

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

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⁷ **Abstract**

⁸ The effects of genetic variants on complex traits are mediated in large part through regulation of gene
⁹ expression. However there has been limited success in explaining variant-trait associations with regulation
¹⁰ of locally encoded genes. There is emerging evidence that distal gene regulation may play an important
¹¹ role in mediating the effect of genetic background on phenotype. Here we investigated the roles of local and
¹² distal gene regulation on complex traits in a mouse model of diet-induced obesity and metabolic disease.
¹³ We measured longitudinal metabolic phenotypes in a population of 500 diversity outbred (DO) mice along
¹⁴ with transcriptome-wide gene expression in four disease-relevant tissues: adipose, pancreatic islets, liver, and
¹⁵ skeletal muscle. We developed a novel high-dimensional mediation analysis (HDMA) to model emergent
¹⁶ transcriptomic states mediating genetic effects on traits. We identified a set of tissue-specific composite
¹⁷ transcriptomic signatures that were heritable and trait-related. These transcriptomic signatures were highly
¹⁸ interpretable in terms of biological processes as well as cell type composition in islets and adipose tissue.
¹⁹ The transcripts that contributed most strongly to the composite transcriptomic signatures had low local
²⁰ heritability and high distal heritability and predicted obesity in an independent population of mice and in
²¹ human study populations with high accuracy. In contrast, local expression quantitative trait loci (eQTL)
²² alone were unable to predict obesity in an independent population. Together these results suggest that
²³ both the tissue used for gene expression analysis as well as distal gene regulation are critically important in
²⁴ identifying transcriptional mediators of the genome on complex traits.

25 **Introduction**

26 In the quest to understand the genetic architecture of complex traits, gene expression is an important mediator
27 between genotype and phenotype. There is ample evidence from genome-wide association studies (GWAS)
28 that regulation of gene expression accounts for the bulk of the genetic effect on complex traits, as most
29 trait-associated variants lie in gene regulatory regions^{1–7}. It is widely assumed that these variants influence
30 local transcription, and methods such as transcriptome-wide association studies (TWAS)^{8–11}, summary
31 data-based Mendelian randomization (SMR)¹⁰, and others capitalize on this idea to identify genes associated
32 with multiple disease traits^{12–15}

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

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

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

45 To assess the role of wide-spread distal gene regulation on complex traits, we investigated diet-induced obesity
46 and metabolic disease as an archetypal example. Diet-induced obesity and metabolic disease are genetically
47 complex with hundreds of variants mapped through GWAS [REFS]. These variants are known to act through
48 multiple tissues that interact dynamically with each other [REFS], including adipose tissue, pancreatic
49 islets, liver, and skeletal muscle. The multi-system etiology of metabolic disease complicates mechanistic
50 dissection of the genetic architecture, requiring large, dedicated data sets that include high-dimensional,
51 clinically relevant phenotyping, dense genotyping in a highly recombined population, and transcriptome-wide
52 measurements of gene expression in multiple tissues. Measuring gene expression in multiple tissues is critical
53 to adequately assess the extent to which local gene regulation varies across multiple tissues and whether such
54 variability might account for previous failed attempts to identify trait-relevant local eQTL. Such data sets
55 are extremely difficult to obtain in human populations, particularly in the large numbers of subjects required

56 for adequate statistical power. Thus, to investigate further the role of local and distal gene regulation on
57 complex traits, we have generated an appropriate data set in a large population of diversity outbred (DO)
58 mice²¹ in a population model of diet-induced obesity and metabolic disease¹².

59 The DO mice were derived from eight inbred founder mouse strains, five classical lab strains, and three
60 strains more recently derived from wild mice²¹. They represent three subspecies of mouse *Mus musculus*
61 *domesticus*, *Mus musculus musculus*, and *Mus musculus castaneus*, and capture 90% of the known variation
62 in laboratory mice²². They are maintained with a breeding scheme that ensures equal contributions from
63 each founder across the genome thus rendering almost the whole genome visible to genetic inquiry²¹. We
64 paired clinically relevant metabolic traits from 500 DO mice [REF], including body weight, plasma levels
65 of insulin and glucose and plasma lipids, with transcriptome-wide gene expression in four tissues related to
66 metabolic disease: adipose tissue, pancreatic islets, liver, and skeletal muscle. Taken together, these data
67 enable a comprehensive view into the genetic architecture of metabolic disease.

68 Results

69 Genetic variation contributed to wide phenotypic variation

70 Although the environment was consistent across all animals, the genetic diversity present in this population
71 resulted in widely varying distributions across physiological measurements (Fig. 1). For example, body
72 weights of adult individuals varied from less than the average adult B6 body weight to several times the body
73 weight of a B6 adult in both sexes (Males: 18.5 - 69.1g, Females: 16.0 - 54.8g) (Fig. 1A). Fasting blood
74 glucose (FBG) also varied considerably (Fig. 1B) although few of the animals had FBG levels that would
75 indicate pre-diabetes (19 animals, 3.8%), or diabetes (7 animals, 1.4%) according to previously developed
76 cutoffs (pre-diabetes: FBG \geq 250 mg/dL, diabetes: FBG \geq 300, mg/dL)²³. Males had higher FBG than
77 females on average (Fig. 1C) as has been observed before suggesting either that males were more susceptible
78 to metabolic disease on the high-fat diet, or that males and females may require different thresholds for
79 pre-diabetes and diabetes.

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

84 The trait correlations (Fig. 1G) shows that most of the metabolic trait pairs were weakly correlated indicating
85 complex relationships among the measured traits. This low level of redundancy suggests a broad sampling of

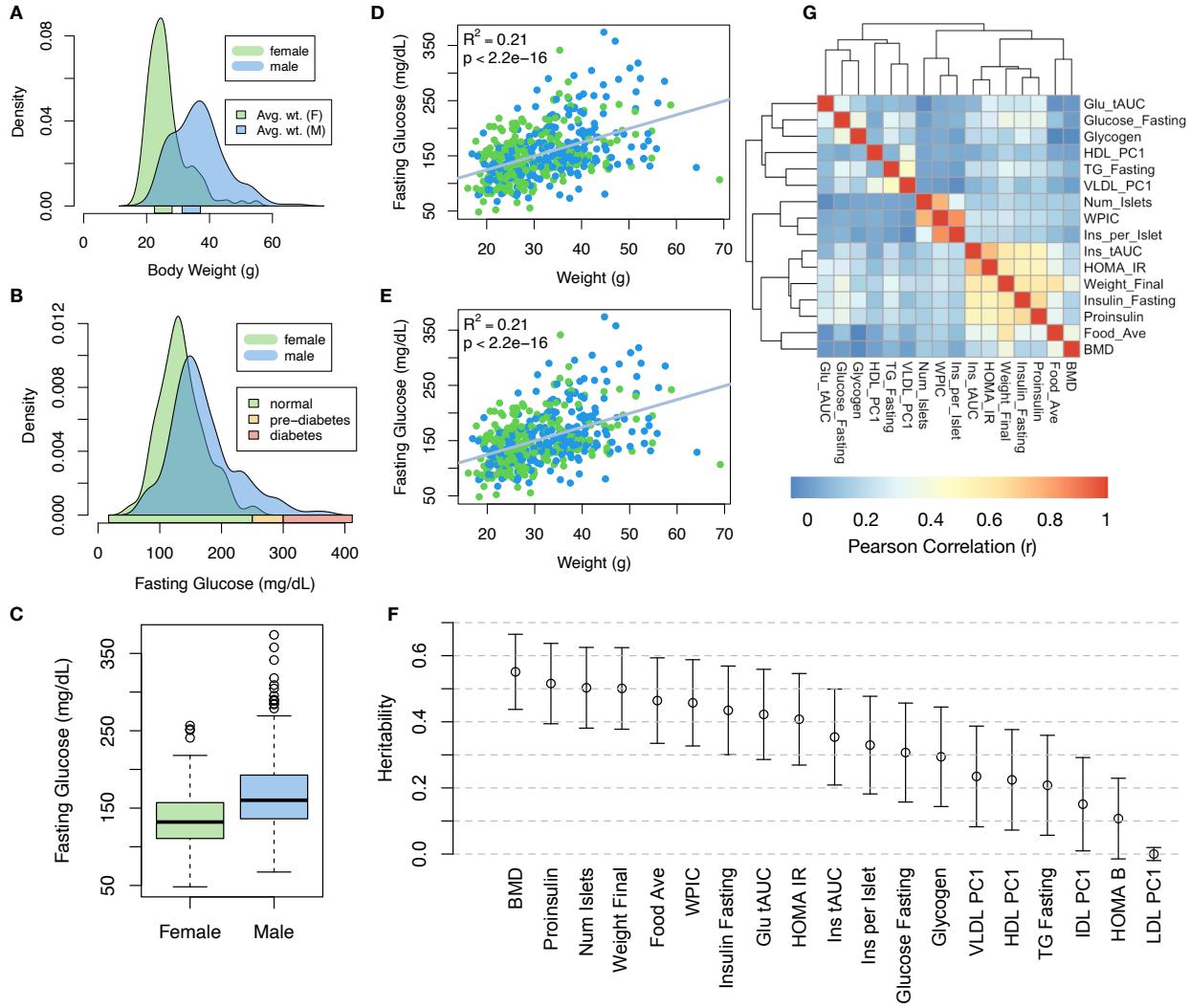


Figure 1: Clinical overview. **A.** Distributions of final body weight in the diversity outbred mice. Sex is indicated by color. The average B6 male and female adult weights at 24 weeks of age are indicated by blue and green bars on the x-axis. **B.** The distribution of final fasting glucose across 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 pairs of physiological traits.

multiple heritable aspects of metabolic disease including overall body weight, glucose homeostasis, pancreatic composition and liver function.

