Title: Omnigenic epistasis regulates complex trait heritability

Anna K Miller[1], Calvin Pan[2], Aldons J Lusis[2], Dana C Crawford[1,3], Scott M Williams[1,3]*, David A Buchner[1,4]*

[1]Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland OH [2]Departments of Medicine, Microbiology, and Human Genetics, University of California, Los Angeles, Los Angeles, CA [3]Department of Population and Quantitative Health Sciences, Case Western Reserve University School of Medicine, Cleveland OH [4]Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland OH

*Notes co-corresponding authors

Abstract

The non-linear components of the genetic architecture of most common traits have been difficult to define. To better understand the architecture of complex traits we detected both main effect (additive) and epistatic QTLs in a sex-stratified manner in the Hybrid Mouse Diversity Panel. A high concordance of effects between males and females was observed for nearly all loci across the genome for both additive and epistatic effects, indicating that identified variants do not represent random noise. Instead, these results demonstrate that most loci contribute to trait heritability, consistent with an omnigenic model of inheritance that includes both additive and epistatic interactions. These findings demonstrate the role of genome-wide genetic interactions in the genetic architecture of complex mammalian traits and can explain why current genome-wide association studies fail to detect a considerable portion of trait heritability.

Introduction

Complex traits are regulated by many genetic variants that together influence the severity, presentation, and prevalence of a phenotype[1,2]. Genome-wide association studies (GWAS) have successfully identified many such variants for complex traits but most of these studies were conducted with the assumption that variant effects are independent. Thus, for nearly all complex traits, most detected variants are modeled as explaining small additive changes in risk. However, these combined effects fail to account for most trait heritability, which led to the term “missing heritability”[3]. Missing heritability may be due to many factors including human ancestry, environmental factors, genetic interactions, and rare variants[3,4]. Others proposed it may be due to many non-significant single nucleotide polymorphisms (SNPs) that collectively account for a substantial proportion of trait heritability[5]. An extension of this hypothesis, known as the omnigenic model, proposes that heritability is explained by the cumulative effects of genetic variation in nearly all genes expressed in relevant cell types, including genes outside of obviously relevant genetic pathways[2].

Characterizing non-linear effects, in particular in the context of an omnigenic model, has been difficult for human phenotypes in part due to limiting computational resources and the diversity of genetic backgrounds expected for outbred populations that are usually studied. To address the latter limitation, we utilized data from the Hybrid Mouse Diversity Panel (HMDP), a population of over 100 inbred and recombinant inbred mouse strains designed to measure and identify the genetic and environmental factors underlying complex traits[6]. Compared to GWAS in humans, the HMDP better controls variation due to environmental factors, has many relevant tissues available for phenotyping, and the strains can be used for the integration of follow-up studies. The HMDP panel also provides an order of magnitude greater mapping resolution compared to traditional linkage analyses in mice[6,7]. As evidence of these advantages, use of the HMDP has successfully identified novel genes for a diverse array of traits, including obesity,

diabetes, atherosclerosis, and heart failure, many of which have been validated using transgenic or knockout models[6,8-10]. Given the outstanding questions related to defining the genetic architecture of complex traits, the HMDP represents a powerful tool for identifying specific variants that regulate complex traits in mice, as well as discovering more complex patterns of how genetic variation influences trait variation and disease susceptibility[11].

In this study, we report a sex-stratified association analysis of several metabolic and blood traits using the HMDP. Given that the vast majority of association studies combine sexes in their analyses and simply adjust for sex as a covariate, this approach can reveal associations hidden in a sex-combined analysis that only adjusts for sex using traditional methods of genome-wide analyses[12]. The detection of marginal and interaction effects separately in females and males also enabled comparisons between the direction of effects in each sex, and testing whether these effects were more often in the same direction than what would be expected by chance. The implication of an enrichment in similar directionality between effects in males and females is that these loci have comparable and functional roles in the regulation of that trait. In our analyses, the direction of effects across sexes were more similar than expected by chance for nearly all loci, including both statistically significant and non-significant loci, as well as for both main effects and epistatic interactions. These findings are consistent with an omnigenic model for both main and epistatic effects, re-defining our understanding of the scope and scale of non-additive interactions and their critical role in the genetics of complex traits and diseases.

Results

Heritability

Narrow sense and broad sense heritability were calculated separately for male and female mice for 8 metabolic-related traits and 3 blood-related traits (Table 1). On average, the

metabolic traits had higher heritability than the blood traits in both sexes. Broad sense heritability was greater than narrow sense heritability in males and females for all traits except adiposity in males. The heritability estimates differed between the two sexes for many of the traits; therefore, all subsequent analyses were stratified by sex.

Detection of loci with main effects

Main effect associations for 8 metabolic traits were tested in females using 694 mice encompassing 98 strains from the HMDP. The Bonferroni-corrected threshold for significant main effects, based on 12,221 haplotype blocks and 2,314 SNPs outside of the haplotype blocks, was $p < 4.13E-6$. At this threshold, there were no significant marginal effects in females (Table 2). However, there was a significant enrichment of main effects as compared to the number expected by chance alone at the nominal $p < 0.05$ significance level for all traits except HDL (Table S2). For HDL, significantly fewer main effects were detected at $p < 0.05$ than expected by chance alone (Table S2). While we were unable to detect statistically significant main effects using a conservative Bonferroni threshold, we identified associations that were previously observed in these data sets using slightly different strain composition and an FDR-based threshold for significance at $p < 1.3E-5$[12]. There were 5 unique SNPs in adiposity and 1 unique SNP in HDL that passed this less conservative threshold (Table S3).

