic factors (Methods). Overall,
104 local and distal genetic factors contributed approximately equally to transcript abundance. In all tissues,
105 both local and distal factors explained between 8 and 18% of the variance in the median transcript (Fig 2A).

106 To assess the importance of genetic regulation transcript levels to organism-level traits, we compared the
107 local and distal heritabilities of transcripts to their trait relevance, defined as the maximum correlation
108 of a transcript across all traits. The local heritability of transcripts was negatively correlated with their
109 trait relevance (Fig. 2B), suggesting that the more local genotype influenced transcript abundance, the
110 less effect this variation had on the measured traits. Conversely, the distal heritability of transcripts was
111 positively correlated with trait relevance (Fig. 2C). That is, transcripts that were more highly correlated
112 with the measured traits tended to be distally, rather than locally, heritable. Importantly, this pattern was
113 consistent across all tissues, strongly suggesting that this is a generic finding. This finding is consistent with
114 previous observations that low-heritability transcripts explain more expression-mediated disease heritability
115 than high-heritability transcripts¹⁹. However, the positive relationship between trait correlation and distal
116 heritability demonstrated further that there are diffuse genetic effects throughout the genome converging on
117 trait-related transcripts.

118 **High-Dimensional Mediation identified a high-heritability composite trait that was mediated
119 by a composite transcript**

120 The above univariate analyses establish the importance of distal heritability for trait-relevant transcripts.
121 However, the number of transcripts dramatically exceeds the number of phenotypes. Thus, we expect the
122 heritable, trait-relevant transcripts to be highly correlated and organized according to coherent, emergent

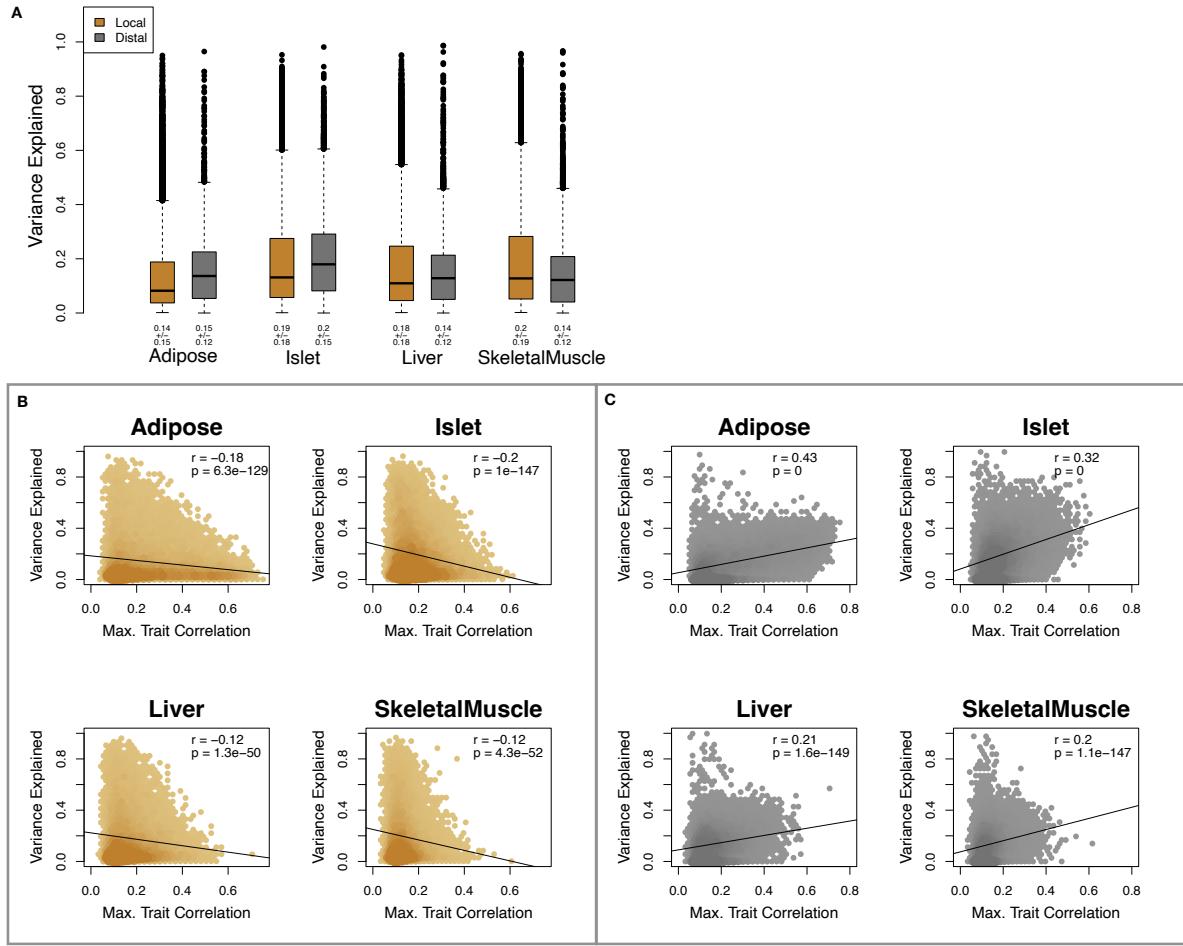


Figure 2: Transcript heritability and trait relevance. **A.** Distributions of distal and local heritability of transcripts across the four tissues. Overall local and distal factors contribute equally to transcript heritability. The relationship between **(B.)** local and **(C.)** distal heritability and trait relevance across all four tissues. Here trait relevance is defined as the maximum correlation between the transcript and all traits. Local heritability was negatively correlated with trait relevance, and distal heritability is positively correlated with trait relevance. Pearson (r) and p values for each correlation are shown in the upper-right of each panel.

123 biological processes representing the mediating endophenotypes driving clinical trait variation. To identify
 124 these endophenotypes in a theoretically principled way, we developed a novel dimension-reduction technique,
 125 HDMA, that uses the theory of causal graphical models to identify a transcriptomic signature that is
 126 simultaneously 1) highly heritable, 2) strongly correlated to the measured phenotypes, and 3) conforms to the
 127 causal mediation hypothesis (Fig. 3). HDMA projects the high-dimensional scores—a composite genome score
 128 (G_C), a composite transcriptome score (T_C), and a composite phenotype score (P_C)—and uses the univariate
 129 theory of mediation to constrain these projections to satisfy the hypotheses of perfect mediation, namely
 130 that upon controlling for the transcriptomic score, the genome score is uncorrelated to the phenotype score.
 131 Formally, perfect mediation implies a constraint on the correlation coefficients among scores as

$$\text{Corr}(G_C, P_C) = \text{Corr}(G_C, T_C)\text{Corr}(T_C, P_C)$$

which is equivalent to the partial correlation of G_C and P_C after controlling for T_C being zero. The value $\text{Corr}(G_C, T_C)\text{Corr}(T_C, P_C)$ is called the path coefficient of the mediation model. The projections of the high-dimensional data matrices in HDMA are designed to satisfy this constraint, and thus conform to the perfect mediation hypothesis, as closely as possible. We stress, however, that validating any causal assertion requires direct experimentation and, thus, that the output of HDMA are scores that are consistent with causal mediation. Thus, HDMA is a strategy for causal hypothesis generation, where the causal mediator is a complex endophenotype learned from a high-dimensional readout.

Operationally, HDMA is closely related to generalized canonical correlation analysis (CCA), for which provably convergent algorithms have recently been developed²⁶. A complete mathematical derivation and implementation details for HDMA are available in **Supp. Methods XXX**.

We used HDMA to identify the major axis of variation in the transcriptome was consistent with mediating the effects of the genome on metabolic traits (Fig 3). Fig. 3A shows the partial correlations (ρ) between the pairs of these composite vectors. The partial correlation between G_C and T_C was 0.42, and the partial correlation between T_C and P_C was 0.78. However, when the transcriptome was taken into account, the partial correlation between G_C and P_C was effectively zero (0.039). P_C captured 30% of the overall trait variance, and its estimated heritability was 0.71 ± 0.084 , which was higher than any of the measured traits (Fig. 1F). Thus, HDMA identified a maximally heritable metabolic composite trait and a highly heritable component of the transcriptome that are correlated as expected in the perfectly mediated model.

As discussed in Supp. Methods XXX, HDMA is related to a generalized form of CCA. Standard CCA is prone to over-fitting because in any two large matrices it can be trivial to identify highly correlated composite vectors [REF]. To assess whether our implementation of HDMA was similarly prone to over-fitting in a high-dimensional space, we performed permutation testing. We permuted the individual labels on the transcriptome matrix 1000 times and recalculated the path coefficient, which is the partial correlation of G_C and T_C multiplied by the partial correlation of T_C and P_C . This represents the strength of the path from G_C to P_C that is putatively mediated through T_C . The null distribution of the path coefficient is shown in Fig. 3B, and the observed path coefficient from the original data is indicated by a red line. The observed path coefficient was well outside the null distribution generated by permutations ($p < 10^{-16}$). Fig. 3C illustrates this observation in more detail. Although we identified high correlations between G_C and T_C , and modest correlations between T_C and P_C in the null data (Fig 3C), these two values could not be