88 Distal Heritability Correlated with Phenotype Relevance

We performed eQTL analysis using R/qtl2²⁴ (Methods) and identified both local and distal eQTLs for transcripts in each of the four tissues (Supp. Fig 1). Significant local eQTLs far outnumbered distal eQTLs

91 (Supp. Fig. 1F) and tended to be shared across tissues (Supp. Fig. 1G) whereas the few significant distal
 92 eQTLs we identified tended to be tissue-specific (Supp. Fig. 1H)

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

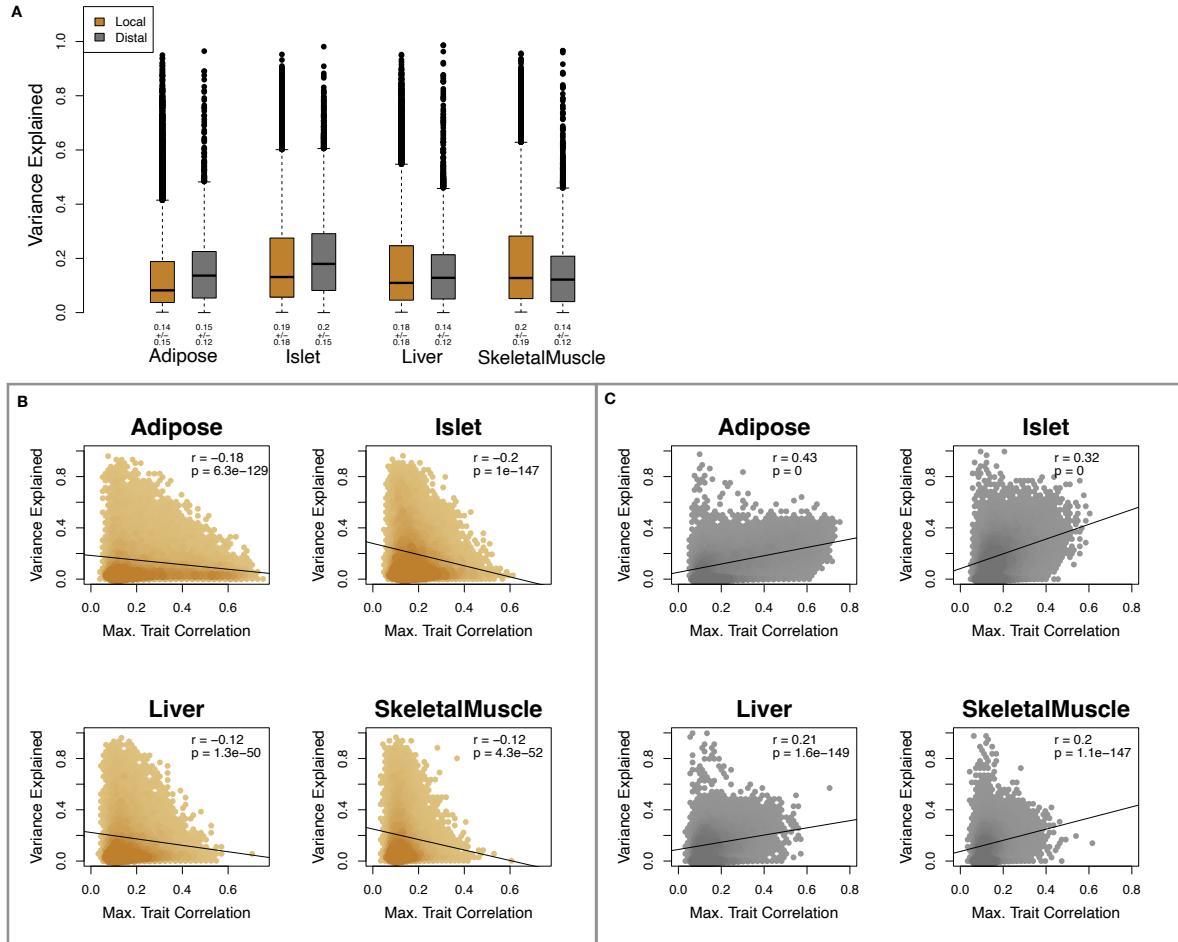


Figure 2: Transcript heritability and trait relevance. **A.** Distributions of distal and local heritability of transcripts across the four tissues. Overall local and distal factors contribute equally to transcript heritability. The relationship between **(B.)** local and **(C.)** distal heritability 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.

96 The local heritability of transcripts was negatively correlated with their trait relevance, defined as the
 97 maximum correlation of a transcript across all traits (Fig. 2B). This suggests that the more local genotype
 98 influenced transcript abundance, the less effect this variation had on the measured traits. Conversely, the

99 distal heritability of transcripts was positively correlated with trait relevance (Fig. 2C). That is, transcripts
100 that were more highly correlated with the measured traits tended to be distally, rather than locally, heritable.
101 Importantly, this pattern was consistent across all tissues, strongly suggesting that this is a generic finding.
102 This finding is consistent with previous observations that low-heritability transcripts explain more expression-
103 mediated disease heritability than high-heritability transcripts¹⁹. However, the positive relationship between
104 trait correlation and distal heritability demonstrated further that there are diffuse genetic effects throughout
105 the genome converging on trait-related transcripts.

106 **High-Dimensional Mediation identified a high-heritability composite trait that was perfectly
107 mediated by a composite transcript**

108 The above univariate analyses establish the importance of distal heritability for trait-relevant transcripts.
109 However, the number of transcripts dramatically exceeds the number of degrees of freedom of the phenotype.
110 Thus, we expect the heritable, trait-relevant transcripts to be highly correlated and organized according
111 to coherent, emergent biological processes representing the mediating endophenotypes driving clinical trait
112 variation. To identify these endophenotypes in a theoretically principled way, we developed a novel dimension-
113 reduction technique, HDMA, that uses the theory of causal graphical models to identify a transcriptomic
114 signature that is simultaneously 1) highly heritable, 2) strongly correlated to the measured phenotypes, and
115 3) conforms to the causal mediation hypothesis (Fig. 3). HDMA projects the high-dimensional scores—a
116 composite genome score (G_C), a composite transcriptome score (T_C), and a composite phenotype score
117 (P_C)—and uses the univariate theory of mediation to constrain these projections to satisfy the hypotheses of
118 perfect mediation. Specifically, perfect mediation implies that upon controlling for the transcriptomic score,
119 the genome score is uncorrelated to the phenotype score, which can also be viewed as a constraint on the
120 correlation coefficients

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

121 which corresponds to the path coefficient in the mediation model [REF]. Operationally, HDMA is closely
122 related to generalized canonical correlation analysis, for which provably convergent algorithms have recently
123 been developed²⁵. Implementation details for HDMA are available in **Supp. Methods XXX**.

124 We used high-dimensioal mediation to identify the major axis of variation in the transcriptome that mediated
125 the effects of the genome on metabolic traits (Fig 3). Fig. 3A shows the partial correlations (ρ) between
126 the pairs of these composite vectors. The partial correlation between G_C and T_C was 0.42, and the partial