Main effects of the same 8 metabolic traits were detected in males using 664 mice from 102 strains from the HMDP. The Bonferroni-corrected threshold for significant main effects, based on 11,385 haplotype blocks and 5,907 SNPs, outside of the haplotype blocks was $p < 2.89E-6$. There were multiple significant marginal associations detected for adiposity (n=58), bodyweight (n=22), and fat mass (n=60), which are the traits with the largest narrow sense heritability among those studied (Table 2, Table S1). As adiposity, body weight, and fat mass are highly related, we examined whether the significant SNPs overlapped between these traits. Overall, 20 haplotype blocks contained SNPs associated with all three traits. Thirty-five

additional haplotype blocks contained SNPs associated with two of the traits. Thirty-three blocks were significantly associated with both adiposity and fat mass, 1 block was significantly associated with both adiposity and body weight, and 1 block was significantly associated with both body weight and fat mass (Fig. S1, Fig. S2, Table S4). Only 10 haplotype blocks were unique to one of the three traits (Fig. S1, Table S4).

Main effects for 3 blood traits were assessed in females using 670 mice encompassing 97 strains and in males using 640 mice encompassing 98 strains from the HMDP. The Bonferroni-corrected threshold for significant main effects was based on 12,193 haplotype blocks and 5,221 SNPs outside of the haplotype blocks in females ($p < 2.87E-6$). In males there were 11,437 haplotype blocks and 5,752 SNPs outside of the haplotype blocks (corrected $p < 2.91E-6$). At these thresholds, there were no significant marginal effects in either males or females (Table 2). However, there was significant enrichment in main effects ($p < 0.05$) for all traits in both sexes (Table S2).

Detection of loci with pairwise epistatic interactions

In addition to main effects, pairwise interactions were detected in both male and female mice of the HMDP for the metabolic traits. In female mice, 8 significant pairwise epistatic interactions were detected for fat mass at a Bonferroni-corrected significance threshold of $p < 6.82E-10$ (Table S1). No interactions were detected for the other 7 traits; however, body weight, fat mass, and HDL had a significantly enriched number of interactions with a nominal p-value < 0.05 compared to the number expected by chance (Table S2). All of the significant interactions for fat mass were inter-chromosomal interactions (Fig. S2).

In male mice, significant pairwise epistatic interactions were detected for adiposity, body weight, cholesterol, fat mass, and triglycerides, but not for glucose, HDL, and insulin (Table 2, Table S1). Additionally, there was a significant enrichment of interactions at a nominal $p < 0.05$

for cholesterol, triglycerides, and HDL, whereas adiposity, body weight, fat mass, glucose, and insulin had significantly fewer interactions than expected by chance (Table S2). Despite the particularly high number of interactions detected for adiposity, body weight, and fat mass, a higher percentage of loci had marginal effect associations that were significant at the Bonferroni level for these traits than was observed for pairwise interacting loci. This is a reflection of the large number of unique possible interactions that were tested (~149 million) as compared to the number for marginal associations (~17,000). These interacting loci were widely distributed throughout the genome (Fig. S4, Fig. S5, Fig. S6). Despite the larger number of interactions detected in males relative to females, there was no enrichment of interacting loci on the X chromosome (Fig. S4, Fig. S5, Fig. S6). As with main effects, many of the significantly interacting pairs were common among related phenotypes. For example, there were also 44,579 interactions common among adiposity, body weight, and fat mass, with many other interacting pairs shared between two of the three traits (Table S6, Fig. S7). Also, there were 58 interactions for cholesterol and 122 for triglycerides, but 35 interacting pairs overlapped between the two traits. Of the significant pairwise interactions detected in metabolic traits, intra-chromosomal interactions comprised 7.3% of interactions for adiposity, 6.7% for body weight, and 6.3% for fat mass, all enriched compared to the ~5% expected by chance alone.

None of the blood traits had significant interactions at the Bonferroni-corrected threshold (Table 2). However, the percent of granulocytes in males and females, as well as monocyte percent and white blood count in males, were significantly enriched for interactions at a $p < 0.05$, relative to the number expected by chance. Monocyte percent and white blood count in females had significantly fewer interactions than expected by chance (Table S2).

Concordant Directionality of Effects in Male and Female Mice

Given the increased number of significant main effect and interacting loci detected in males relative to females, we hypothesized that associations in females would have the same

direction of effect on each trait as that in males, even if they did not reach the level of statistical significance in females. For example, SNP rs48316748 was the most significant SNP associated with body weight in male mice in the HMDP ($p < 1.27E-11$), with the “A” allele being associated with increased body weight. The “A” allele at this SNP was also associated with an increase in body weight in the analysis of body weight in females, although the p-value was not statistically significant ($p < 0.11$). To test the hypothesis of concordance of direction of effect among statistically significant loci, the direction of effect of each SNP was assessed in each sex and compared. If there were no effects shared between male and female mice, the null hypothesis was that the direction of effects in the two sexes should be the same 50% of the time, based on random equivalence. However, among the three traits for which significant main effects were detected in males (adiposity, body weight, and fat mass), the allelic direction of effect was the same in males and females more frequently than by chance alone (range: 63-100%) (Table 4). This was also seen for the Bonferroni significant interactions, with 79-100% of significant interactions in males having the same direction of effects in females (Table 4). Therefore, although the significant main effects and interaction effects may not be statistically significant in both sexes, they nonetheless had a consistent effect in both male and female mice. The conserved directionality of effects between the sexes was not simply due to most SNPs throughout the genome having the same direction of effect, as the percentage of positive and negative betas for all main effects and interactions were nearly equivalent, with slightly more negative than positive betas (Table S5).