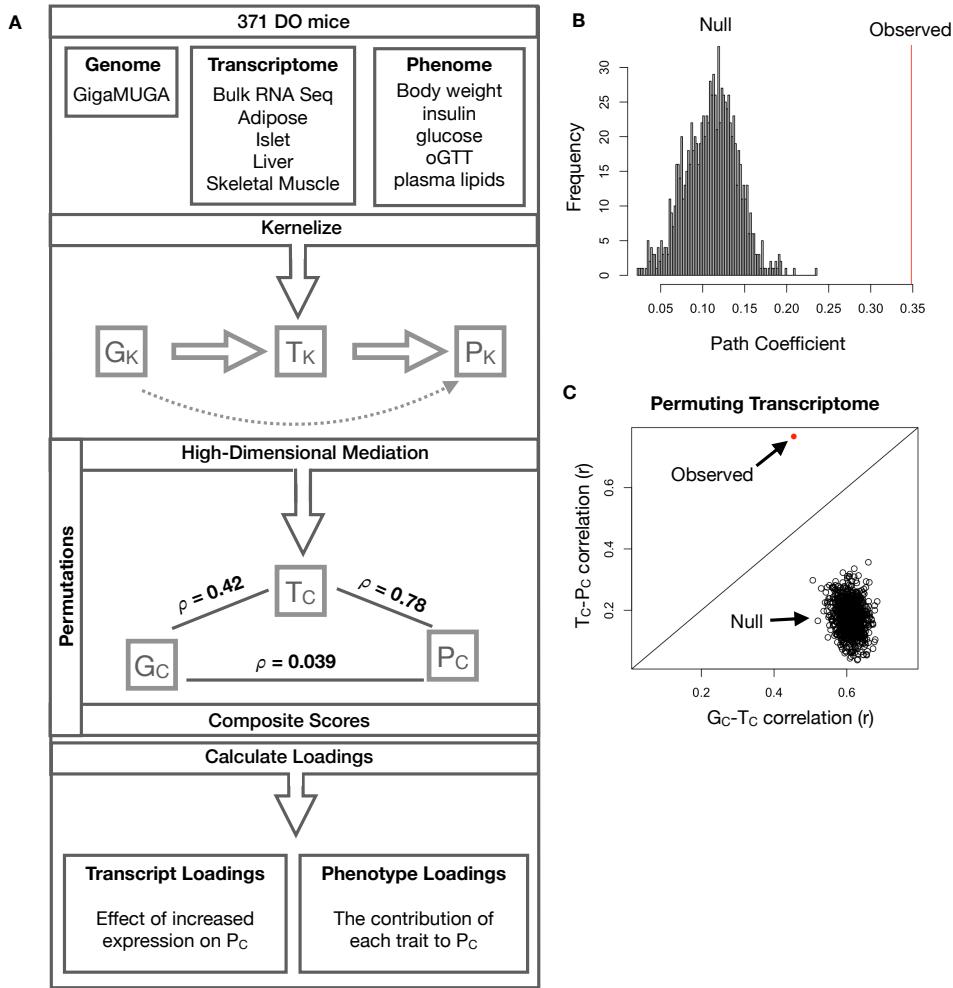


Figure 3: High-dimensional mediation. **A.** Workflow indicating major steps of high-dimensional mediation. The genotype, transcriptome, and phenotype matrices were independently normalized and converted to kernel matrices representing the pairwise relationships between individuals for each data modality (K_G = genome kernel, K_T = transcriptome kernel; K_P = phenome kernel). High-dimensional mediation was applied to these matrices to maximize the direct path $G \rightarrow T \rightarrow P$, the mediating pathway (arrows), while simultaneously minimizing the direct $G \rightarrow P$ pathway (dotted line). The composite vectors that resulted from high-dimensional mediation were G_C , T_C , and P_C . The partial correlations ρ between these vectors indicated perfect mediation. Transcript and trait loadings were calculated as described in the methods. **B.** The null distribution of the path coefficient derived from 10,000 permutations compared to the observed path coefficient (red line). **C.** The null distribution of the G_C-T_C correlation vs. the T_C-P_C correlation compared with the observed value (red dot).

161 maximized simultaneously in the null data. In contrast, the red dot shows that in the real data both the
 162 G_C-T_C correlation and the T_C-P_C correlation could be maximized simultaneously suggesting that the path
 163 from genotype to phenotype through transcriptome is highly non-trivial and identifiable in this case. These
 164 results suggest that these composite vectors represent genetically determined variation in phenotype that is
 165 mediated through genetically determined variation in transcription.

166 **Body weight and insulin resistance were highly represented in the expression-mediated com-**
167 **posite trait**

168 Each composite score is simply a weighted combination of the measured variables and the magnitude and
169 sign of the weights, called loadings, correspond the relative importance and directionality of each variable in
170 the composite score. The loadings of each measured trait onto P_C indicate how much each contributed to
171 the composite phenotype. Final body weight contributed the most (Fig. 4), followed by homeostatic insulin
172 resistance (HOMA_IR) and fasting plasma insulin levels (Insulin_Fasting). We can thus interpret P_C as
173 an index of metabolic disease (Fig. 4B). Individuals with high values of P_C have a higher metabolic index
174 and greater metabolic disease, including higher body weight and higher insulin resistance. We refer to P_C
175 as the metabolic index going forward. Traits contributing the least to the metabolic index were measures
176 of cholesterol and pancreas composition. Thus, when we interpret the transcriptomic signature identified
177 by HDMA, we are explaining primarily the putative transcriptional mediation of body weight and insulin
178 resistance, as opposed to cholesterol measurements.

179 **High-loading transcripts have low local heritability, high distal heritability, and were linked**
180 **mechanistically to obesity**

181 We interpreted large loadings onto transcripts as indicating strong mediation of the effect of genetics on
182 metabolic index. Large positive loadings indicate that higher expression was associated with a higher
183 metabolic index (i.e. higher risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). Conversely,
184 large negative loadings indicate that high expression of these transcripts was associated with a lower metabolic
185 index (i.e. lower risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). We used gene set
186 enrichment analysis (GSEA)^{27;28} to look for biological processes and pathways that were enriched at the top
187 and bottom of this list (Methods).

188 In adipose tissue, both GO processes and KEGG pathway enrichments pointed to an axis of inflammation
189 and metabolism (Supp. Fig. 2 and Fig. 11). GO terms and KEGG pathways associated with inflammation,
190 particularly macrophage infiltration, were positively associated with metabolic index, indicating that increased
191 expression in inflammatory pathways was associated with a higher metabolic index. It is well established that
192 adipose tissue in obese individuals is inflamed [cite] and infiltrated by macrophages [cite], and the results
193 here suggest that this may be a dominant heritable component of metabolic disease.

194 The strongest negative enrichments in adipose tissue were related to mitochondrial activity in general, and
195 thermogenesis in particular (Supp. Fig. 2 and Fig. 11). It has been shown mouse strains with greater
196 thermogenic potential are also less susceptible to obesity on a high-fat diet [cite].

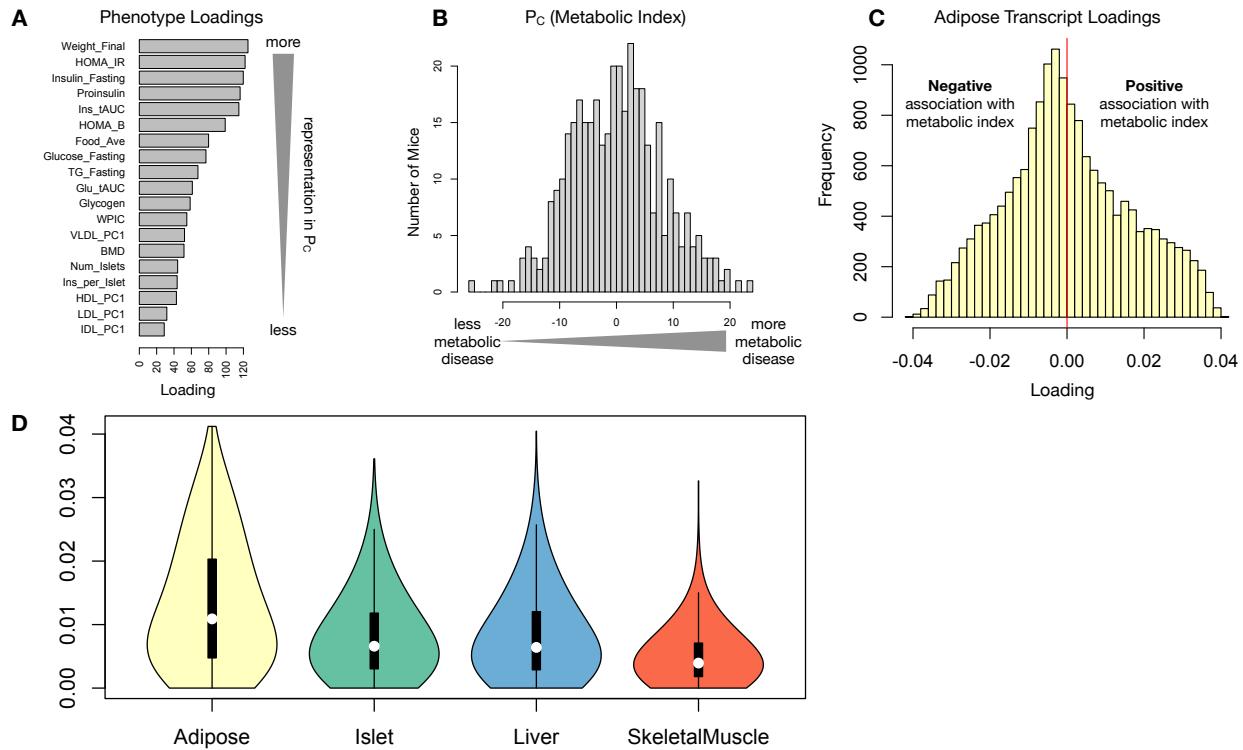


Figure 4: Interpretation of loadings. **A.** Loadings across traits. Body weight and insulin resistance contributed the most to the composite trait. **B.** Phenotype scores across individuals. Individuals with large positive phenotype scores had higher body weight and insulin resistance than average. Individuals with large negative phenotype scores had lower body weight and insulin resistance than average. **C.** Distribution of transcript loadings in adipose tissue. For transcripts with large positive loadings, higher expression was associated with higher phenotype scores. For transcripts with large negative loadings, higher expression was associated with lower phenotype scores. **D.** Distribution of absolute value of transcript loadings across tissues. Transcripts in adipose tissue had the largest loadings indicating that adipose tissue gene expression was a strong mediator of genotype on body weight and insulin resistance.