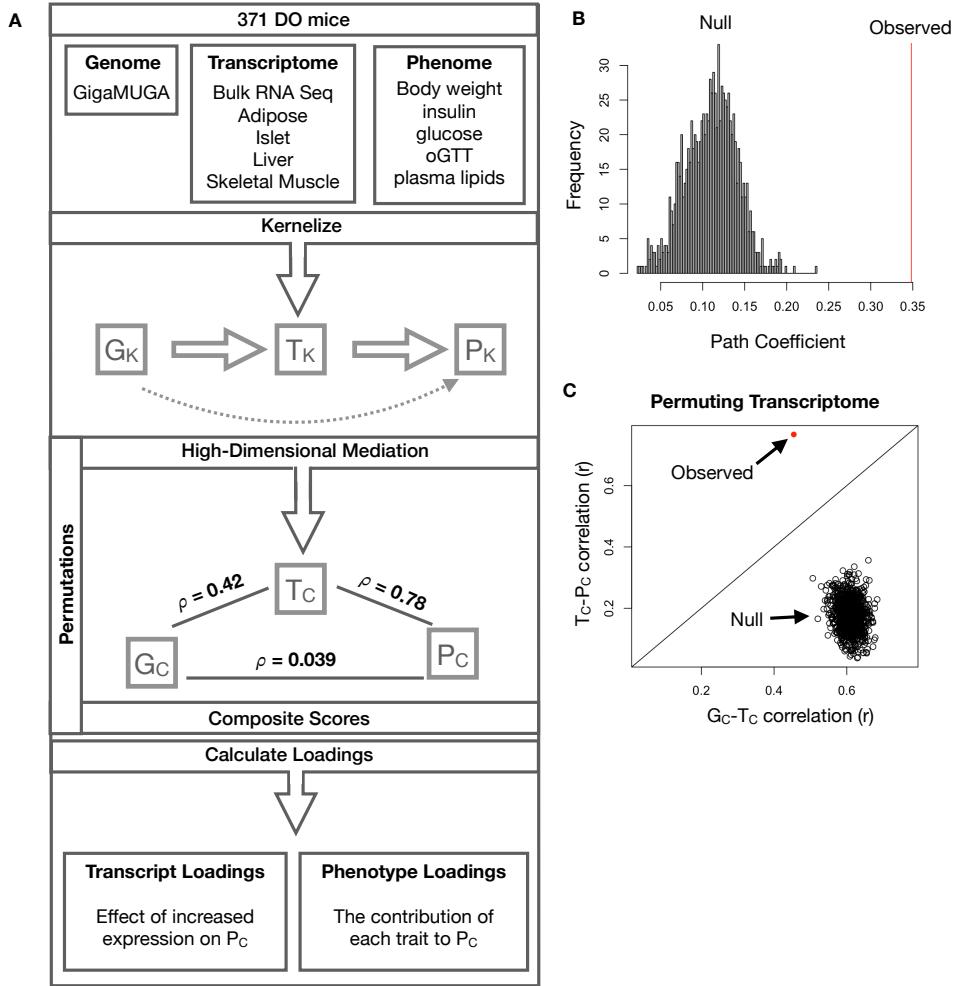


Figure 3: High-dimensional mediation. **A.** Workflow indicating major steps of high-dimensional mediation. The genotype, transcriptome, and phenotype matrices were kernelized to yield single matrices representing the relationships between all individuals for each data modality (G_K = genome kernel, T_K = transcriptome kernel; P_K = phenotype 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).

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 that was perfectly mediated by a highly heritable component of the transcriptome.

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

Body weight and insulin resistance were highly represented in the expression-mediated composite trait

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

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

We interpreted large loadings onto transcripts as indicating strong mediation of the effect of genetics on metabolic index. Large positive loadings indicate that higher expression was associated with a higher

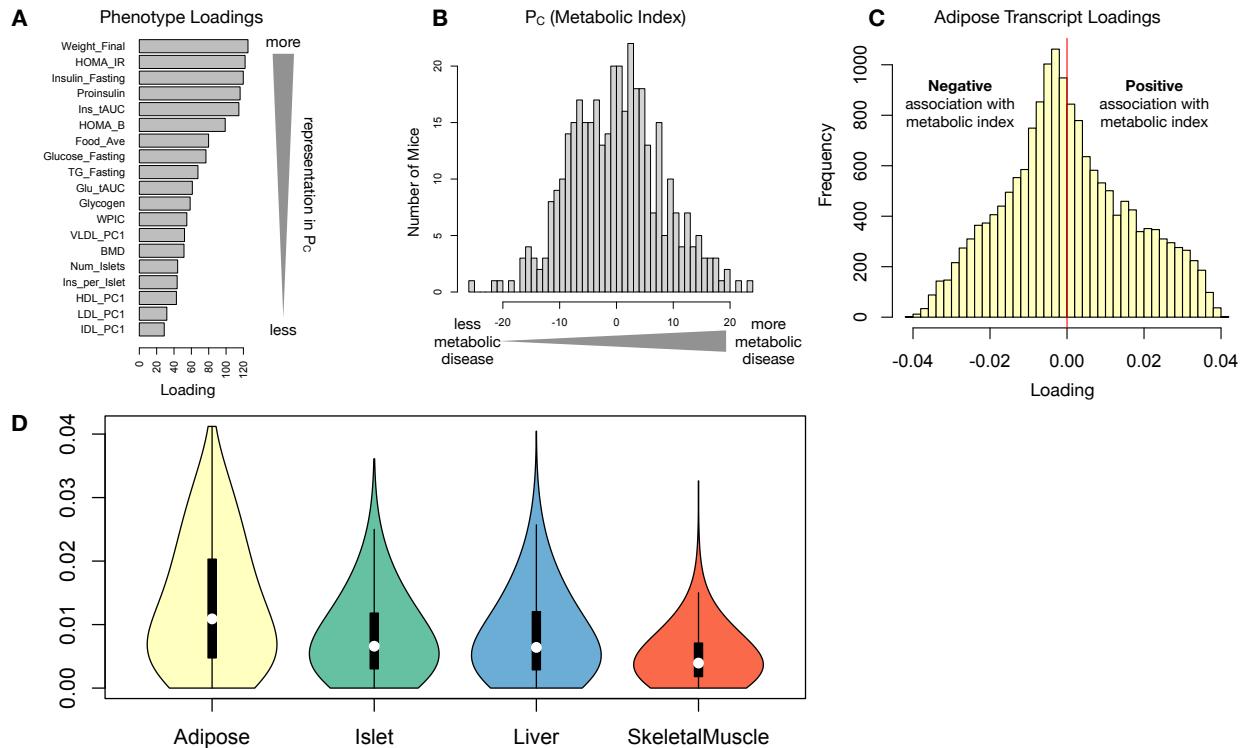


Figure 4: Interpretation of loadings. **A.** Loadings across traits. Body weight and insulin resistance contributed the most to the composite trait. **B.** Phenotype scores across individuals. Individuals with large positive phenotype scores had higher body weight and insulin resistance than average. 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 transcripts in adipose tissue were the best mediators of the genetic effects on body weight and insulin resistance.

metabolic index (i.e. higher risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). Conversely, large negative loadings indicate that high expression of these transcripts was associated with a lower metabolic index (i.e. lower risk of obesity and metabolic disease on the high-fat diet) (Fig. 4C). We used gene set enrichment analysis (GSEA)^{26;27} to look for biological processes and pathways that were enriched at the top and bottom of this list (Methods).

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

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

178 Transcripts associated with the citric acid (TCA) cycle as well as the catabolism of the branched-chain amino
179 acids (BCAA) (valine, leucine, and isoleucine) were strongly enriched with negative loadings in adipose
180 tissue (Supp. Fig. 3). Expression of genes in both pathways (for which there is some overlap) has been
181 previously associated with insulin sensitivity^{12;28;29}, suggesting that heritable variation in regulation of these
182 pathways may influence risk of insulin resistance.

183 Looking at the 10 most positively and negatively loaded transcripts from each tissue, it is apparent that
184 transcripts in the adipose tissue had the largest loadings, both positive and negative, of all tissues (Fig. 5A
185 bar plot) This suggests that much of the effect of genetics on body weight and insulin resistance is mediated
186 through gene expression in adipose tissue. The strongest loadings in liver and pancreas were comparable,
187 and those in skeletal muscle were the weakest (Fig. 5A), suggesting that less of the genetic effects were
188 mediated through transcription in skeletal muscle. Heritability analysis showed that transcripts with the
189 largest loadings had higher distal heritability than local heritability (Fig. 5A heat map and box plot). This
190 pattern contrasts with transcripts nominated by TWAS (Fig. 5B), which tended to have lower loadings,
191 higher local heritability and lower distal heritability. Transcripts with the highest local heritability in each
192 tissue (Fig. 5C) had the lowest loadings.

193 We performed a literature search for the genes in each of these groups along with the terms “diabetes”,
194 “obesity”, and the name of the expressing tissue to determine whether any of these genes had previous
195 associations with metabolic disease in the literature (Methods). Multiple genes in each group had been
196 previously associated with obesity and diabetes (Fig. 5 bolded gene names). Genes with high loadings were
197 most highly enriched for previous literature support. They were 2.25 more likely than TWAS hits and 3.6
198 times more likely than genes with high local heritability to be previously associated with obesity or diabetes.

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

200 Clustering of transcripts with top loadings in each tissue showed tissue-specific functional modules associated
201 with obesity and insulin resistance (Fig. 6A) (Methods). The clustering highlights the importance of immune
202 activation particularly in adipose tissue. Except for the “mitosis” cluster, which had large positive loadings
203 in three of the four tissues, all clusters were strongly loaded in only one or two tissues. For example, the lipid
204 metabolism cluster was loaded most heavily in liver. The positive loadings suggest that high expression of
205 these genes particularly in the liver was associated with increased metabolic disease. This cluster included