To extend these findings beyond the loci with p-values that met the conservative Bonferroni-adjusted threshold for significance, we next tested whether the direction of effects was similar in males and females for all main effects and interactions tested, regardless of statistical significance. Concordance between males and females were analyzed and sorted based on the p-value for association in males. Male p-values were used as most of the

significant main effects and interactions were found in males. For all traits examined, the percentage of loci with effects in the same direction in males and females was far greater than the 50% expected by chance for both main effects (Fig. 1) and interactions (Fig. 2) across nearly all levels of statistical significance. The enrichment relative to chance was greatest among the most significant loci, and decreased towards the 50% expected by chance as the p-values increased, asymptotically approaching 50% as p-values approached 1. These data demonstrate that the marginal and interaction effects detected for most loci throughout the genome are not simply random noise, and instead indicate that most loci are in fact contributing to trait heritability via both marginal and interaction effects.

Interactions comprising of similar levels of positive and negative epistasis

Having demonstrated the widespread nature of genetic interactions underlying genetic architecture, it remained to be determined the directional nature of these interactions in terms of positive or negative epistasis, with important implications for evolutionary selective pressures[13]. Interactions are positive where genotype combinations show increased effects relative to the sum of independent additive effects, and are negative when effects of the interacting loci decrease the effect of alleles in comparison to their independent additive effects[14]. To assess the relative levels of positive and negative epistasis among the interacting loci, we first selected the interactions with the same direction of effect in both sexes and a p-value < 0.5 as these are more likely to represent true interactions. These interactions are however unlikely to include those with sexually dimorphic effects. The pairwise interactions were further pruned to include only those for which both individual SNPs had a marginal effect with the same direction of effect in order to more easily infer the direction of the non-additive component. Of the remaining interactions, those with interaction effects in the same direction as the individual SNP marginal effects were considered positive epistasis, because the non-additive component is enhancing the effects of the marginal associations. Those where the interaction effects are in the opposite

direction as the individual SNP marginal effects were considered negative epistasis. For each trait, on average 45.89% [range: 40.23- 51.72%] of the interactions were positive epistasis and 54.11% were negative epistasis [range: 48.23-59.77%] (Table 3). Thus, of the interactions analyzed, approximately half were positive epistasis and half were negative epistasis, with a slight but consistent bias towards negative epistasis (Table 3).

Hidden impact of genetic interactions on apparently additive effects

We sought to test whether the most significant SNPs underlying main effects and interactions were the same. Towards this end, we selected the 1,000 most significant interactions for each trait in males and females (based on p-values) and examined where they ranked in terms of statistical signficance among all loci for main effect assocations. For male adiposity, body weight, and fat mass, the most significantly interacting SNPs were enriched among the most significant marginal associations (Fig. 3A-C). However, this same enrichment was not observed for any other trait in males nor in any of the traits in females. In fact, among the other traits, the most significant interacting SNPs were underrepresented among the most significant marginal associations. Of note, male adiposity, body weight, and fat mass were the traits with the greatest abundance of highly significant interacting loci, and were also the only traits demonstrating an enrichment of interacting loci among the top marginal hits. This indicates that the genetic architecture of these traits includes interactions with large enough effects that they are detectable as main effects. Based on these findings, we hypothesized that in other traits with highly significant interactions, a similar pattern might exist. To test this hypothesis, we examined only the 122 Bonferroni significant triglyceride interactions and similarly found that these loci were overrepresented among the most significant marginal associations (Fig. 3I). Thus, when highly significant interactions underly the genetic architecture of a given trait, it is possible that these loci will be detected as main effect loci, whereas in the absence of highly significant interactions, the loci underlying the most significant additive and non-additive effects

appear to be distinct (Fig. 3D-H, Fig. S8, Fig. S9). It is not clear how to *a priori* distinguish between these two possibilities without directly and exhaustively testing for non-additive interactions for each trait.

Discussion

In the current study, we utilized the HMDP to measure the sex-specific heritability of several metabolic and blood traits. As expected, broad sense heritability was higher than narrow sense heritability across almost all traits and in both sexes, indicating a contribution from dominance or non-additive factors to total heritability. However, the additive component was always the largest component of total heritability. The contribution of non-additive or dominance factors to total heritability is consistent with many prior studies, as is the smaller contribution relative to additive effects[12,15-18]. However, it remains possible that a portion of the additive effects are in fact due to their underlying effect in interactions[19,20]. This is supported by our observation that among the traits for which tens of thousands of highly significant interactions were discovered (adiposity, body weight, fat mass), the most significant loci were also among the most significant additive loci. It is thus likely that the “additive” effects of these loci are at least in part due to the pairwise interactions involving these loci. However, this was clearly not the case for all traits, as those for which thousands of significant interactions were not detected, there was no evidence of enrichment of interacting loci among the most significant additive loci. It is worth noting that adiposity, body weight, and fat mass, exhibited the highest levels of narrow sense heritability relative to all other traits, perhaps reflecting the increased contribution of non-additive effects masquerading as additive.

