Title: Sex-specific Associations in the Hybrid Mouse Diversity Panel help define genetic architecture

Running title (50 characters or less including spaces): Sex-specific effects on genetic architecture

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

Abstract

A better understanding of the role of sex in studies of genetic architecture for complex traits and diseases will help translate genetic data into improved precision-based medicine and clinical care. Towards this end, we explored the use of sex-stratified versus sex-combined analyses for several metabolic and blood traits in the Hybrid Mouse Diversity Panel (HMDP). Traits such as body weight and glucose levels exhibited a high degree of genetic correlation between males and females whereas other traits such as HDL levels and white blood count did not. Nonetheless, even with the high genetic correlation between males and females for body weight, the use of sex-stratified analyses enabled the identification of dozens of loci regulating adiposity not identified in sex-combined analyses or sex-stratified analyses in the opposite sex. In addition, comparisons of the direction of allelic effects in males and females detected in the sex-stratified analyses demonstrated a high concordance, even among loci that were not statistically significant using a conventional p-value threshold. Simulation studies indicated that these elevated concordance rates were consistent with a genetic architecture consisting of hundreds of additive loci regulating every trait analyzed, including those for which no statistically significant loci were identified. These findings demonstrate the importance of stratifying by sex and suggest a method for identifying biologically rather than statistically significant associations. Applying these methods to GWAS data broadly may result in the identification of many additional loci contributing to the genetic architecture of complex traits that were missed using conventional sex-adjusted GWAS methods.

Introduction

Complex traits are regulated by many genetic variants that together influence their severity, presentation, and prevalence (Fu et al. 2013; Boyle et al. 2017). Genome-wide association studies (GWAS) have successfully identified many such variants for a broad range of complex traits. However, for many traits there are important differences between males and females that may reflect underlying differences in their respective genetic architectures. Most association studies combine sexes in their analyses and adjust for sex as a covariate, but this approach can conceal differences that a sex-stratified approach can reveal. The likelihood that such sex differences exist is consistent with the frequent sex differences often seen in disease prevalence, severity, age of onset, or outcomes (Khramtsova et al. 2019). For example, the prevalence of autism spectrum disorder is 4 times higher in males than females, mortality rates associated with COVID-19 infection are elevated in males, and females have double the likelihood of developing multiple sclerosis (Pradhan and Olsson 2020; Walton et al. 2020; Zeidan et al. 2022). Although factors beyond genetics likely contribute to these gender differences, it is becoming increasingly clear that genetic factors, both autosomal and sex-linked, contribute to the sexually dimorphic effects seen for many complex traits and diseases. A better understanding of the sex differences underlying complex traits and diseases between males and females may improve the accuracy and efficacy of precision medicine-based clinical care as well as reduce health care disparities resulting from the historical relative lack of attention given to women's health issues.

Although sex is typically accounted for as a variable in sex-combined association studies, a number of studies in both humans and model organisms have successfully improved the identification of genetic variants associated with a range of complex traits and diseases by performing sex-stratified analysis or testing for Genotype x Sex interactions (Nuzhdin et al.

1997; Parks et al. 2015; Graham et al. 2019; Nievergelt et al. 2019; Choquet et al. 2021; Johnston et al. 2021; Ciesielski et al. 2022; Silveira et al. 2023). These studies have often identified associations between specific variants or loci and their respective phenotype that were otherwise not identified in a sex-combined analysis or in the analyses of the opposite sex. In one study of major depression comparing sex-specific and combined-sex analyses of major depression based on the UK Biobank datasets, sex-specific polygenic risk scores were better at predicting major depressive disorder than sex-combined polygenic risk score, indicating the importance of considering sex in genomic analyses for precision-based medicine going forward (Silveira et al. 2023). Similar findings were observed with testosterone levels, interactions between polygenic risk scores and sex for a number of blood cell traits and coronary artery disease risk, and cognitive function based on a polygenic risk score for schizophrenia, (Flynn et al. 2021; Koch et al. 2021; Xu et al. 2022; Surakka et al. 2023). These studies highlight the importance of considering sex as a variable, and in certain instances performing sex-specific analyses of genetic data to identify variants associated with risk or calculating polygenic risk scores that do not transcend sex.