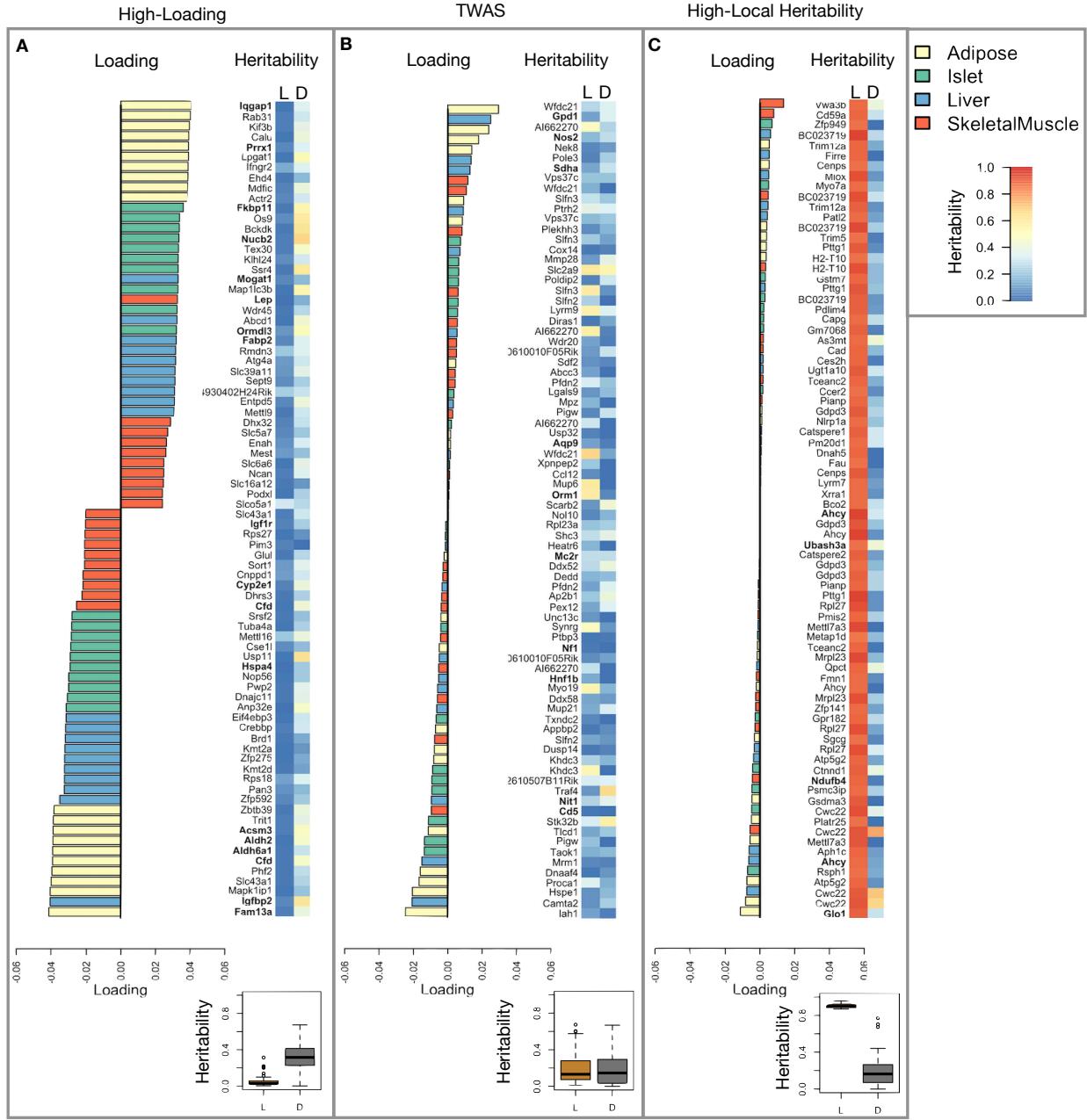


Figure 5: Transcripts with high loadings have high distal heritability and literature support. Each panel has a bar plot showing the loadings of transcripts selected by different criteria. Bar color indicates the tissue of origin. The heat map shows the local (L - left) and distal (D - right) heritability 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.

the gene *Pparg*, whose primary role is in the adipose tissue where it is considered a master regulator of adipogenesis³⁰. Agonists of *Pparg*, such as thiazolidinediones, which are FDA-approved to treat type II diabetes, reduce inflammation and adipose hypertrophy³⁰. Consistent with this role, the loading for *Pparg*

209 in adipose tissue was negative, suggesting that higher expression was associated with leaner mice (Fig. 6B).
210 In contrast, *Pparg* had a large positive loading in liver, where it is known to play a role in the development of
211 hepatic steatosis, or fatty liver. Mice that lack *Pparg* specifically in the liver, are protected from developing
212 steatosis and show reduced expression of lipogenic genes^{31;32}. Overexpression of *Pparg* in the livers of mice
213 with a *Ppara* knockout, causes upregulation of genes involved in adipogenesis³³. In the livers of both mice
214 and humans high *Pparg* expression is associated with hepatocytes that accumulate large lipid droplets and
215 have gene expression profiles similar to adipocytes^{34;35}.

216 The local and distal heritability of *Pparg* is low in adipose tissue suggesting its expression in this tissue is
217 highly constrained in the population (Fig. 6B). However, the distal heritability of *Pparg* in liver is relatively
218 high suggesting it is complexly regulated and has sufficient variation in this population to drive variation in
219 phenotype. Both local and distal heritability of *Pparg* in the islet are relatively high, but the loading is low,
220 suggesting that variability of expression in the islet does not drive variation in metabolic index. These results
221 highlight the importance of tissue context when investigating the role of heritable transcript variability in
222 driving phenotype.

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

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

225 To test whether the transcript loadings identified in the DO could be translated to another population, we
226 tested whether they could predict metabolic phenotype in an independent population of CC-RIX mice, which
227 were F1 mice derived from multiple pairings of Collaborative Cross (CC) [cite] strains (Fig. 7) (Methods).
228 We tested two questions. First, we asked whether the loadings identified in the DO mice were relevant to
229 the relationship between the transcriptome and the phenome in the CC-RIX. We predicted body weight (a
230 surrogate for metabolic index) in each CC-RIX individual using measured gene expression in each tissue and
231 the transcript loadings identified in the DO (Methods). The predicted body weight and acutal body weight
232 were highly correlated in all tissues (Fig. 7B left column). The best prediction was achieved for adipose
233 tissue, which supports the observation in the DO that adipose expression was the strongest mediator of the
234 genetic effect on metabolic index. This result also confirms the validity and translatability of the transcript
235 loadings and their relationship to metabolic disease.

236 The second question related to the source of the relevant variation in gene expression. If local regulation was
237 the predominant factor influencing gene expression, we should be able to predict phenotype in the CC-RIX
238 using transcripts imputed from local genotype (Fig. 7A). The DO and the CC-RIX were derived from the
239 same eight founder strains and so carry the same alleles throughout the genome. We imputed gene expression

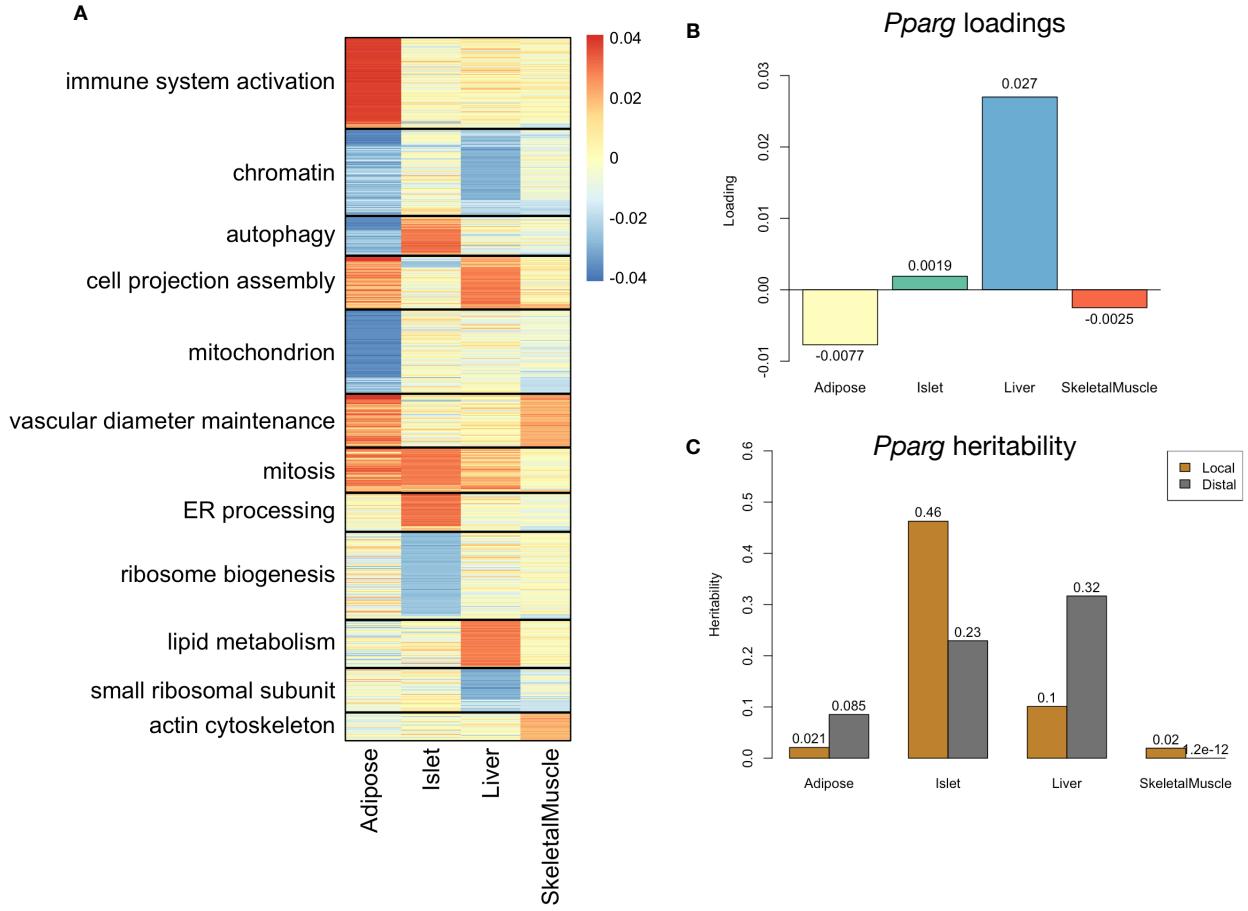


Figure 6: Tissue-specific transcriptional programs were associated with obesity and insulin resistance. **A** Heat map showing the loadings of all transcripts with loadings greater than 2.5 standard deviations from the mean in any tissue. The heat map was clustered using k medoid clustering. Functional 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.