197 Transcripts associated with the citric acid (TCA) cycle as well as the catabolism of the branched-chain amino
 198 acids (BCAA) (valine, leucine, and isoleucine) were strongly enriched with negative loadings in adipose
 199 tissue (Supp. Fig. 3). Expression of genes in both pathways (for which there is some overlap) has been
 200 previously associated with insulin sensitivity^{12;29;30}, suggesting that heritable variation in regulation of these
 201 pathways may influence risk of insulin resistance.
 202 Looking at the 10 most positively and negatively loaded transcripts from each tissue, it is apparent that
 203 transcripts in the adipose tissue had the largest loadings, both positive and negative, of all tissues (Fig. 5A
 204 bar plot) This suggests that much of the effect of genetics on body weight and insulin resistance is mediated
 205 through gene expression in adipose tissue. The strongest loadings in liver and pancreas were comparable,
 206 and those in skeletal muscle were the weakest (Fig. 5A), suggesting that less of the genetic effects were
 207 mediated through transcription in skeletal muscle. Heritability analysis showed that transcripts with the

208 largest loadings had higher distal heritability than local heritability (Fig. 5A heat map and box plot). This
209 pattern contrasts with transcripts nominated by TWAS (Fig. 5B), which tended to have lower loadings,
210 higher local heritability and lower distal heritability. Transcripts with the highest local heritability in each
211 tissue (Fig. 5C) had the lowest loadings, consistent with our findings above (Fig. 2B).

212 We performed a literature search for the genes in each of these groups along with the terms “diabetes”,
213 “obesity”, and the name of the expressing tissue to determine whether any of these genes had previous
214 associations with metabolic disease in the literature (Methods). Multiple genes in each group had been
215 previously associated with obesity and diabetes (Fig. 5 bolded gene names). Genes with high loadings were
216 most highly enriched for previous literature support. They were 2.375 more likely than TWAS hits and 3.8
217 times more likely than genes with high local heritability to be previously associated with obesity or diabetes.

218 **Tissue-specific transcriptional programs were associated with metabolic traits**

219 Clustering of transcripts with top loadings in each tissue showed tissue-specific functional modules associated
220 with obesity and insulin resistance (Fig. 6A) (Methods). The clustering highlights the importance of immune
221 activation particularly in adipose tissue. The “mitosis” cluster had large positive loadings in three of the
222 four tissues potentially suggesting system-wide hypertrophy. Otherwise, all clusters were strongly loaded in
223 only one or two tissues. For example, the lipid metabolism cluster was loaded most heavily in liver. The
224 positive loadings suggest that high expression of these genes particularly in the liver was associated with
225 increased metabolic disease. This cluster included the gene *Pparg*, whose primary role is in the adipose tissue
226 where it is considered a master regulator of adipogenesis³¹. Agonists of *Pparg*, such as thiazolidinediones, are
227 FDA-approved to treat type II diabetes, and reduce inflammation and adipose hypertrophy³¹. Consistent
228 with this role, the loading for *Pparg* in adipose tissue was negative, suggesting that higher expression was
229 associated with leaner mice (Fig. 6B). In contrast, *Pparg* had a large positive loading in liver, where it is
230 known to play a role in the development of hepatic steatosis, or fatty liver. Mice that lack *Pparg* specifically
231 in the liver, are protected from developing steatosis and show reduced expression of lipogenic genes^{32;33}.
232 Overexpression of *Pparg* in the livers of mice with a *Ppara* knockout, causes upregulation of genes involved in
233 adipogenesis³⁴. In the livers of both mice and humans high *Pparg* expression is associated with hepatocytes
234 that accumulate large lipid droplets and have gene expression profiles similar to that of adipocytes^{35;36}.

235 The local and distal heritability of *Pparg* is low in adipose tissue suggesting its expression in this tissue is
236 highly constrained in the population (Fig. 6B). However, the distal heritability of *Pparg* in liver is relatively
237 high suggesting it is complexly regulated and has sufficient variation in this population to drive variation in
238 phenotype. Both local and distal heritability of *Pparg* in the islet are relatively high, but the loading is low,

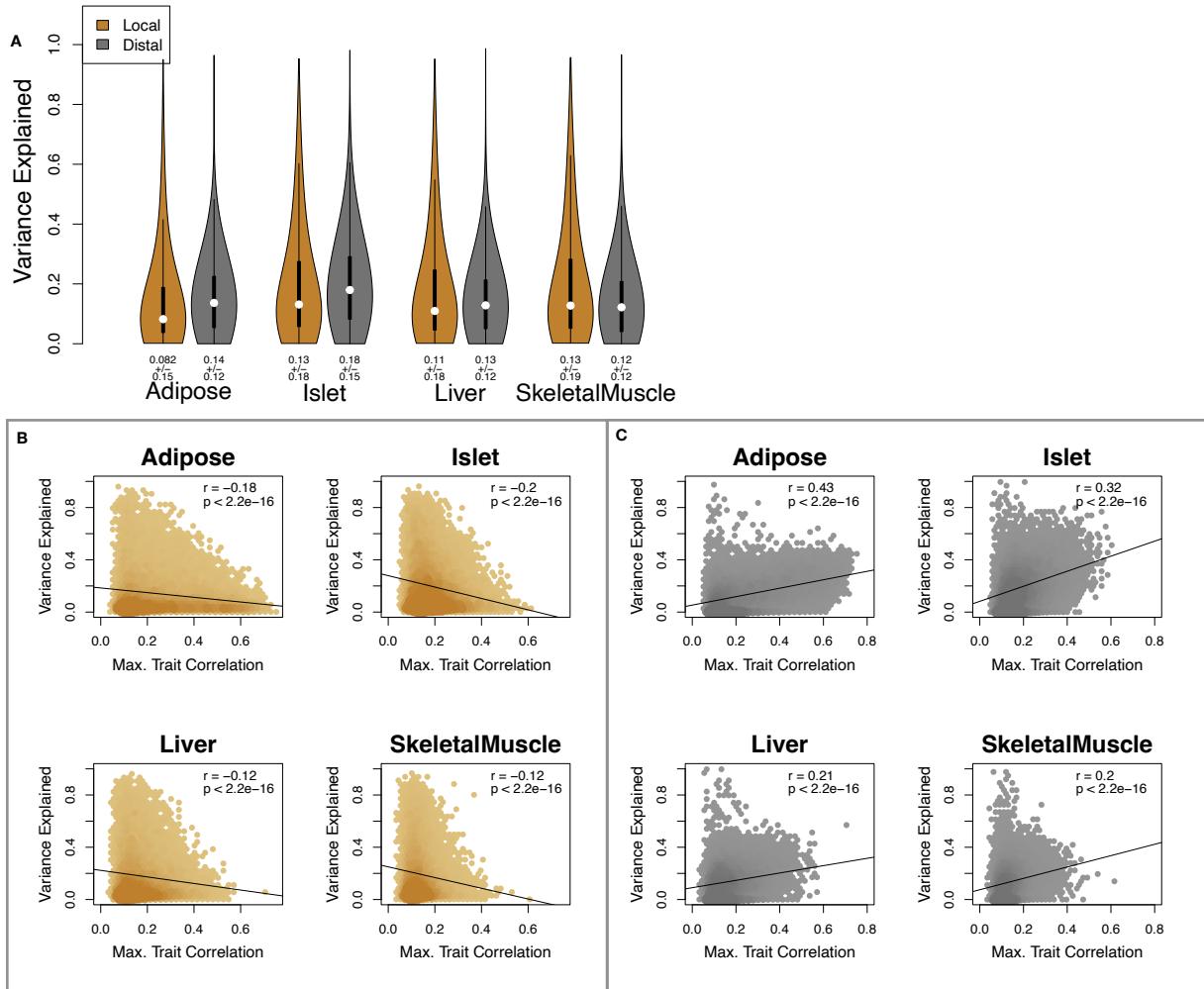


Figure 5: Transcripts with high loadings have high distal heritability and literature support. Each panel has a bar plot showing the loadings of transcripts selected by different criteria. Bar color indicates the tissue of origin. The heat map shows the local (L - left) and distal (D - right) heritability of each transcript. **A.** Loadings for the 10 transcripts with the largest positive loadings and the 10 transcripts with the largest negative loadings for each tissue. **B.** Loadings of TWAS candidates with the 10 largest positive correlations with traits and the largest negative correlations with traits across all four tissues. **C.** The transcripts with the largest local heritability (top 20) across all four tissues.

suggesting that variability of expression in the islet does not drive variation in metabolic index. These results highlight the importance of tissue context when investigating the role of heritable transcript variability in driving phenotype.

Gene lists for all clusters are available in Supplemental File XXX.