In addition to the sexually dimorphic loci that are identified in sex-stratified genomic analyses, we reasoned that these studies provide the opportunity for large scale comparisons between the estimated effects of genetic variants on a given trait or disease between males and females. Under the null hypothesis that GWAS variants with a p-value larger than an arbitrary threshold (typically $5x10^{-8}$ in human studies) have no effect on a phenotype, the expectation would be that there should be no relationship between the effects attributed to a particular variant in both independent male and female analyses. However, if there is a real biological association between a genetic variant and a given phenotype, then one would hypothesize that these variants might be enriched for similar effects in separate male and female analyses relative to that expected by chance. Applying this comparison across all genetic variants can

provide an additional means of estimating the true number of variants that contribute to trait heritability, independent of the arbitrary p-value threshold for significance that is used in most GWAS studies. Recent proposals to modify p-value thresholds, apply other measures of confidence, or even abandon p-values, highlight the growing sense that p-values alone are not reliable measures of biological significance and should instead be integrated with other information such as prior knowledge, mechanistic support, and data quality (Wasserstein and Lazar 2016; McShane et al. 2019; Hirschauer et al. 2022). Alternative methods of incorporating biological information into statistical thresholds for significance have also been proposed and include measures of biological function such as chromatin accessibility or enhancer status or interactions with promoter regions (Kichaev et al. 2019; Hammond et al. 2021), applying pathway-based approaches as opposed to single variant analyses (White et al. 2019), machine learning algorithms to interpret GWAS results (Kolosov et al. 2021), and simply relaxing significance thresholds (Williams and Haines 2011; Chen et al. 2021).

To examine the role of sex-specific analyses on the ability to detect associations between genotypic and phenotypic variation, as well as test the hypothesis that large-scale comparison between the sex-specific analyses could reveal novel insights into genetic architecture, we compared a series of sex-specific and sex-combined analyses on metabolic and blood-related traits in the Hybrid Mouse Diversity Panel (HMDP). Prior studies have had initial success detecting associations for a wide range of mouse complex traits utilizing the 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 (Lusis et al. 2016). 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 (Bennett

et al. 2010; Lusis et al. 2016). 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 (Farber et al. 2011; Orozco et al. 2012; Parks et al. 2013; Lusis et al. 2016). In addition, previous studies of the HMDP using sex-specific analyses demonstrated an improved ability to identify novel loci related to metabolic disease (Parks et al. 2015). Given the outstanding questions related to defining the genetic architecture of complex traits in both males and females, 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 (Rau et al. 2020).

In this study, we report a comparison between sex-stratified and sex-combined association analyses of several metabolic and blood traits using the HMDP. The male and female genotype and trait data were published by Bennett et al, but only a sex-stratified analysis of female mice was previously described (Bennett et al. 2015). The findings in this study demonstrate an improved ability for detecting associations in a sex-stratified analysis in the HMDP. Additionally, they suggest that hundreds of additional loci throughout the genome contribute to the genetic architecture of complex traits, far beyond those identified using conservative and arbitrary p-value cutoffs, even for complex traits for which not even a single statistically significant locus was identified.

Results

Heritability

To determine the additive and non-additive heritability components for a series of complex traits in the HMDP, narrow sense and broad sense heritability were calculated

separately for male and female mice for 8 metabolic 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. For example, the narrow sense heritability of adiposity was larger in males (0.740) than in females (0.566) and the narrow sense heritability of body weight was larger in females (0.598) than males (0.463). However, on average heritability were similar between the sexes.

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**

Genetic Correlation of Traits

Given that heritability estimates differed between sexes for some of the traits, sex-stratified genetic correlations were calculated to measure the genetic trait variability within and

between sexes. The genetic correlations between pairs of traits were first compared within each sex. The absolute value of the correlation between traits within the same sex was on average ± 0.34 (SD = 0.23) in males and ± 0.29 (SD = 0.21) in females. Traits with related phenotypes were highly correlated in both sexes. For example, cholesterol and triglycerides were highly correlated in both males (0.88) and in females (0.65) and body weight and insulin were highly correlated in both males (0.63) and females (0.76) (Table S1). Some traits were more strongly correlated in one sex than the other. For example, fat mass and insulin were more correlated in females (0.83) than in males (0.43) (Table S1). Additionally, body weight and monocyte % were more correlated in males (0.52) than in females (0.13). Some traits were not correlated such as adiposity and insulin in both males (-0.06) and females (-0.04). Additionally, adiposity and cholesterol were not correlated in either males (-0.02) or females (-0.08).