Given that heritability estimates strongly indicated a role for non-additive interactions in the complex trait heritability, this study aimed to better understand their role in the underlying

genetic architecture. Studies were performed using standard linear regression analyses to identify significant marginal associations and non-additive epistatic interactions, and many such loci were discovered (Table 2). However, it is widely appreciated that many statistical hurdles limit the ability to identify pairwise interactions that meet conservative thresholds for statistical significance. Thus, we reasoned that many of the non-significant loci identified may nonetheless represent bona-fide interactions that contribute to trait heritability. Based on the hypothesis that interaction effects are likely to be conserved between males and females, we utilized a novel means to test whether SNP-SNP interactions are real based on enrichment of directional effects between males and females relative to that expected by chance, as opposed to an arbitrary p-value threshold applied to each interaction test. These results overwhelmingly demonstrated that tens of millions of pairwise interactions are contributing to trait heritability, which is orders of magnitude greater than prior demonstrations of epistasis in mammalian systems, and is consistent with an omnigenic model that includes epistasis underlying the genetic architecture of complex traits. In addition to the omnigenic epistasis discovered, omnigenic marginal effects were also detected for each trait. The detection of many marginal effects likely contributing to the genetic architecture echoes previous findings[2], although a different statistical method was used, different traits were examined, and a different organism was studied. Together the results support the robustness of the omnigenic model as a key feature in the genetic architecture of all complex traits.

The scope of the genetic interactions detected here is extraordinary. Dating back to Fisher, many thought that interactions existed, but given the difficulty associated with their detection, they were thought to contribute little towards the heritability of complex traits[15,21-24]. In contrast, these data indicate that most variants in the genome are likely associated with both main effects and interactions and are therefore contributing to trait heritability under an omnigenic model. Despite the small effects associated with each genetic interaction, the sheer

number of interactions implies that collectively they may account for a large proportion of trait heritability[25]. Thus, accounting for the omnigenic nature of the genetic architecture may be important for improving the modeling of trait heritability, and incorporation of these loci may improve the ability to predict disease risk via more accurate polygenic risk scores (PRS). As argued previously, we suggest PRS can be used to efficiently model the complex traits using many main effects and interactions[26].

The omnigenic model has not been identified in previous tests for genetic interactions, despite many statistical attempts to discover epistatic loci more efficiently. One such method for reducing the statistical penalties associated with epistasis, instead of a genome x genome search for interacting loci, is to instead focus only on those loci that have significant marginal effects. Many interactions have detected interactions with this method. [27,28] However, in this study, many of the statistically significant interactions detected included SNPs with a non-significant marginal effect. Thus, some of the detected interactions would have been missed using this statistical shortcut. It was of note that for certain traits (adiposity, body weight, fat mass in males), it would have been useful to prioritize the most significant main affect loci, whereas for all other traits, it would not have helped to identify interactions. Unfortunately, we know of no way *a priori* to ascertain whether for a given trait, the interactions are likely to be enriched or not. This suggests that it is critical to test genome-wide for genetic interactions. However, given that we have shown that there are millions of interactions outside of the conservative p-value for significance, perhaps the most efficient method for detecting epistatic loci is to reduce the threshold for significance. Although further evidence will likely be required to demonstrate a functional role for any specific interaction identified at a relaxed p-value threshold, en masse it is likely to identify millions of putative interactions that may be further validated in other populations or tested within genetic models.

In conclusion, we presented simple but novel analyses applied to the HMDP to detect the characteristics of omnigenic epistasis regulating several complex traits, even those traits that lacked statistically significant marginal and interaction effects. This indicates that loci spanning the genome are contributing to trait heritability, and strongly argues that current conservative p-value thresholds may not be the best means to approach association studies. Integration of this omnigenic model may lead to more accurate genetic models for complex traits, for which current models fail to accurately account for most heritability and similarly fail to accurately predict trait outcomes based on polygenic risk scored in distinct and diverse populations[15-17,29-31]. These studies were made possible by performing sex-stratified analyses, instead of adjusting for sex as is typically done in most GWAS models. Future studies may more effectively model a trait's heritability and complex genetic architecture by performing sex-dependent analyses as well as including the many main effects and interactions that play a role in heritability but are not statistically significant. With a more comprehensive understanding of the many main and interaction effects required to model genetic architecture, a clearer understanding of cause of disease will hopefully be detected for many complex traits. Continued advances towards a better understanding of the genetic architecture of complex traits and disease promises to improve disease risk prediction, help unravel disease pathophysiology, and guide the use of personalized medications and treatments with improved targeted precision.