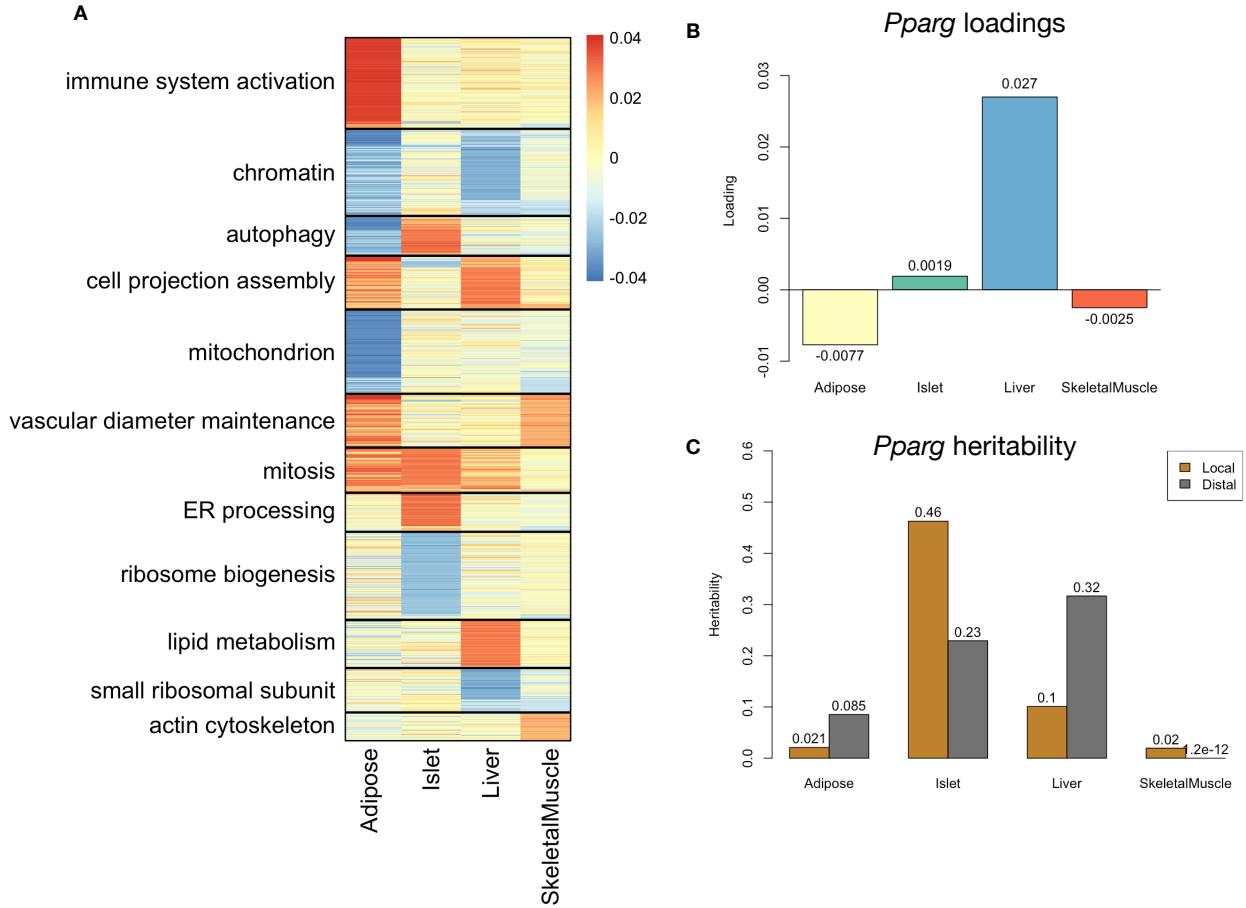


Figure 6: Tissue-specific transcriptional programs were associated with obesity and insulin resistance. **A** Heat map showing the loadings of all transcripts with loadings greater than 2.5 standard deviations from the mean in any tissue. The heat map was clustered using k medoid clustering. Functional enrichments of each cluster are indicated along the left margin. **B** Loadings for *Pparg* in different tissues. **C** Local and distal of *Pparg* expression in different tissues.

243 **Gene expression, but not local eQTLs, predicted body weight in an independent population**

244 To test whether the transcript loadings identified in the DO could be translated to another population, we
 245 tested whether they could predict metabolic phenotype in an independent population of CC-RIX mice, which
 246 were F1 mice derived from multiple pairings of Collaborative Cross (CC) [cite] strains (Fig. 7) (Methods).
 247 We tested two questions. First, we asked whether the loadings identified in the DO mice were relevant to
 248 the relationship between the transcriptome and the phenotype in the CC-RIX. We predicted body weight (a
 249 surrogate for metabolic index) in each CC-RIX individual using measured gene expression in each tissue and
 250 the transcript loadings identified in the DO (Methods). The predicted body weight and acutal body weight
 251 were highly correlated in all tissues (Fig. 7B left column). The best prediction was achieved for adipose
 252 tissue, which supports the observation in the DO that adipose expression was the strongest mediator of the

253 genetic effect on metabolic index. This result also confirms the validity and translatability of the transcript
 254 loadings and their relationship to metabolic disease.

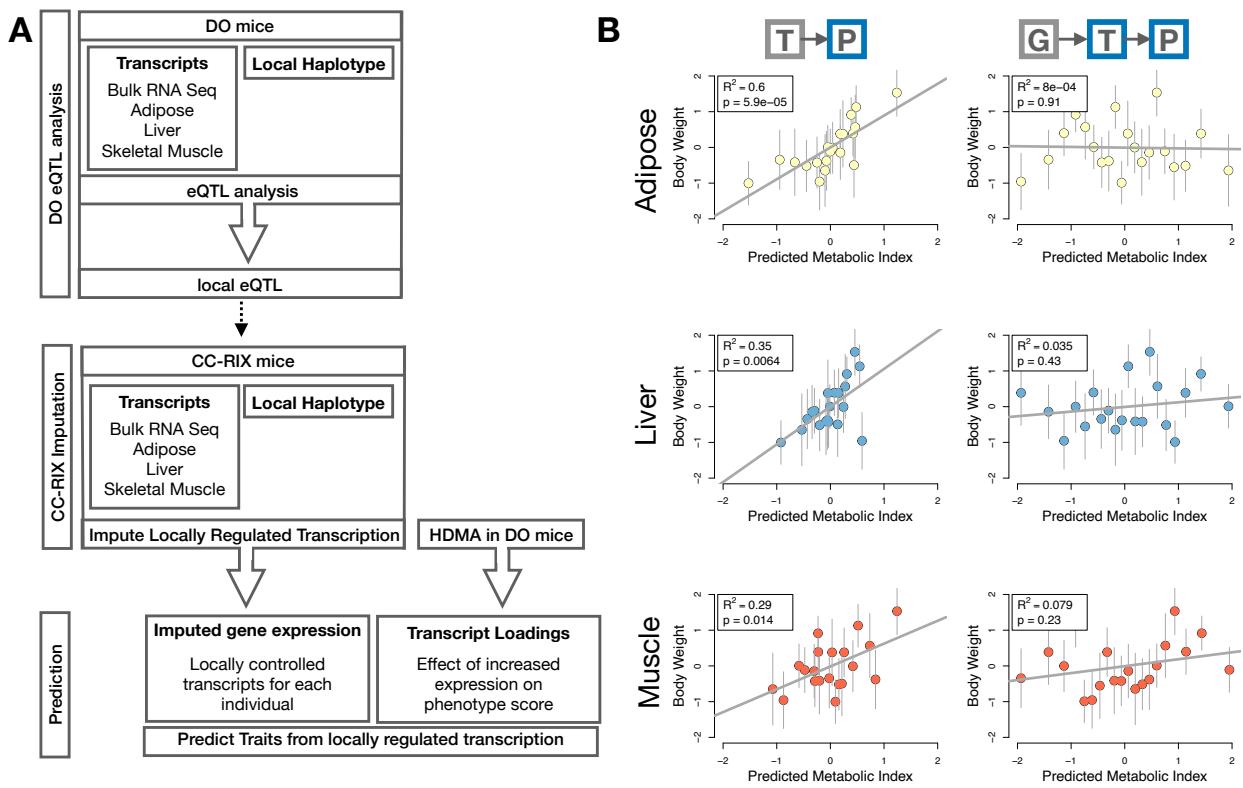


Figure 7: Transcription, but not local genotype, predicts phenotype in the CC-RIX. **A.** Workflow showing procedure for translating HDMA results to an independent population of mice. **B.** Relationships between the predicted metabolic index and measured body weight. The left column shows the predictions using measured transcripts. The right column shows the prediction using transcript levels imputed from local genotype. Gray boxes indicate measured quantities, and blue boxes indicate calculated quantities. The dots in each panel represent individual CC-RIX strains. The gray lines show the standard deviation on body weight for the strain.

255 The second question related to the source of the relevant variation in gene expression. If local regulation was
 256 the predominant factor influencing gene expression, we should be able to predict phenotype in the CC-RIX
 257 using transcripts imputed from local genotype (Fig. 7A). The DO and the CC-RIX were derived from the
 258 same eight founder strains and so carry the same alleles throughout the genome. We imputed gene expression
 259 in the CC-RIX using local genotype and were able to estimate variation in gene transcription robustly (Supp.
 260 Fig. 4). However, these imputed values failed to predict body weight in the CC-RIX when weighted with the
 261 loadings from HDMA. (Fig. 7B right column). This result suggests that local regulation of gene expression is
 262 not the primary factor driving heritability of complex traits, consistent with our findings in the DO population
 263 that distal heritability was a major driver of trait-relevant variation and that high-loading transcripts had
 264 comparatively high distal and low local heritability.

265 **Distally heritable transcriptomic signatures reflected variation in composition of adipose tissue
266 and islets**

267 The interpretation of global genetic influences on gene expression and phenotype is potentially more challenging
268 than the interpretation and translation of local genetic influences, as genetic effects cannot be localized to
269 individual gene variants or transcripts. However, there are global patterns across the loadings that can
270 inform mechanism. For example, heritable variation in cell type composition can be inferred from transcript
271 loadings. We observed above that immune activation in the adipose tissues was a highly enriched process
272 correlating with obesity in the DO population. For example, in humans, it has been extensively observed that
273 macrophage infiltration in adipose tissue is a marker of obesity and metabolic disease [REF]. To determine
274 whether the immune activation reflected a heritable change in cell composition in adipose tissue in DO mice,
275 we compared loadings of cell-type specific genes in adipose tissue (Methods). Consistent with human results,
276 the mean loading of macrophage-specific genes was significantly greater than 0 (Fig. 8A), indicating that
277 obese mice were genetically predisposed to have high levels of macrophage infiltration in adipose tissue in
278 response to the high-fat, high-sugar diet. Loading for marker genes for other cell types were not statistically
279 different from zero, indicating that changes in the abundance of those cell types is not a mediator of metabolic
280 index.

281 We also compared loadings of cell-type specific transcripts in islet (Methods). The mean loadings for alpha-cell
282 specific transcripts were significantly greater than 0, while the mean loadings for delta- and endothelial-cell
283 specific genes were significantly less than 0 (Fig. 8B). These results suggest either that mice with higher
284 metabolic index had inherited a higher proportions of alpha cells, and lower proportions of endothelial and
285 delta cells in their pancreatic islets, that such compositional changes were induced by the HFHS diet in a
286 heritable way, or both. In either case, these results support the hypothesis that alterations in islet composition
287 drive variation in metabolic index.