240 in the CC-RIX using local genotype and were able to estimate variation in gene transcription robustly (Supp.
 241 Fig. 4). However, these imputed values failed to predict body weight in the CC-RIX when weighted with the
 242 loadings from HDMA. (Fig. 7B right column). This result suggests that local regulation of gene expression is
 243 not the primary factor driving heritability of complex traits, consistent with our findings in the DO population
 244 that distal heritability was a major driver of trait-relevant variation and that high-loading transcripts had
 245 comparatively high distal and low local heritability.

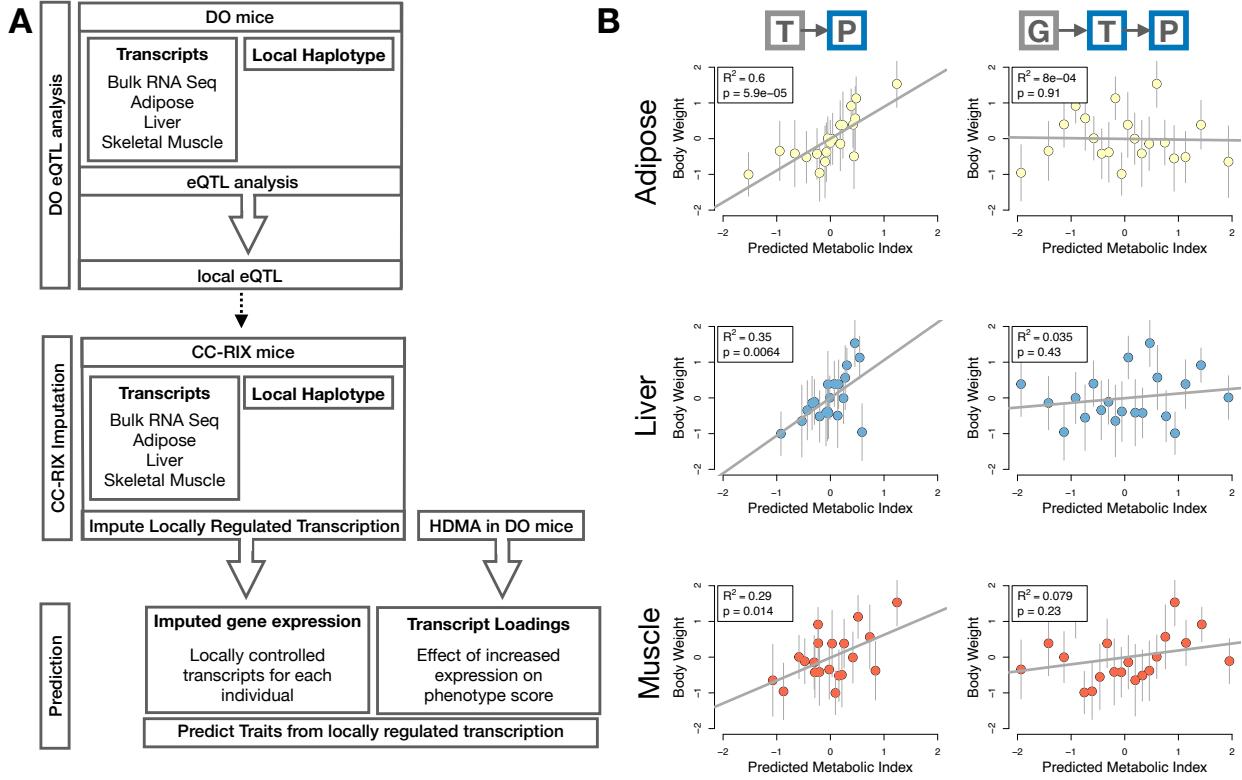


Figure 7: Transcription, but not local genotype, predicts phenotype in the CC-RIX. **A.** Workflow showing procedure for translating HDMA results to an independent population of mice. **B.** Relationships between the predicted metabolic index and measured body weight. The left column shows the predictions 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.

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

247 The interpretation of global genetic influences on gene expression and phenotype is potentially more challenging
248 than the interpretation and translation of local genetic influences, as genetic effects cannot be localized to
249 individual gene variants or transcripts. However, there are global patterns across the loadings that can
250 inform mechanism. For example, heritable variation in cell type composition can be derived from transcript
251 loadings. We observed above that immune activation in the adipose tissues was an important driver of obesity
252 in the DO population. To determine whether this is reflected as an increase in macrophages in adipose
253 tissue, we compared loadings of cell-type specific genes in adipose tissue (Methods). The mean loading
254 of macrophage-specific genes was significantly greater than 0 (Fig. 8A), indicating that obese mice were
255 genetically predisposed to have high levels of macrophage infiltration in adipose tissue in response to the
256 high-fat, high-sugar diet.

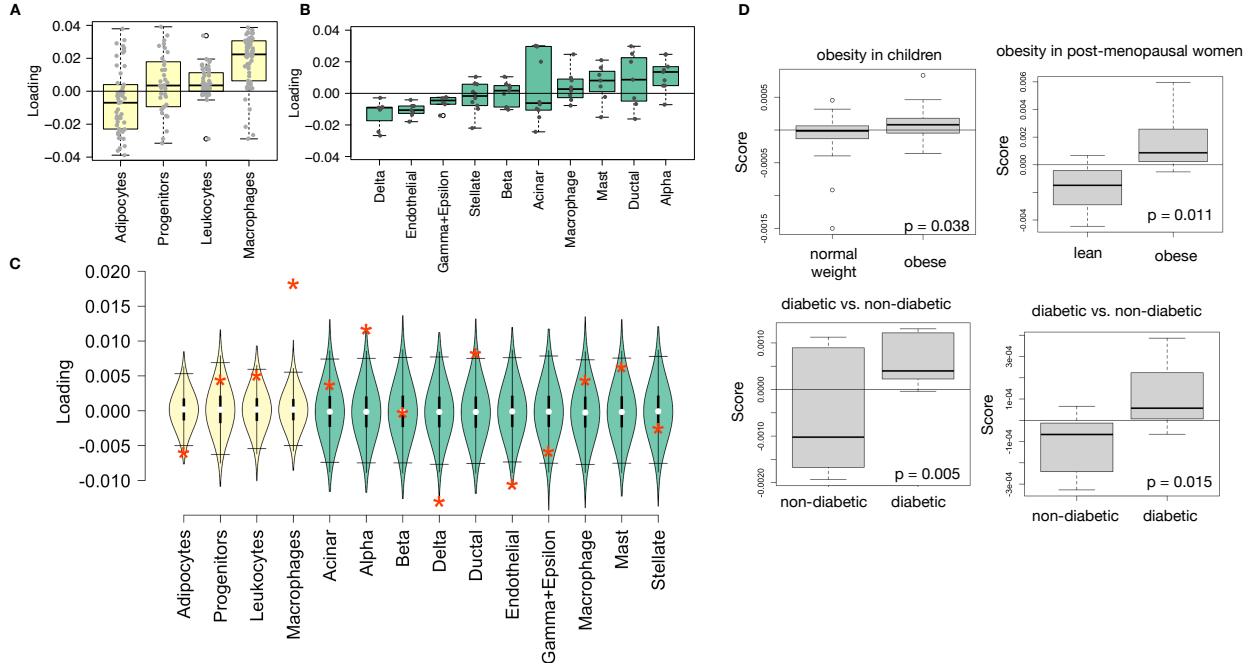


Figure 8: HDMA results translate to humans. **A.** Distribution of loadings for cell-type-specific transcripts in adipose tissue. **B.** Distribution of loadings for cell-type-specific transcripts in pancreatic islets (green). **C.** Null distributions for the mean loading of randomly selected transcripts 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.

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

265 Notably, the loadings for pancreatic beta cell-type specific loadings was not significantly different from zero.
 266 This is not necessarily reflective of the function of the beta cells in the obese mice, but rather suggests that
 267 any variation in the number of beta cells in these mice was unrelated to obesity and insulin resistance. This
 268 is further consistent with the islet composition traits having small loadings in the phenotype score (Fig. 4).

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

270 Ultimately, the heritable transcriptomic signatures that we identified in DO mice will be useful if they inform
271 pathogenicity and treatment of human disease. To investigate the potential for translation of the gene
272 signatures identified in DO mice, we compared them to transcriptional profiles in obese and non-obese human
273 subjects (Methods). We limited our analysis to adipose tissue because the adipose tissue signature had the
274 strongest relationship to obesity and insulin resistance in the DO.