Methods

Mice:

HMDP genotype and trait data are from Bennett et al. and included 8 metabolic traits (adiposity, bodyweight, cholesterol (unspecified), fat mass, glucose (unspecified), HDL, insulin, and triglycerides) and 3 blood traits (granulocyte %, monocyte %, and white blood count) and

were obtained from the Mouse Phenome Database (https://phenome.jax.org/)[12,32]. These traits were selected for analysis because they had the largest sample sizes available among all HMDP datasets. Males and females were analyzed separately (Fig. S10). A total of 694 female mice from 98 strains and 664 male mice from 102 strains were used in the metabolism trait analyses. Strain BXD24/TyJ-Cep290<rd16>/J was removed from both male and female analysis as it carries a mutation that affects vision[33] and four additional strains were removed due to missing phenotype or genotype data (Table S7). The female analyses also excluded strain BXD29-Tlr4<lps-2J>/J and the male analyses excluded strains AXB13/PgnJ and NON/ShiLtJ due to missing phenotype or genotype data (Table S7).[12] The blood trait analyses used 97 strains and 670 female mice and 98 strains and 640 male mice. Both the female blood trait and metabolism trait analyses used the same strains. The male blood trait strains were the same as the male metabolism strains except for 3 fewer strains (BXD31/TyJ, BXD6/TyJ, and CXB8/HiAJ were excluded) (Table S7).

Genotype analysis:

The HMDP strains were previously genotyped using the Mouse Diversity Genotyping Array[32]. The number of genotyped SNPs in females was 200,885 and in males was 199,910 (Fig. S11)[12]. This genotype data excluded multiallelic SNPs; thus, only biallelic SNPs were included in these numbers and used in the analysis. SNPs were further pruned from the HMDP data using the PLINK linkage disequilibrium (LD)-based SNP pruning command using an R^2 of 0.8[34] (Fig. S11). Haplotype blocks were calculated in PLINK from the remaining SNPs using a max block size of 15,000 kb[34]. For the female metabolic traits, 12,221 haplotype blocks plus 2,314 SNPs located outside of the blocks were used in the analysis. For the male metabolic traits, 11,385 haplotype blocks plus 5,907 SNPs located outside of the blocks were used in the analysis (Fig. S11a). For the female blood traits, 12,193 haplotype blocks plus 5,221 SNPs located outside of the blocks were used for analyses. For the male blood traits, 11,437

haplotype blocks plus 5,752 SNPs located outside of the blocks were used in the analysis (Fig. S11b). The total number of haplotype blocks and SNPs for each trait were used to calculate the Bonferroni significance thresholds for female metabolism main effects (4.13E-6), male metabolism main effects (2.89E-6), female blood main effects (2.87E-6), male blood main effects (2.91E-6), female metabolic interactions (6.82E-10), male metabolic interactions (3.34E-10), female blood interactions (3.30E-10), and male blood interactions (3.38E-10). When determining significant marginal associations within a haplotype block, only the SNP with the most significant p-value within the block was used. Similarly, only the most significant SNP-SNP interaction was included in the results for the sets of SNPs that were in the same pair of haplotype blocks. The thresholds for significance applied to main effects varied between this analysis and that of Bennett et al.[12], due to the different number of strains analyzed and the FDR-based threshold previously used as opposed to the Bonferroni-based threshold in this study[12].

Statistical Analysis:

Trait heritability was estimated using two approaches, “narrow sense heritability” and “broad sense heritability”[5]. Narrow sense heritability, which refers to heritability due to additive genetic variance, was calculated using the Genome-wide Complex Trait Analysis (GCTA) software[35]. It estimates the proportion of phenotypic variance explained by all genome-wide SNPs for a complex trait and the narrow sense heritability estimate is based on sharing genomic regions identical by descent[35]. Broad sense heritability, which includes additive effects as well as dominance and non-additive effects[35], was calculated using the R package “heritability” and was estimated via reproducibility of trait measures in individuals of the same strain[36].

FaST-LMM (version 0.6.1), a linear mixed model method that accounts for population structure, was used for single locus main effects and pairwise interaction association tests[37]. A kinship matrix was constructed for the SNPs on all chromosomes except the one being tested to

improve power and allows main effect SNPs to be tested only once for association in the regression equation. Not every main effect and interaction was measured in both sexes as differences in haplotype block structure excluded different SNPs. Both interchromosomal and intrachromosomal SNP-SNP interactions were calculated[37]. FaST-LMM calculated the effect size for each SNP as the fixed-effect weight for main effects and as the Beta for each SNP-SNP interaction for epistasis tests[37]. The enrichment of associations identified at p-value < 0.05 were calculated using chi-square tests.

Positive and negative epistasis were calculated using only the interactions with the same beta directionality in males and females. Additionally, only interactions with individual SNP marginal effects with the same sign were included. Of those, the tests with an interaction beta in the same direction of the individual SNP marginal effects were considered positive epistasis. The tests with an interaction beta in the opposite direction of the individual SNP marginal effects were considered negative epistasis.

Data Visualization

The Circos plot (Fig. S2) was created using the command line Circos program (version 0.69-6). All other visualizations were created in R using the Tidyverse (version 1.3.2) with data filtering by dplyr (version 1.0.9).