288 Notably, the loadings for pancreatic beta cell-type specific loadings was not significantly different from zero.
289 We stress that this is not necessarily reflective of the function of the beta cells in the obese mice, but rather
290 suggests that any variation in the number of beta cells in these mice was unrelated to obesity and insulin
291 resistance, the major contributors to metabolic index. This is further consistent with the islet composition
292 traits having small loadings in the phenome score (Fig. 4).

293 **Heritable transcriptomic signatures translated to human disease**

294 Ultimately, the heritable transcriptomic signatures that we identified in DO mice will be useful if they inform
295 pathogenicity and treatment of human disease. To investigate the potential for translation of the gene

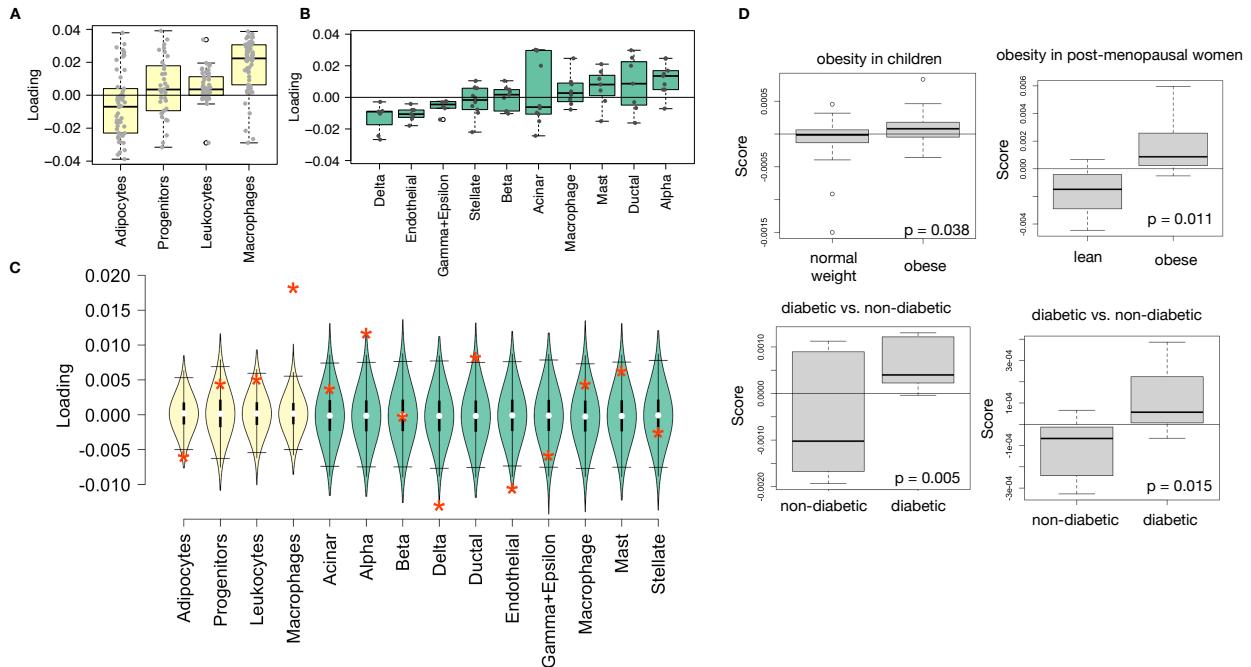


Figure 8: HDMA results translate to humans. **A.** Distribution of loadings for cell-type-specific transcripts in adipose tissue. **B.** Distribution of loadings for cell-type-specific transcripts in pancreatic islets (green). **C.** Null distributions for the mean loading of randomly selected transcripts in each cell type compared with the observed mean loading of each group of transcripts (red asterisk). **D.** Predictions of metabolic phenotypes in four adipose transcription data sets downloaded from GEO. In each study the obese/diabetic patients were predicted to have greater metabolic disease than the lean/non-diabetic patients based on the HDMA results from DO mice.

296 signatures identified in DO mice, we compared them to transcriptional profiles in obese and non-obese human
 297 subjects (Methods). We limited our analysis to adipose tissue because the adipose tissue signature had the
 298 strongest relationship to obesity and insulin resistance in the DO.

299 We calculated a predicted obesity score for each individual in the human studies based on their adipose
 300 tissue gene expression (Methods) and compared the predicted scores for obese and non-obese groups as well
 301 as diabetic and non-diabetic groups. In all cases, the predicted obesity scores were higher on average for
 302 individuals in the obese and diabetic groups compared with the lean and non-diabetic groups (Fig. 8D). This
 303 indicates that the distally heritable signature of metabolic index identified in DO mice is relevant to obesity
 304 and diabetes in human subjects.

305 Existing therapies are predicted to target mediator gene signatures

306 Another potential application of the transcript loading landscape is in ranking potential drug candidates
 307 for the treatment of metabolic disease. Although high-loading transcripts may be good candidates for
 308 understanding specific biology related to obesity, the transcriptome overall is highly interconnected and

309 redundant, and focusing on individual transcripts for treatment may be less effective than using broader
310 transcriptomic signatures that capture the emergent biology [cite or remove]. The ConnectivityMap (CMAP)
311 database³⁷ developed by the Broad Institute allows querying thousands of compounds that reverse or enhance
312 the extreme ends of transcriptomic signatures in multiple different cell types. By identifying drugs that
313 reverse pathogenic transcriptomic signatures, we can potentially identify compounds that have favorable
314 effects on gene expression.

315 To test this hypothesis, we queried the CMAP database through the CLUE online query tool (<https://clue.io/query/>, version 1.1.1.43) (Methods). We identified top anti-correlated hits across all cell types. To
316 get more tissue-specific results, we also looked at top results in cell types that most closely resembled our
317 tissues. We looked at results in adipocytes (ASC) as well as pancreatic tumor cells (YAPC) regardless of *p*
318 value (Supplemental Figure XXX and XXX).

320 Looking across all cell types, the notable top hits from the adipose tissue loadings included mTOR inhibitors
321 and glucocorticoid agonists (Supplemental Figure XXX). It is thought that metformin, which is commonly
322 used to improve glycemic control, acts, at least in part, by inhibiting mTOR signaling^{38;39}. However,
323 long-term use of other mTOR inhibitors, such as rapamycin, are known to cause insulin resistance and
324 β -cell toxicity^{39–41}. Glucocorticoids are used to reduce inflammation, which was a prominent signature in
325 the adipose tissues, but these drugs also promote hyperglycemia and diabetes^{42;43}. Acute treatment with
326 glucocorticoids has further been shown to reduce thermogenesis in rodent adipocytes^{44–46}, but increase
327 thermogenesis in human adipocytes^{47;48}. Thus, the pathways identified by CMAP across all cell types were
328 highly related to the transcript loading profiles, but the relationship was not a simple reversal.

329 The top hit for the adipose composite transcript in CMAP adipocytes was a PARP inhibitor (Supplemental
330 Figure XXXB). PARPs play a role in lipid metabolism and are involved in the development of obesity and
331 diabetes⁴⁹. PARP1 inhibition increases mitochondrial biogenesis⁵⁰. Inhibition of PARP1 activity can further
332 prevent necrosis in favor of the less inflammatory apoptosis⁵¹, thereby potentially reducing inflammation in
333 stressed adipocytes. Other notable hits among the top 20 were BTK inhibitors, which have been observed
334 to suppress inflammation and improve insulin resistance⁵² as well as to reduce insulin antibodies in type I
335 diabetes⁵³. IKK inhibitors have been shown to improve glucose control in type II diabetes^{54;55}.

336 Among the top most significant hits for the transcript loadings from pancreatic islets (Fig. XXX), was
337 suppression of T cell receptor signaling, which is known to be involved in Type 1 diabetes⁵⁶, as well as
338 TNFR1, which has been associated with mortality in diabetes patients⁵⁷. Suppression of NOD1/2 signaling
339 was also among the top hits. NOD1 and 2 sense ER stress^{58;59}, which is associated with β -cell death in type

340 1 and type 2 diabetes⁶⁰. This cell death process is dependent on NOD1/2 signaling⁵⁸, although the specifics
341 have not yet been worked out.

342 We also looked specifically at hits in pancreatic tumor cells (YAPC) regardless of significance level to get a
343 transcriptional response more specific to the pancreas. Hits in this list included widely used diabetes drugs,
344 such as sulfonylureas, PPAR receptor agonists, and insulin sensitizers. Rosiglitazone is a PPAR- γ agonist
345 and was one of the most prescribed drugs for type 2 diabetes before its use was reduced due to cardiac
346 side-effects⁶¹. Sulfonylureas are another commonly prescribed drug class for type 2 diabetes, but also have
347 notable side effects including hypoglycemia and accelerated β -cell death⁶².

348 Discussion

349 Here we used a novel high-dimensional mediation analysis (HDMA) to investigate the relative contributions of
350 local and distal gene regulation to heritable trait variation in genetically diverse mouse models of diet-induced
351 obesity and metabolic disease. We identified tissue-specific composite transcripts that are predicted to
352 mediate the effect of genetic background on metabolic traits. Transcripts contributing most strongly to these
353 composite transcripts were distally, but not locally heritable, and composite transcripts were able to predict
354 obesity in a large, independent mouse population with divergent population structure, whereas models using
355 local eQTL only could not. Moreover, the composite transcript from mouse adipose tissue translated to
356 predict obesity and diabetes status in human cohorts with measured adipose gene expression. Taken together,
357 these results support the hypothesis that gene expression mediating the effect of genetic background on
358 metabolic phenotypes is primarily distally regulated, and that the heritable endophenotypes defined by
359 gene expression signatures translate between mice and humans. We speculate that the central importance of
360 distal heritability found in this study is likely to a generic finding for complex common diseases and could
361 have significant consequences for the development of therapies for these diseases.