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

281 **Targeting gene signatures**

282 Another potential application of the transcript loading landscape is in ranking potential drug candidates
283 for the treatment of metabolic disease. Although high-loading transcripts may be good candidates for
284 understanding specific biology related to obesity, the transcriptome overall is highly interconnected and
285 redundant, and focusing on individual transcripts for treatment may be less effective than using broader
286 transcriptomic signatures that capture the emergent biology [cite or remove]. The ConnectivityMap (CMAP)
287 database [cite] developed by the Broad Institute allows us to query thousands of compounds that reverse or
288 enhance the extreme ends of transcriptomic signatures in multiple different cell types. By identifying drugs
289 that reverse pathogenic transcriptomic signatures, we can potentially identify compounds that have favorable
290 effects on gene expression.

291 To test this hypothesis, we queried the CMAP database through the CLUE online query tool [cite] (Methods).
292 We identified top anti-correlated hits both across all cell types, as well as in adipocytes and pancreatic tumor
293 cells (Supplemental Figure XXX and XXX).

294 Looking broadly across cell types, the notable top hits from the adipose tissue loadings included mTOR
295 inhibitors and glucocorticoid agonists (Supplemental Figure XXX). It is thought that metformin, which
296 is commonly used to improve glycemic control, acts, at least in part, by inhibiting mTOR signaling^{36,37}.
297 However, long-term use of other mTOR inhibitors, such as rapamycin, are known to cause insulin resistance
298 and β -cell toxicity³⁷⁻³⁹. Glucocorticoids are used to reduce inflammation, which was a prominent signature

299 in the adipose tissues, but these drugs also promote hyperglycemia and diabetes^{40;41}. Accute treatment
300 with glucocorticoids has further been shown to reduce thermogenesis in rodent adipocytes^{42–44}, but increase
301 thermogenesis in human adipocytes^{45;46}. Thus, the pathways identified by CMAP across all cell types were
302 highly related to the transcript loading profiles, but the relationship was not a simple reversal.

303 The top hit in adipocytes was a PARP inhibitor (Supplemental Figure XXXB). PARPs play a role in lipid
304 metabolism and are involved in the development of obesity and diabetes⁴⁷. PARP1 inhibition increases
305 mitochondrial biogenesis⁴⁸. Inhibition of PARP1 activity can further prevent necrosis in favor of the less
306 inflammatory apoptosis⁴⁹, thereby potentially reducing inflammation in stressed adipocytes. Other notable
307 hits in the top 20 were BTK inhibitors, which have been observed to suppress inflammation and improve
308 insulin resistance⁵⁰ as well as to reduce insulin antibodies in type I diabetes⁵¹, and IKK inhibitors have been
309 shown to improve glucose control in type II diabetes^{52;53}.

310 The CMAP database includes assays in multiple cell types. Among the top hits for the query with transcript
311 loadings from pancreatic islets (Fig. XXX), was suppression of T cell receptor signaling, which is known to
312 be involved in Type 1 diabetes⁵⁴, as well as TNFR1, which has been associated with mortality in diabetes
313 patients⁵⁵. Suppression of NOD1/2 signaling was also among the top hits. NOD1 and 2 sense ER stress^{56;57},
314 which is associated with β -cell death in type 1 and type 2 diabetes⁵⁸. This cell death process is dependent
315 on NOD1/2 signaling⁵⁶, although the specifics have not yet been worked out.

316 Among the top hits in pancreatic tumor cells were known diabetes drugs, including sulfonylureas, PPAR
317 receptor agonists, and insulin sensitizers. Rosiglitazone is a PPAR- γ agonist and was one of the most
318 prescribed drugs for type 2 diabetes before its use was reduced due to cardiac side-effects⁵⁹. Sulfonylureas
319 are another commonly prescribed drug class for type 2 diabetes, but also have notable side effects including
320 hypoglycemia and accellerated β -cell death⁶⁰.

321 Discussion

322 Here we used a novel high-dimensional mediation analysis (HDMA) to investigate the relative contributions
323 of local and distal gene regulation to heritable trait variation in a genetically diverse mouse model of diet-
324 induced obesity and metabolic disease. We found that distally heritable transcripts dominated the composite
325 transcripts mediating the effect of genetic background on metabolic traits, and that the composite transcripts,
326 but not local eQTL, were able to predict obesity in an independent mouse population with divergent allelic
327 structure. Moreover, the composite transcript from adipose tissue predicted obesity and diabetes status in
328 human cohorts with measured adipose gene expression. Taken together, these results support the hypothesis

329 that gene expression mediating the effect of genetic background on phenotype are primarily distally regulated,
330 and that the gene regulatory networks influencing metabolic disease are conserved across mice and humans.

331 It has frequently been assumed that gene regulation in *cis* is the primary driver of genetically associated
332 trait variation, but attempts to use local gene regulation to explain phenotypic variation have had limited
333 success^{16;17}. In recent years, evidence has mounted that distal gene regulation may be an important mediator
334 of trait heritability^{19;18;61}. It has been observed that transcripts with high local heritability do not explain
335 as much expression-mediated disease heritability as transcripts with low local heritability¹⁹. Consistent
336 with these observations, genes located near GWAS hits tend to be enriched with functional annotations and
337 complexly regulated¹⁸. In contrast, genes with high local heritability (simple regulation) tend to be depleted
338 of functional annotations suggesting they are less likely to be directly involved in disease traits¹⁸.

339 Consistent with these observations, we found here that transcript loading in the composite transcripts was
340 negatively correlated with local heritability and positively correlated with distal heritability. Together these
341 observations suggest that distal gene regulation was the dominant mode through which gene expression
342 mediated the effect of genetic background on phenotype.

343 These observations and others led Liu et al.⁶¹ to suggest that most heritability of complex traits is driven by
344 weak distal eQTLs. They proposed a framework of understanding heritability of complex traits in which
345 massive polygenicity is distributed across common variants in both functional “core genes”, as well as more
346 peripheral genes that may not seem obviously related to the trait.

347 The transcripts that contributed the most to the composite transcripts were strongly distally regulated
348 and only weakly locally regulated, suggesting that distal gene regulation plays a primary role in driving
349 heritable trait variation. In support of this hypothesis, we demonstrated that the transcriptomic signatures
350 we identified predicted obesity in an independent population of CC-RIX mice and in humans, whereas a
351 model using local gene regulation alone was unable to predict obesity in the mice. Finally, we showed that
352 these transcriptomic signatures have multiple applications, including analyses of tissue composition from bulk
353 RNA-Seq and drug prioritization.

354 The mediating composite transcript included expression from four tissues known to be involved in metabolic
355 disease. Gene expression in adipose tissue was the strongest mediator of genetic effects on metabolic disease.
356 Further analysis of the loadings onto transcripts in each tissue revealed that the mediating signatures
357 were tissue-specific transcriptional programs, many of which were previously known to be involved in the
358 pathogenesis of metabolic disease. We showed here that regulation of these programs is heritable and mediated
359 a large proportion of disease risk.

360 This approach contrasts with traditional univariate approaches in several important respects. First, in contrast
361 to univariate approaches, which assume independence of genetic variants and transcripts, HDMA allows for
362 arbitrarily complex gene regulation, as well as the interconnectedness and redundancy of the transcriptome.
363 Second, rather than assuming a single, large genetic effect as univariate approaches do, HDMA assumes that
364 traits are highly polygenic, and that genetic effects are weak and are distributed across the genome. HDMA
365 generates a weighted vector of transcripts that can be analyzed as a whole, or dissected to identify transcripts
366 with stronger and weaker effects.

367 This method explicitly models a central proposal of the omnigenic model which posits that once the expression
368 of the core genes (i.e. trait-mediating genes) is accounted for, there should be no residual correlation between
369 the genome and the phenotype.

370 These ideas are consistent with principles of robustness in complex systems^{62–64}. If a transcript were both
371 important to a trait and subject to strong local regulation, a population would be susceptible to extremes
372 in phenotype that might frequently cross the threshold to disease. Indeed, strong disruption of highly
373 trait-relevant genes is the cause of Mendelian disease.

374 The transcripts with the highest loadings are similar to the core genes of the omnigenic model [REF]. These
375 were transcripts of moderate local heritability that were highly functionally related to the traits. Transcripts
376 with small loadings are more peripheral to the traits measured in this experiment. There was no clear
377 demarcation between the core and peripheral genes as far as loading, but a clear separation should not be
378 expected given the complexity of gene regulation and the genotype-phenotype map⁶⁵.

379 We saw further that the distal heritability was weak and spread across the genome, consistent with the
380 prediction by Liu *et al.*⁶¹ that trait heritability is mediated through weak distal eQTLs. Most strongly
381 mediating transcripts had modest distal heritability, and even for those whose expression was strongly
382 regulated by distal factors, these factors were multiple and spread across the genome. For example, *Nucb2*,
383 was a strongly mediating transcript in islet and was also strongly distally regulated (66% distal heritability).
384 This gene is expressed in pancreatic β cells and is involved in insulin and glucagon release^{66–68}. Although its
385 transcription was highly heritable in islets, that regulation was distributed across the genome, with no clear
386 distal eQTL (Supp. Fig. 5). Thus, although distal regulation of some genes may be strong, this regulation is
387 likely to be highly complex and not easily localized.