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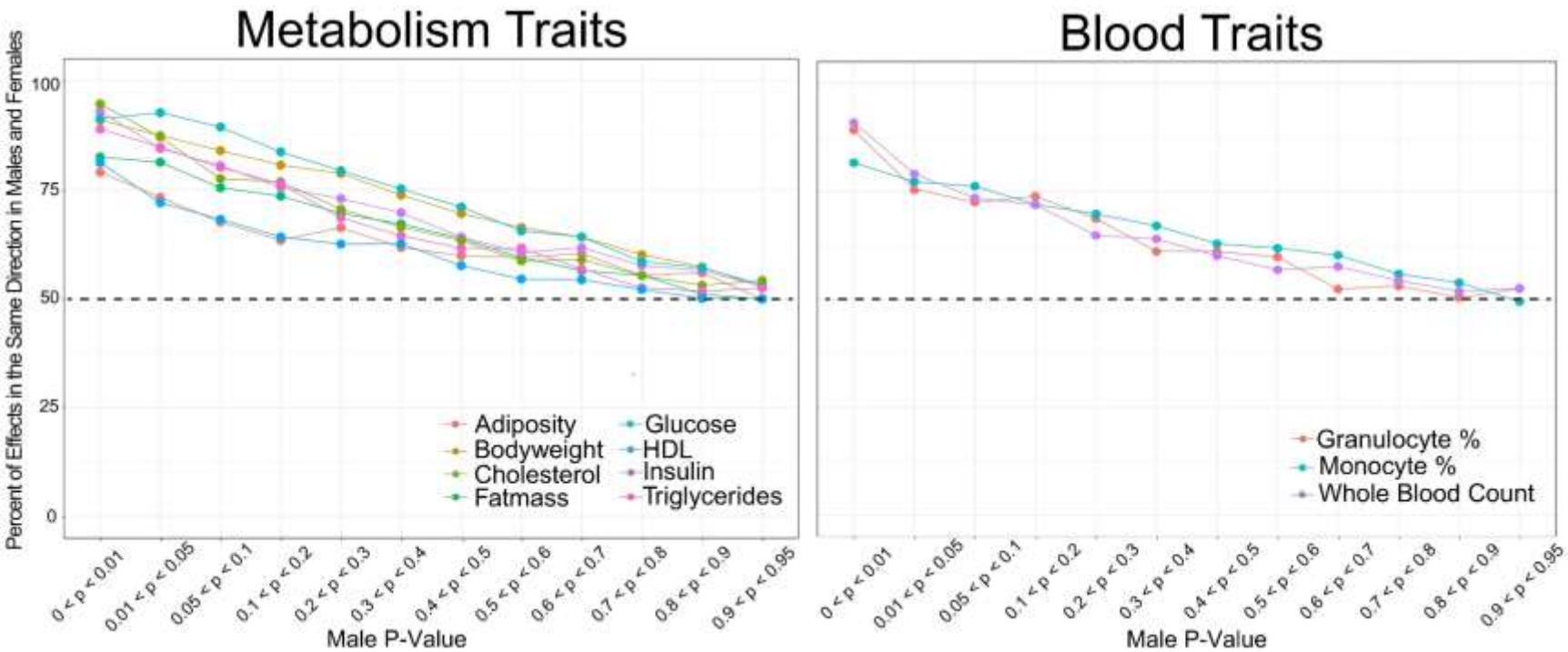
Metabolism Traits
Blood Traits
Percent of Effects in the Same Direction in Males and Females
100
75
50
25
0
Adiposity
Bodyweight
Cholesterol
Fatmass
Glucose
HDL
Insulin
Triglycerides
Granulocyte %
Monocyte %
Whole Blood Count
0 < p < 0.01
0.01 < p < 0.05
0.05 < p < 0.1
0.1 < p < 0.2
0.2 < p < 0.3
0.3 < p < 0.4
0.4 < p < 0.5
0.5 < p < 0.6
0.6 < p < 0.7
0.7 < p < 0.8
0.8 < p < 0.9
0.9 < p < 0.95
Male P-Value