362 Genetics is indispensable for the dissection of disease mechanisms because it is one of the only data modalities
363 that supports causal inferences about molecules and disease outcomes [REF]. It has frequently been assumed
364 that gene regulation in *cis* is the primary driver of genetically associated trait variation, but attempts to use
365 local gene regulation to explain phenotypic variation have had limited success^{16;17}. In recent years, evidence
366 has mounted that distal gene regulation may be an important mediator of trait heritability^{19;18;63}. It has
367 been observed that transcripts with high local heritability explain less expression-mediated disease heritability
368 than transcripts with low local heritability¹⁹. Consistent with this observation, genes located near GWAS
369 hits tend to be complexly regulated¹⁸. They also tend to be enriched with functional annotations, in contrast
370 to genes with simple local regulation, which tend to be depleted of functional annotations suggesting they

371 are less likely to be directly involved in disease traits¹⁸. These observations are consistent with principles of
372 robustness in complex systems^{64–66}. If a transcript were both important to a trait and subject to strong
373 local regulation, a population would be susceptible to extremes in phenotype that might frequently cross the
374 threshold to disease. Indeed, strong disruption of highly trait-relevant genes is the cause of Mendelian disease.
375 Our results are consistent, instead, with a more complex picture where genes whose expression can drive
376 trait variation are buffered from local genetic variation but are extensively influenced indirectly by genetic
377 variation in the regulatory networks converging on those genes.

378 Recently, the omnigenic model of complex traits has been proposed, which posits that complex traits are
379 massively polygenic and that their heritability is spread out across the genome⁶⁷. In the omnigenic model,
380 genes are classified either as “core genes,” which directly impinge on the trait, or “peripheral genes,” which
381 are not directly trait-related, but influence core genes through the complex gene regulatory network. HDMA
382 explicitly models a central proposal of the omnigenic model which posits that once the expression of the
383 core genes (i.e. trait-mediating genes) is accounted for, there should be no residual correlation between the
384 genome and the phenome. Here, when the composite transcript was taken into account there was no residual
385 correlation between the composite genome and composite phenome (Fig. 3A).

386 Thus, the transcript loadings can be interpreted as indicating higher “core-ness” of a transcript. Unlike in the
387 omnigenic model, we did not observe a clear demarcation between the core and peripheral genes in loading
388 magnitude, but we do not necessarily expect a clear separation given the complexity of gene regulation and
389 the genotype-phenotype map⁶⁸.

390 An extension of the omnigenic model proposed that most heritability of complex traits is driven by weak
391 distal eQTLs that are potentially below detection threshold in feasible sample sizes⁶³. This is consistent with
392 what we observed here. The transcripts with the largest loadings were strongly distally regulated and only
393 weakly locally regulated, suggesting that distal gene regulation plays a primary role in driving heritable trait
394 variation. We saw further that the patterns of distal heritability were complex and spread across the genome,
395 even for transcripts whose expression was strongly regulated by distal factors, rather than localizing to a
396 few detectable distal eQTLs. For example, *Nucb2*, had a high loading in islets and was also strongly distally
397 regulated (66% distal heritability) (Fig. 5). This gene is expressed in pancreatic β cells and is involved in
398 insulin and glucagon release^{69–71}. Although its transcription was highly heritable in islets, that regulation was
399 distributed across the genome, with no clear distal eQTL (Supp. Fig. 5). Thus, although distal regulation of
400 some genes may be strong, this regulation is likely to be highly complex and not easily localized.

401 We stress that HDMA is a method for causal hypothesis generation. As with any causal inference approach,

402 the output of HDMA can only be said to be consistent with causal mediation but does not prove it, which
403 requires experimentation with direct control over the mediating variable [REF]. The issue of experimentation,
404 however, is subtle. The dimension-reduction in HDMA is distinguished by the fact that the putative causal
405 intermediates can be emergent states defined by the expression of thousands of genes. This is a strength,
406 because the mediating variable can be a higher-order process such as “macrophage activation and infiltration”,
407 but, in contrast to univariate hypotheses at the level of individual transcripts, the relevant validation
408 experiment may be technologically infeasible, unknowable a priori, or both. Nevertheless, downstream
409 analyses of the composite transcripts strongly supports a causal interpretation. Indeed, the composite
410 transcripts identified by HDMA are richly interpretable in both tissue- and gene-specific manners. The
411 transcripts with the strongest loadings were enriched in biological functions previously known to be involved
412 in the pathogenesis of metabolic disease, such as inflammation in adipose tissue. That these processes
413 were identified in this analysis suggests additionally that they have a heritable component, and that some
414 individuals are genetically susceptible to greater adipose inflammation on a high-fat, high-sugar diet.

415 Individual high-loading transcripts also demonstrated biologically interpretable, tissue-specific patterns. We
416 highlighted *Pparg*, which is known to be protective in adipose tissue³¹ where it was negatively loaded, and
417 harmful in the liver^{32–36}, where it was positively loaded. Such granular patterns may be useful in generating
418 hypotheses for further testing, and prioritizing genes as therapeutic targets. The tissue-specific nature of
419 the loadings also may provide clues to tissue-specific effects, or side effects, of targeting particular genes
420 system-wide.

421 We showed further that these composite transcripts can be used as weighted vectors in multiple types of
422 analysis, such as drug prioritization using gene set enrichment analysis (GSEA) and the CMAP database. In
423 particular, the CMAP analysis identified drugs which have been demonstrated to reverse insulin resistance
424 and other aspects of metabolic disease. This finding supports the causal role of these full gene signatures in
425 pathogenesis of metabolic disease and thus their utility in prioritizing drugs and gene targets as therapeutics.

426 Another useful application of the composite transcripts is to pair them with cell-type specific genes to generate
427 causal hypotheses about changes in cell composition in individual tissues. Combining the multi-tissue,
428 transcriptome-wide weighted vectors with public databases and data sets thus provides a path for generating a
429 wide range of testable hypotheses. Moreover, each data set presented here was derived from human tissues or
430 cell lines, thus demonstrating the translatability of these results. That the mouse-derived adipose composite
431 transcript was able to classify human adipose gene expression in terms of obesity and diabetes status further
432 supports the direct translatability of these findings, the utility of HDMA, and the continued importance of
433 mouse models of human disease in which it is possible to obtain complete transcriptomes in multiple tissues

434 across large numbers of individuals.

435 Altogether, our results have shown that both tissue specificity and distal gene regulation are critically
436 important to understanding the genetic architecture of complex traits. We identified important genes and
437 gene signatures that were heritable, plausibly causal of disease, and translatable to other mouse populations
438 and to humans. Finally, we have shown that by directly acknowledging the complexity of both gene regulation
439 and the genotype-to-phenotype map, we can gain a new perspective on disease pathogenesis and develop
440 actionable hypotheses about pathogenic mechanisms and potential treatments.

441 Old discussion

442 Here we used a novel high-dimensional mediation analysis (HDMA) to investigate the relative contributions of
443 local and distal gene regulation to heritable trait variation in a genetically diverse mouse model of diet-induced
444 obesity and metabolic disease. We identified tissue-specific composite transcripts mediating the effect of
445 genetic background on metabolic traits. Transcripts contributing most strongly to these composite transcripts
446 were distally heritable. These composite transcripts, but not local eQTL, were able to predict obesity in
447 an independent mouse population with divergent allelic structure. Moreover, the composite transcript from
448 adipose tissue predicted obesity and diabetes status in human cohorts with measured adipose gene expression.
449 Taken together, these results support the hypothesis that gene expression mediating the effect of genetic
450 background on phenotype is primarily distally regulated, and that the gene regulatory networks influencing
451 metabolic disease are conserved across mice and humans.

452 It has frequently been assumed that gene regulation in *cis* is the primary driver of genetically associated
453 trait variation, but attempts to use local gene regulation to explain phenotypic variation have had limited
454 success^{16;17}. In recent years, evidence has mounted that distal gene regulation may be an important mediator
455 of trait heritability^{19;18;63}. It has been observed that transcripts with high local heritability explain less
456 expression-mediated disease heritability than transcripts with low local heritability¹⁹. Consistent with this
457 observation, genes located near GWAS hits tend to be complexly regulated¹⁸. They also tend to be enriched
458 with functional annotations, in contrast to genes with simple local regulation, which tend to be depleted
459 of functional annotations suggesting they are less likely to be directly involved in disease traits¹⁸. These
460 observations are consistent with principles of robustness in complex systems^{64–66}. If a transcript were both
461 important to a trait and subject to strong local regulation, a population would be susceptible to extremes
462 in phenotype that might frequently cross the threshold to disease. Indeed, strong disruption of highly
463 trait-relevant genes is the cause of Mendelian disease.

464 The composite transcripts we identified here supported the hypothesis that distally regulated gene expression

465 is the dominant mediator of trait variation. Transcript loadings (the degree to which they contributed to
466 the composite transcript) were negatively correlated with local heritability and positively correlated with
467 distal heritability. The most strongly loaded transcripts were enriched for functional annotations associated
468 with metabolic disease. These distally regulated composite transcripts were highly heritable and explained a
469 high proportion of disease risk, further supporting their role as mediators. The composite transcripts were
470 moreover able to predict obesity in an independent cohort of mice whereas models using local eQTL only
471 could not. Together these observations suggest that distal gene regulation was the dominant mode through
472 which gene expression mediated the effect of genetic background on complex metabolic traits.

473 Identification of this distally heritable signature depended on the high-dimensional approach we used. Because
474 HDMA uses a kinship matrix rather than genotypes at individual loci, it allows for arbitrarily complex
475 gene regulation, as well as the interconnectedness and redundancy of the transcriptome. This feature also
476 means that HDMA assumes that traits are highly polygenic and distributed across the genome. In contrast,
477 one-dimensional, univariate approaches assume a large, localized genetic effect. Thus, the HDMA approach
478 is consistent with the omnigenic model of complex traits which posits that complex traits are massively
479 polygenic and that their heritability is spread out across the genome⁶⁷. In the omnigenic model, genes
480 are classified either as “core genes,” which directly impinge on the trait, or “peripheral genes,” which are
481 not directly trait-related, but influence core genes through the complex gene regulatory network. HDMA
482 explicitly models a central proposal of the omnigenic model which posits that once the expression of the
483 core genes (i.e. trait-mediating genes) is accounted for, there should be no residual correlation between the
484 genome and the phenotype. Here, when the composite transcript was taken into account there was no residual
485 correlation between the composite genome and composite phenotype (Fig. 3A).