388 The high complexity of gene regulation combined with a systems-level analysis yields continuous results
389 that do not necessarily implicate individual transcripts or genetic loci in disease pathogenesis. Most studies
390 have focused on pinpointing individual loci whose mechanistic roles can be clearly dissected through further

391 experiments and exploited at therapeutic targets. In this analysis, too, it is possible to focus on individual
392 genes and their context in both tissues and pathways. For example, the loadings on *Pparg* were tissue-specific
393 in a way that comports with known biology, i.e. it is known to be protective in adipose tissue where it was
394 negatively loaded, and harmful in the liver, where it was positively loaded. However, whole transcriptomic
395 signatures can also be informative in their own right. Combined with increasing amounts of high-dimensional
396 data in public databases, weighted vectors can be useful for generating hypotheses and potential drug
397 treatments. We showed that weighted vectors of genes can be analyzed for enriched biological functions
398 and pathways using GSEA. These vectors can also be paired with data about cell-type specific genes to
399 generate hypotheses about cell composition in individual tissues. Gene expression derived from patient
400 biopsies confirmed that the transcriptional signatures we identified in mice predict obesity status in humans,
401 further supporting the translatability of these results. Finally, we used the CMAP database to show that the
402 transcriptomic signatures we identified in mice could be translated into human drug targets, as currently
403 used diabetes drugs and targets were among the top hits for reversing the disease-associated signatures. That
404 these drugs are known to reverse diabetes pathogenesis supports the causal role of these gene signatures in
405 disease risk as modeled by high-dimensional mediation.

406 In conclusion, we have shown that both tissue specificity and distal gene regulation are critically important to
407 understanding the genetic architecture of complex traits. We identified important genes and gene signatures
408 that were heritable, causal of disease, and translatable to other mouse populations and to humans. Finally,
409 we have shown that by directly acknowledging the complexity of both gene regulation and the genotype-to-
410 phenotype map, we can gain a new perspective on disease pathogenesis and develop actionable hypotheses
411 about pathogenic mechanisms and potential treatments.

412 Potentially comment on: 1) Mediating on other endophenotypes 2) Limitation that we only find signatures
413 “consistent with” mediation and that our approach is hypothesis generating 3) Is the kinship matrix the
414 sum of all local QTLs, or is it something more inclusive, including distal regulation, development, etc. 4)
415 endophenotypes don’t need to be gene expression. Can be anything you think is causally related to phenotype
416 and can be manipulated

417 Data Availability

418 Here we tell people where to find the data

419 Acknowledgements

420 Here we thank people

421 **Supplemental Figures**

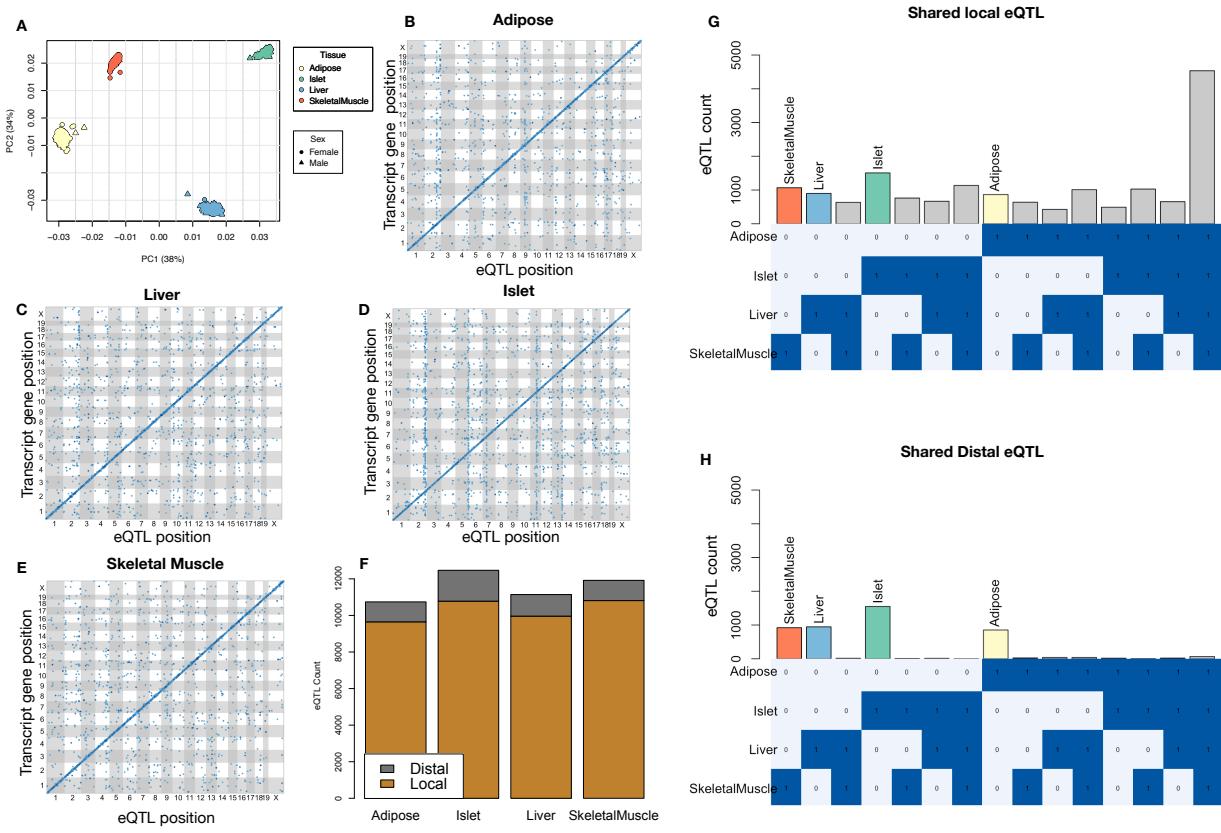


Figure 9: Overview of eQTL analysis in DO mice. **A.** RNA seq samples from the four different tissues clustered by tissue. **B.-E.** eQTL maps are shown for each tissue. The *x*-axis shows the position of the mapped eQTL, and the *y*-axis shows the physical position of the gene encoding each mapped 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

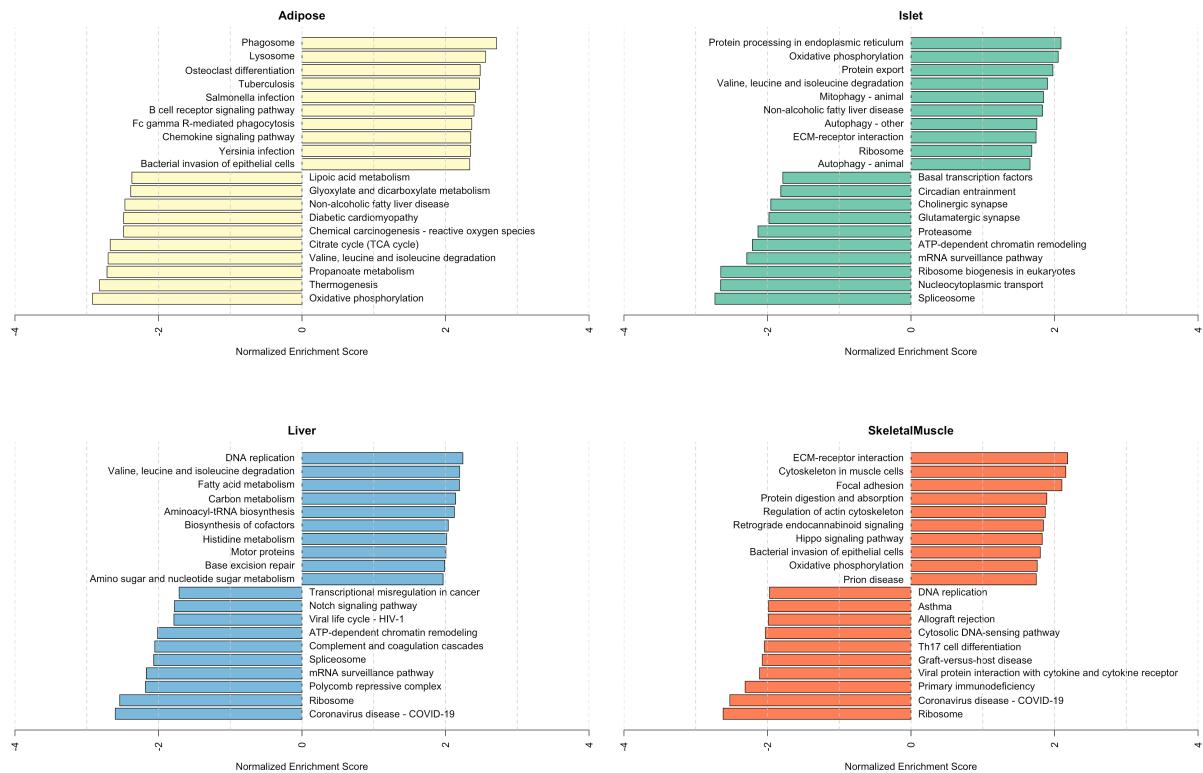


Figure 10: Bar plots showing normalized enrichment scores (NES) for KEGG pathways as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched KEGG pathway.