Figure 1

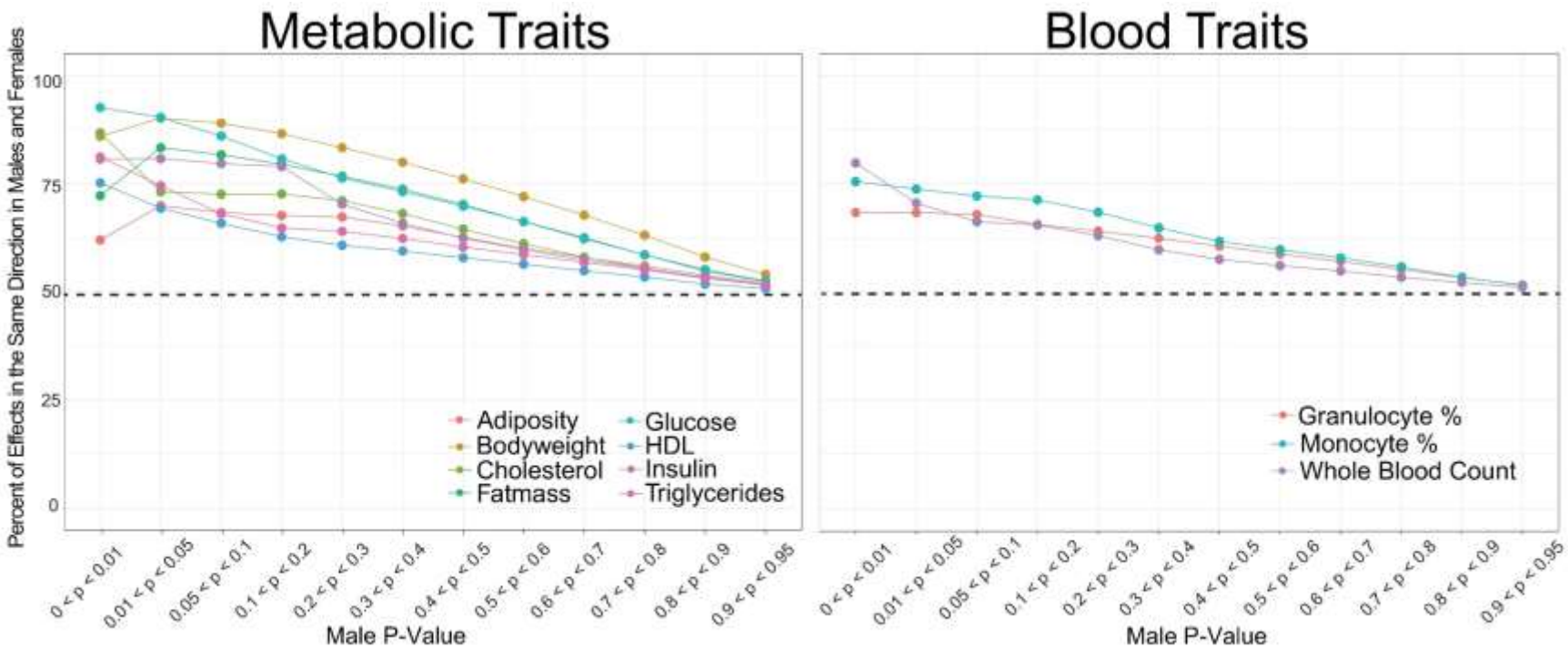
Metabolic Traits
Blood Traits
Percent of Effects in the Same Direction in Males and Females
100
75
50
25
0
Adiposity
Bodyweight
Cholesterol
Fatmass
Glucose
HDL
Insulin
Triglycerides
Granulocyte %
Monocyte %
Whole Blood Count
0 < p < 0.01
0.01 < p < 0.05
0.05 < p < 0.1
0.1 < p < 0.2
0.2 < p < 0.3
0.3 < p < 0.4
0.4 < p < 0.5
0.5 < p < 0.6
0.6 < p < 0.7
0.7 < p < 0.8
0.8 < p < 0.9
0.9 < p < 0.95
Male P-Value

Figure 2

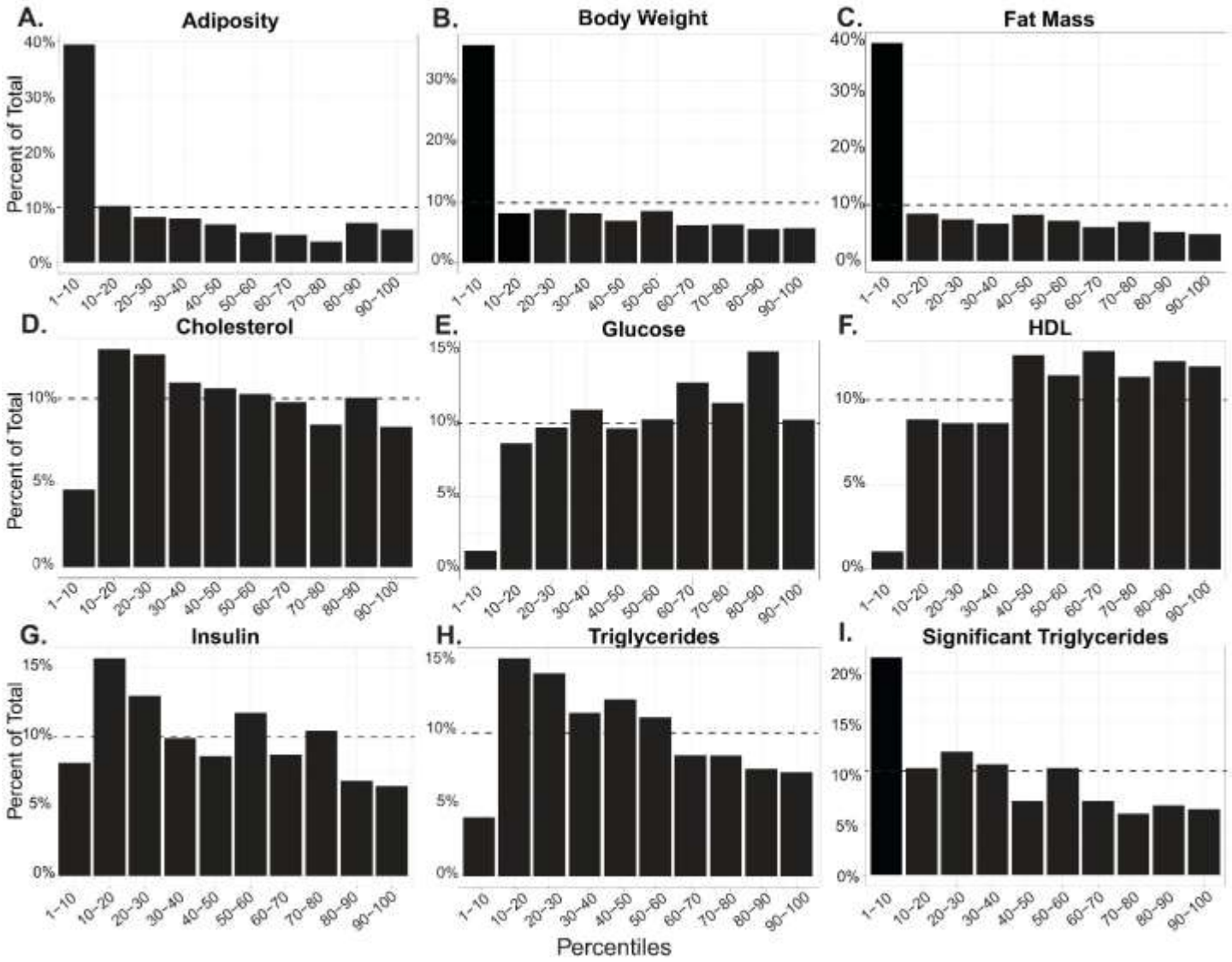
A.
Adiposity
B.
Body Weight
C.
Fat Mass
D.
Cholesterol
E.
Glucose
F.
HDL
G.
Insulin
H.
Triglycerides
I.
Significant Triglycerides
Percent of Total
Percentiles
1–10
10–20
20–30
30–40
40–50
50–60
60–70
70–80
80–90
90–100