486 Thus, the composite transcript is essentially a weighted vector with larger weights (loadings) indicating higher
487 “core-ness” of a transcript. There was no clear demarcation between the core and peripheral genes in loading
488 magnitude, but we do not necessarily expect a clear separation given the complexity of gene regulation and
489 the genotype-phenotype map⁶⁸. Still, the transcripts with the largest loadings had high distal heritability,
490 low local heritability, and were enriched for biological processes related to metabolic traits, as we would
491 predict for core genes.

492 An extension of the omnigenic model proposed that most heritability of complex traits is driven by weak
493 distal eQTLs⁶³. This is consistent with what we observed here. The transcripts with the largest loadings
494 were strongly distally regulated and only weakly locally regulated, suggesting that distal gene regulation
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497 distal factors, these factors were multiple and spread across the genome. For example, *Nucb2*, was a strongly
498 mediating transcript in islet and was also strongly distally regulated (66% distal heritability) (Fig. 5). This
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507 individuals are genetically susceptible to greater adipose inflammation on a high-fat, high-sugar diet.

508 Individual transcripts also demonstrated biologically interpretable, tissue-specific patterns. We highlighted
509 *Pparg*, which is known to be protective in adipose tissue³¹ where it was negatively loaded, and harmful in the
510 liver^{32–36}, where it was positively loaded. Such granular patterns may be useful in generating hypotheses for
511 further testing, and prioritizing genes as therapeutic targets. The tissue-specific nature of the loadings also
512 may provide clues to tissue-specific effects, or side effects, of targeting particular genes system-wide, since
513 antagonists of *Pparg* may reduce fatty liver disease, but exacerbate adipose tissue inflammation.

514 We showed further that these composite transcripts can be used as weighted vectors in multiple types of
515 analysis, such as drug prioritization using gene set enrichment analysis (GSEA) and the CMAP database. In
516 particular, the CMAP analysis identified drugs which have been demonstrated to reverse insulin resistance
517 and other aspects of metabolic disease. This finding supports the causal role of these gene signatures in
518 pathogenesis of metabolic disease and thus their utility in prioritizing drugs and gene targets as therapeutics.

519 Another useful application of the composite transcripts is to pair them with cell-type specific genes to generate
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528 In conclusion, we have shown that both tissue specificity and distal gene regulation are critically important to
529 understanding the genetic architecture of complex traits. We identified important genes and gene signatures
530 that were heritable, causal of disease, and translatable to other mouse populations and to humans. Finally,
531 we have shown that by directly acknowledging the complexity of both gene regulation and the genotype-to-
532 phenotype map, we can gain a new perspective on disease pathogenesis and develop actionable hypotheses
533 about pathogenic mechanisms and potential treatments.

534 **Data Availability**

535 Here we tell people where to find the data

536 **Acknowledgements**

537 Here we thank people

538 **Supplemental Figures**

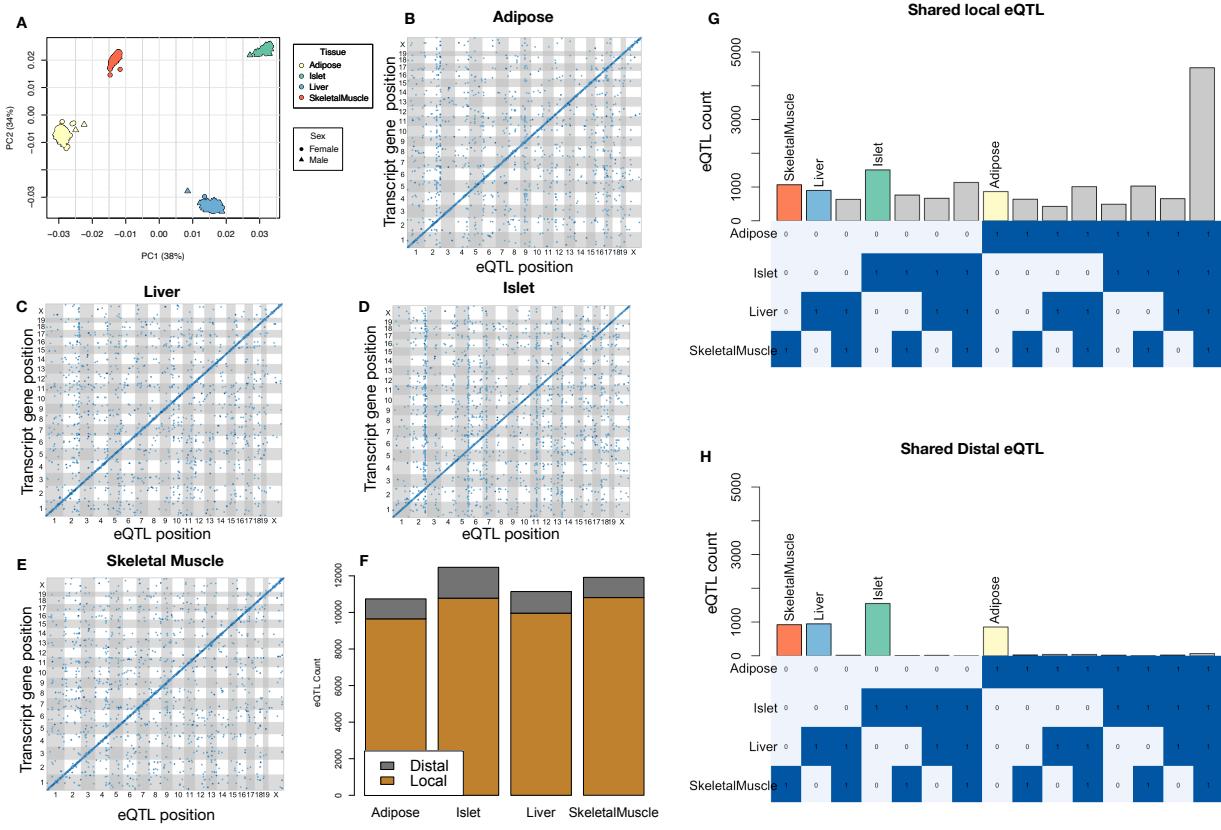


Figure 9: Overview of eQTL analysis in DO mice. **A.** RNA seq samples from the four different tissues clustered by tissue. **B.-E.** eQTL maps are shown for each tissue. The *x*-axis shows the position of the mapped eQTL, and the *y*-axis shows the physical position of the gene encoding each mapped transcript. Each dot represents an eQTL with a minimum LOD score of 8. The dots on the diagonal are locally regulated eQTL for which the mapped eQTL is at the within 4Mb of the encoding gene. Dots off the diagonal are distally regulated eQTL for which the mapped eQTL is distant from the gene encoding the transcript. **F.** Comparison of the total number of local and distal eQTL with a minimum LOD score of 8 in each tissue. All tissues have comparable numbers of eQTL. Local eQTL are much more numerous than distal eQTL. **G.** Counts of transcripts with local eQTL shared across multiple tissues. The majority of local eQTL were shared across all four tissues. **H.** Counts of transcripts with distal eQTL shared across multiple tissues. The majority of distal eQTL were tissue-specific and not shared across multiple tissues. For both G and H, eQTL for a given transcript were considered shared in two tissues if they were within 4Mb of each other. Colored bars indicate the counts for individual tissues for easy of visualization.

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KEGG pathway enrichments by GSEA

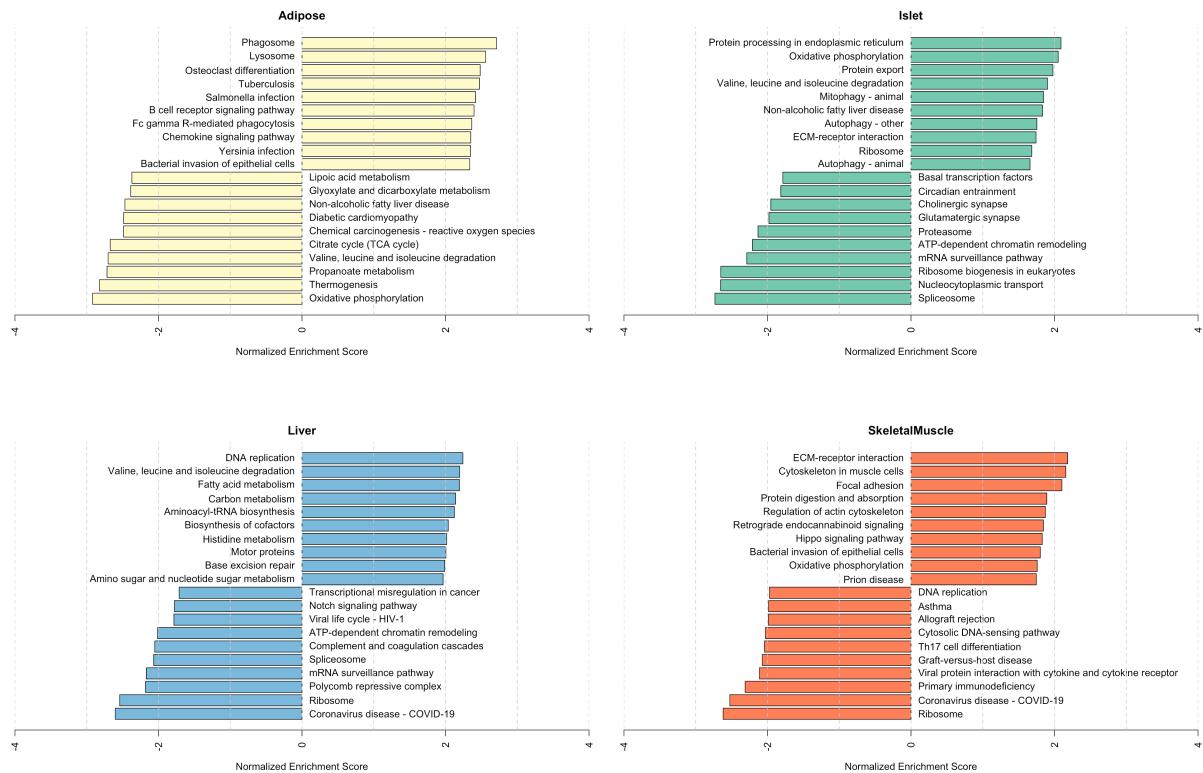


Figure 10: Bar plots showing normalized enrichment scores (NES) for KEGG pathways as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched KEGG pathway.