The genetic correlation of each trait was also calculated between male and female mice. The average of the absolute value of the correlations was 0.58 (SD = 0.22). Some traits had strong correlations across sexes including body weight (0.88) and glucose (0.86) (Table S2). Other traits had much lower correlations across sexes, including HDL (0.17) and white blood count (0.35) (Table S2). While traits were on average more correlated across sexes than between traits of the same sex, some traits were more correlated to other traits within the same sex than to the same trait across sexes. For example, HDL is not very correlated across sexes (0.17) but is much more strongly correlated with triglycerides in males (-0.69) and with glucose in females (-0.46) (Table S2). A similar trend is seen for white blood count where the trait is not very correlated across sexes (0.35) yet is highly correlated with insulin in males (-0.60) and with granulocyte % in females (-0.76) (Table S2).

Detection of Loci with Main Effects

Given that the heritability estimates and genetic correlations underlying some metabolic traits were variable between males and females, main effects were determined with sex-stratified analyses. 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 the number of haplotype blocks and independent SNPs not within any of the haplotype blocks tested, was $p < 4.13E-6$. At this threshold, there were no significant marginal effects in females (Table 2, Table S4). While we were unable to detect statistically significant main effects using a conservative Bonferroni threshold, we identified associations that were previously observed by Bennett et al. in this HMDP dataset. That study used a slightly different strain composition and an FDR-based threshold for significance at $p < 1.3E-5$ (Bennett et al. 2015). Using the less conservative threshold, we replicated 5 unique SNPs in adiposity and 1 unique SNP in HDL that were discovered as significant in the Bennett et al. analysis (Table S5).

Table 2. Detection of Marginal Effects in Female and Male Stratified Mice and in Combined Sexes in the HMDP

	Trait	Female Main Effects	Male Main Effects	Combined Main Effects
Metabolic	Adiposity	0	58	1
	Body Weight	0	22	6
	Cholesterol	0	0	0
	Fat Mass	0	60	5
	Glucose	0	0	0
	HDL	0	0	0
	Insulin	0	0	0

	Triglycerides	0	0	0

While these traits were previously analyzed in female mice by Bennett et al, the same traits were not analyzed in males. Thus, main effects of the same 8 metabolic traits were assessed in males using 664 mice from 102 strains from the HMDP. The Bonferroni-corrected threshold for significant main effects was based on the number of haplotype blocks and SNPs independent of the haplotype blocks tested. The main effect significance threshold 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 S3, Table S4). 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, including thirty-three blocks associated with both adiposity and fat mass, 1 block 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 S6). Only 10 haplotype blocks were unique to one of the three traits (Fig. S1, Table S6). Among the loci identified, there was little overlap with a similar analysis of the HMDP mice when fed a different obesogenic diet (Table S7) (Parks et al. 2015), suggesting a strong Gene x Environmental component to the sex-specific loci identified in each study.

In addition to studies of metabolic traits, and to test the generalizability of any findings to complex traits more broadly, main effects for 3 blood-related traits were assessed in females using 670 mice encompassing 97 strains and in males using 640 mice encompassing 98 strains, using the HMDP. The Bonferroni-corrected threshold for significant main effects was

again based on the number of haplotype blocks and SNPs outside of the haplotype blocks tested. The main effect significance threshold was $p < 2.87E-6$ in females and $p < 2.91E-6$ in males. At these thresholds, there were no significant marginal effects detected in either males or females (Table 2, Table S4).

