October XXX, 2024

Dear Editors,

My co-authors and I are writing to submit the manuscript entitled “Transcripts with high distal heritability mediate genetic effects on complex metabolic traits”, as a research article for *Nature Genetics.*

This work studies the genetic architecture of complex traits using diet-induced obesity and metabolic disease as an archetypal example. We combined two multi-tissue, clinically relevant data sets from independent, genetically diverse mouse populations with a novel high-dimensional mediation analysis (HDMA) to investigate the relationship between expression quantitative trait loci (eQTLs) and the heritability of complex traits. Such data are essentially impossible in human subjects, making our study a unique opportunity to test foundational questions of genetic architecture. Using these data, we provide the first experimental validation of an emerging theory about the critical importance of distal gene regulation for complex trait heritability that has recently developed through an ongoing conversation in *Nature Genetics*, *Cell*, and other top journals.

Evidence from GWAS suggests that genetic variants influence complex traits through regulation of gene expression; however, identifying disease-associated eQTLs has had minimal success. Recent studies published in *Nature Genetics* have critically examined this shortcoming with an emerging possible explanation that distal effects may be more relevant to complex traits than local eQTLs (Yao *et al.* 2020; Vosa *et al.* 2021; Mostavi *et al.,* 2023). In two highly cited papers in *Cell*, Pritchard and colleagues have proposed the *omnigenic model*, a controversial hypothesis positing that highly diffuse distal effects converge on core, trait-driving genes that are themselves buffered against genetic variation (Boyle, *et al.* 2017; Liu, *et al.* 2019). However, as these studies were performed in human data, they are limited in their ability to comment on causal relationships between eQTLs and complex traits. In contrast, our data on genetically diverse mice allow us to rigorously differentiate between local and distal genetic effects.

In a comprehensive series of analyses, we demonstrate definitively that distal regulation of gene expression is the primary driver of trait variation and that the corresponding transcriptomic signatures generalize between mouse populations and to humans, while locally predicted signatures do not. Our novel HDMA framework allows us to rigorously detect this distal signal, thereby functionalizing the trait-driving components of gene expression that cannot be mapped. These findings have profound implications for the ongoing conversation surrounding the role of gene regulation in the heritability of complex traits. Recognizing that distal, rather than local, regulation is the primary mediator of complex trait variation will completely shift the strategy for identifying molecular drivers of pathological traits. We offer solutions, in the form of HDMA, for both identifying and interpreting causal mechanisms of trait variation.

With this manuscript, we provide free public access to unique data sets consisting of genotypes, phenotypes, and gene expression from two genetically diverse mouse populations. We also provide free public access to all code used to perform HDMA and downstream analyses. These data and code provide important resources for further investigation of causal mechanisms of trait heritability in both humans and model organisms.

We believe that this manuscript will be of broad interest to geneticists. The manuscript includes main text, eight figures, thirteen supplementary figures, and a supplementary file. We are aware that the text needs to be shortened for final publication, and we have a plan in place for reducing the word count. No author has any financial, personal, or professional interests that could be construed to have influenced the paper. Thank you for your consideration of this manuscript.

Sincerely,