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Top GO term enrichments by GSEA

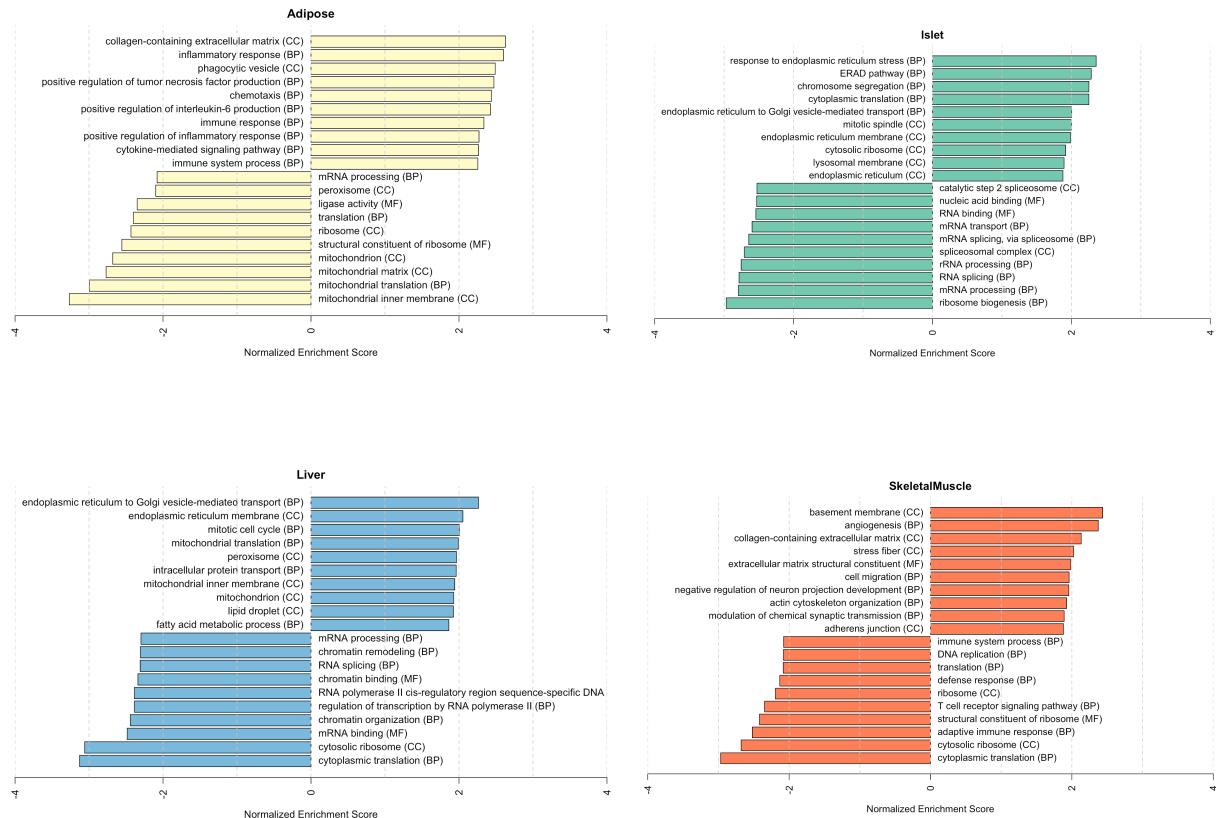


Figure 11: Bar plots showing normalized enrichment scores (NES) for GO terms as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched GO term. The letters in parentheses indicate whether the term is from the biological process ontology (BP), the molecular function ontology (MF), or the cellular compartment ontology (CC).

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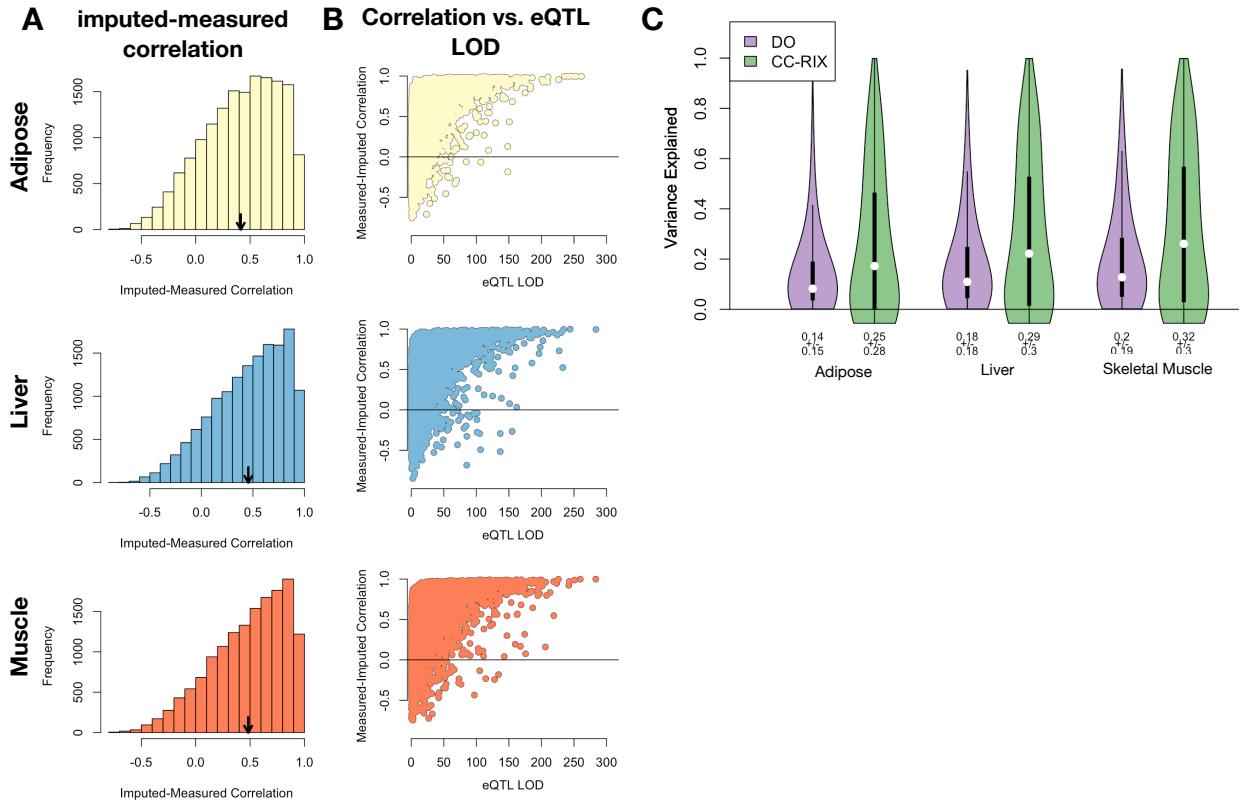


Figure 12: Validation of transcript imputation in the CC-RIX. **A.** Distributions of correlations between imputed and measured transcripts in the CC-RIX. The mean of each distribution is shown by the red line. All distributions were skewed toward positive correlations and had positive means near a Pearson correlation (r) of 0.5. **B.** The relationship between the correlation between measured and imputed expression in the CC-RIX (x-axis) and eQTL LOD score. As expected, imputations are more accurate for transcripts with strong local eQTL. **C.** Variance explained by local genotype in the DO and CC-RIX.

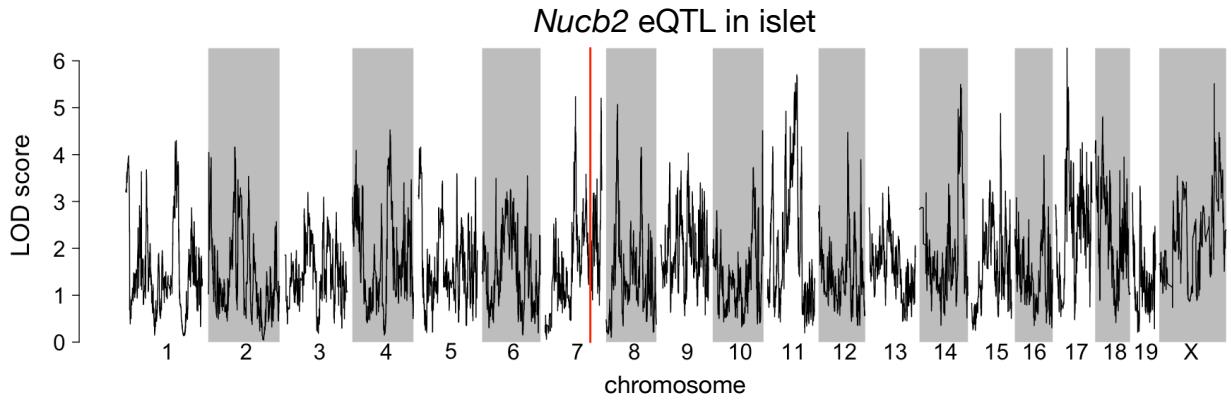


Figure 13: Regulation of *Nucb2* expression in islet. *Nucb2* is encoded on mouse chromosome 7 at 116.5 Mb (red line). In islets the heritability of *Nucb2* expression levels is 69% heritable. This LOD score trace shows that there is no local eQTL at that position, nor any strong distal eQTL anywhere else in the genome.

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