Figure 3

Figure 1. Omnigenic Detection of Main Effects in the HMDP. The percentage of all HMDP main effect loci with consistent direction of effects in the two sexes were plotted according to the male derived p-values. Interaction p-values > 0.95 were excluded due to many low frequency alleles. Directionality was determined using the sign of the effect size of each main effect loci.

Figure 2. Omnigenic Detection of Pairwise Epistasic Interactions in the HMDP. The percentage of all HMDP pairwise interacting loci with consistent direction of effects in the two sexes were plotted according to the male derived p-values. Interaction p-values > 0.95 were excluded due to many low frequency alleles. Directionality was determined using the sign of the Beta value for each interaction.

Figure 3. Enrichment of Highly Significant Interacting Loci Among Significant Additive Loci. The 1,000 most significant interacting loci were selected and tested for the corresponding significance level among main effects for male mice. Main effect significance is binned by decile, with the 1-10% bin corresponding to the most significant loci and the 90-100% bin corresponding to the least significant loci. Data is shown for male HMDP mice for (A) Adiposity, (B) Body Weight, (C) Fat Mass, (D) Cholesterol, (E) Glucose, (F) HDL, (G) Insulin, and (H) Triglycerides. (I) Panel I includes only the 122 Bonferroni-corrected significant male triglyceride interactions, which is a subset of the top 1,000 interactions shown in (H).

Table 1. Narrow Sense and Broad Sense Heritability of Metabolic Traits in HMDP

	Females		Males	
Trait	Narrow Sense	Broad Sense	Narrow Sense	Broad Sense
Metabolic Traits				
Adiposity	0.566	0.681	0.740	0.718
Body Weight	0.598	0.691	0.463	0.598
Cholesterol	0.400	0.438	0.257	0.352
Fat mass	0.574	0.691	0.626	0.688
Glucose	0.175	0.290	0.122	0.211
HDL	0.140	0.206	0.144	0.211
Insulin	0.212	0.285	0.407	0.521
Triglycerides	0.238	0.321	0.273	0.368
Metabolic Average	**0.363**	**0.450**	**0.379**	**0.458**
Blood Traits				
Granulocyte %	0.262	0.358	0.239	0.298
Monocyte %	0.199	0.325	0.121	0.208
White Blood Count	0.118	0.205	0.044	0.101
Blood Average	**0.212**	**0.314**	**0.135**	**0.200**
Overall Average	**0.317**	**0.408**	**0.312**	**0.407**

Table 2. Detection of Marginal and Interaction Effects in Female and Male Mice in the HMDP

	Trait	Female Main Effects	Male Main Effects	Female Interactions	Male Interactions
Metabolic	Adiposity	0	58	0	56,056
	Body Weight	0	22	0	66,296
	Cholesterol	0	0	0	58
	Fat Mass	0	60	8	111,749
	Glucose	0	0	0	0
	HDL	0	0	0	0
	Insulin	0	0	0	0
	Triglycerides	0	0	0	122
Blood	Granulocyte %	0	0	0	0
	Monocyte %	0	0	0	0
	White Blood Count	0	0	0	0

Table 3. Percentage of Positive and Negative Epistasis in the HMDP

Trait	Positive Epistasis	Negative Epistasis
Adiposity	40.23%	59.77%
Body Weight	45.56%	54.44%
Cholesterol	42.97%	57.03%
Fat Mass	43.47%	56.53%
Glucose	46.37%	53.63%
HDL	51.72%	48.23%
Insulin	45.60%	54.40%
Triglycerides	47.02%	52.98%
Metabolic Average	***45.37%***	***54.63%***
Granulocyte %	47.20%	52.80%
Monocyte %	47.47%	52.53%
White Blood Count	47.19%	52.81%
Blood Average	***47.29%***	***52.71%***
Total Average	***45.89%***	***54.11%***

Table 4. Bonferroni Significant Main Effect and Interaction Beta Values Compared Between Sexes

Test	Trait	Sex	N	% of Betas with the Same Sign
Main Effect	Adiposity	Male	58	62.86%
	Body Weight	Male	22	100%
	Fat Mass	Male	60	82.22%
Interaction	Adiposity	Male	56,112	78.99%
	Body Weight	Male	66,296	96.65%
	Cholesterol	Male	58	93.55%
	Fat mass	Male	111,749	79.92%
	Triglycerides	Male	122	100%
	Fat mass	Female	6	100%

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