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Top GO term enrichments by GSEA

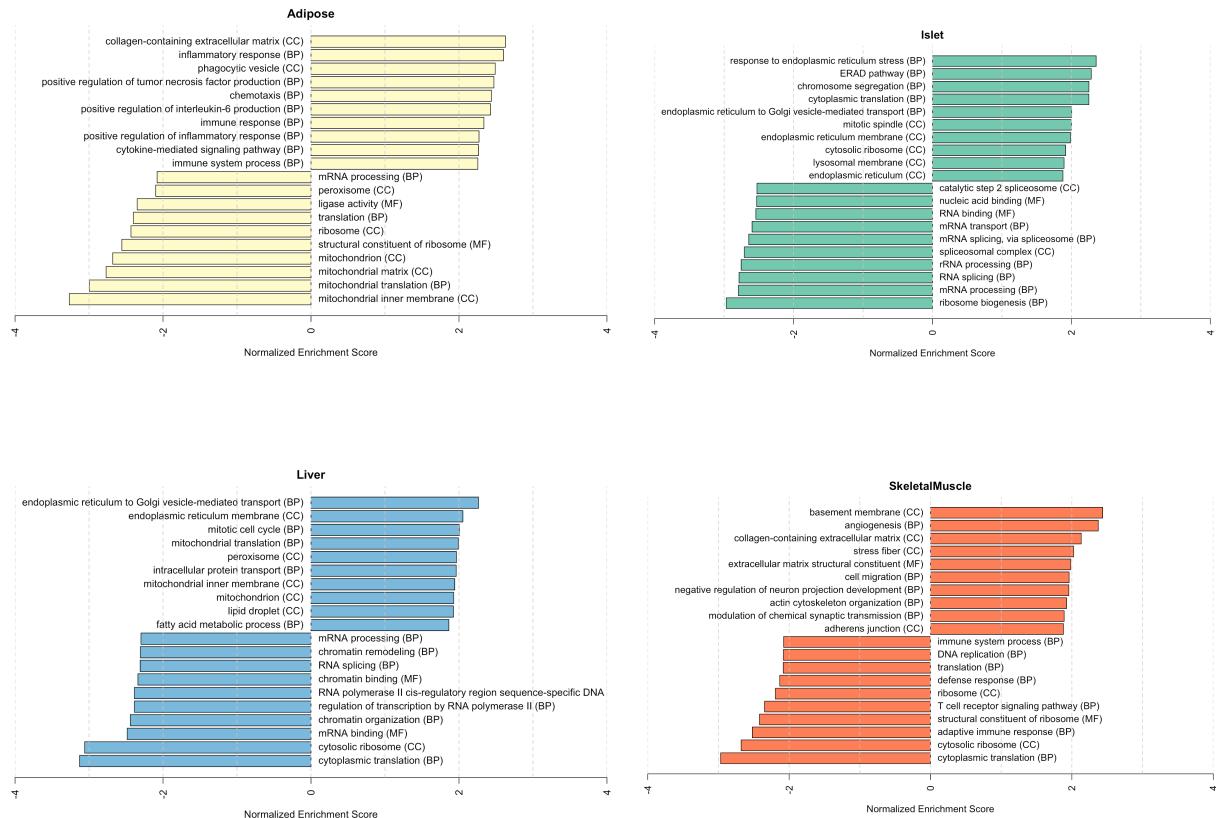


Figure 11: Bar plots showing normalized enrichment scores (NES) for GO terms as determined by fast gene score enrichment analysis (fgsea). Only the top 10 positive and top 10 negative scores are shown. Colors indicate tissue. The name beside each bar shows the name of each enriched GO term. The letters 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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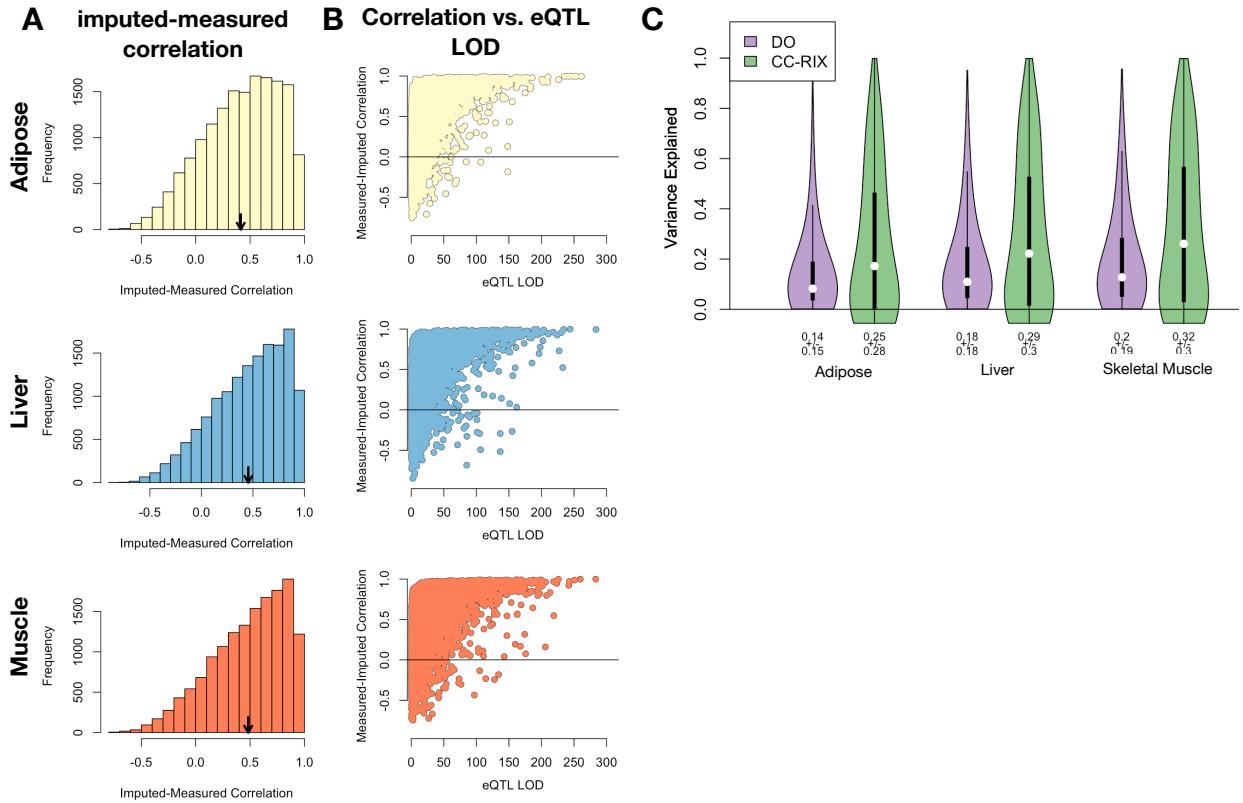


Figure 12: Validation of transcript imputation in the CC-RIX. **A.** Distributions of correlations between imputed and measured transcripts in the CC-RIX. The mean of each distribution is shown by the red line. All distributions were skewed toward positive correlations and had positive means near a Pearson 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.

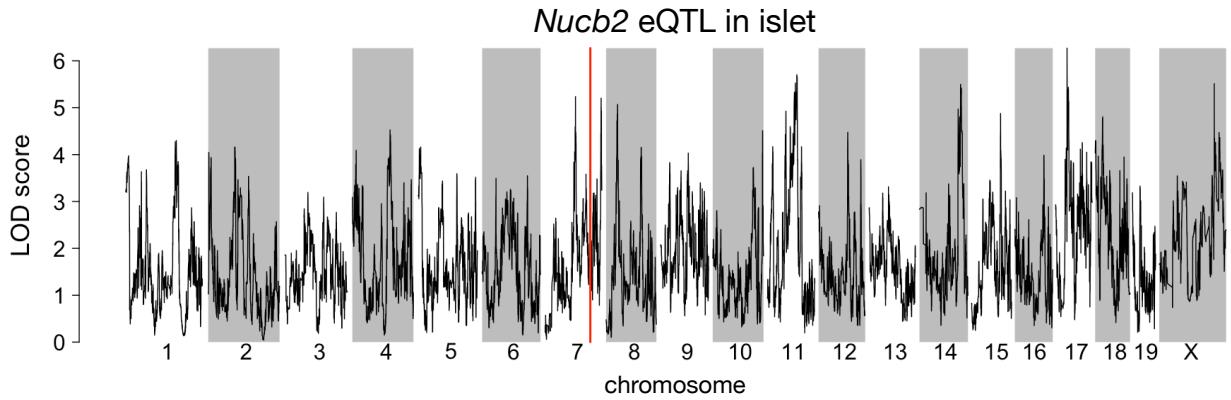


Figure 13: Regulation of *Nucb2* expression in islet. *Nucb2* is encoded on mouse chromosome 7 at 116.5 Mb (red line). In islets the heritability of *Nucb2* expression levels is 69% heritable. This LOD score trace shows that there is no local eQTL at that position, nor any strong distal eQTL anywhere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

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