GWAS often only measure main effects using a sex-combined analysis with an adjustment for sex. Thus, in addition to the sex-stratified analyses, we also analyzed the HMDP data using a combined sex approach to assess if similar or different main effects could be detected. Main effects for the 8 metabolic traits and 3 blood traits were assessed in the sex-combined analysis with both male and female samples, adjusting for sex as a variable in the regression analyses. The Bonferroni-corrected threshold for significant main effects was $p < 2.87E-6$, and thus similar to that for each of the sex-stratified analyses. At this threshold, there was 1 main effect locus detected for adiposity. However, this region was not one of the 58 loci detected in the male-only analysis. There were also 6 main effect loci detected for body weight, with 4 of these also observed in the male-only analysis. There were 5 main effect loci detected for fat mass, with 4 of these loci also observed in the male-only analysis. There was also 1 main effect locus detected for the blood trait granulocyte % that was not detected in the male-only analysis (Table 2, Table S4). Thus, in total, of the 11 total marginal effect loci detected in the sex-combined analyses, only 3 were not also detected in the male-specific analysis. Conversely, among the 140 loci detected across all traits in the male-specific analyses, only 8 were detected in the combined sex analyses (Table 2, Table S4). These results indicates that the power to detect QTLs in the HMDP is diminished in sex-combined analyses that adjust for sex as a variable, rather than conducting separate sex-stratified analyses.

Normalization of Trait Data Did Not Significantly Alter QTL Detection

Metabolic and blood traits were tested for normality using the Shapiro-Wilk's method to measure if a non-normal distribution could be driving the male-specific main effects. Nearly every trait in both sexes was non-normally distributed (Table S8). Therefore, to test whether the significant loci identified above were artifacts due to a non-normal distribution of the trait data, phenotypes were normalized using both a rank-based normalization and a Box-Cox normalization. Performing QTL detection for adiposity using the rank-based normalized data resulted in the identification of 54 significant QTLs, whereas using the Box-Cox normalized data resulted in the identification of 63 significant QTLs. All but 1 of the rank-based normalized QTLs were also detected in the unadjusted model (Table S9). There were more Box-Cox significant QTLs detected than were found in the unadjusted model, but all of the QTLs detected based on the unadjusted data were also identified in the Box-Cox normalized data. Thus, there were nearly identical results, regardless of whether the regression analyses were performed on unadjusted or normalized trait data. In addition to these results for adiposity, similar findings were observed for all other traits studied for both the sex-stratified and sex-combined analyses (Table S9). Collectively, this demonstrates that the QTL mapping findings were robust to transformation of the trait data.

Removal of Outliers Did Not Significantly Alter QTL Detection

In addition to testing whether normalization of the trait data significantly altered the QTL mapping studies, we sought to test whether the QTL detection results were being driven by either a few outlier mice or outlier strains of mice. To test this, outliers were removed from the phenotypic data that were greater than three standard deviations from the mean. Outliers were removed either by removing individual mice that met this criterion or removing strains that collectively met this criterion. When individual mouse outliers were removed from male mice, there were 35 adiposity main effects detected and all 35 of these effects were previously found

in the unadjusted male only model (Table S9). For body weight, 24 significant main effects were detected, again, with all these loci already detected in the unadjusted male only model (Table S9). There were 55 significant main effects detected for fat mass with 53 of these effects detected in the unadjusted male only model (Table S9). Of the traits that detected main effects in the unadjusted model, none of the adiposity traits (adiposity, body weight, or fat mass), had outlier strains that were removed due to their average strain values and the criteria described above. Insulin and granulocyte % had strain outliers, but both traits failed to detect QTLs using the unadjusted, normalized, or outlier removed data. Thus, as with data normalization, removal of outliers had only minor effects on the detection of loci that control these metabolic traits in the HMDP.

Randomization of Sex Prevented Detection of Most Sexually Dimorphic QTLs

After applying various quality control measures to the trait data in the HMDP prior to QTL detection, one of the most striking findings, which was robust to this data manipulation, was the difference in the number of QTLs that associate with adiposity-related traits in male mice relative to the lack of QTLs identified that associate with adiposity-related traits in female mice. This was surprising, as the overall heritability across all three of the adiposity-traits was similar between males and females, and the traits were highly genetically correlated across sexes (0.55-0.88) (Table S2). Thus, to test the robustness of these findings, the sex of each mouse was randomized as either a male or a female and the linear regression analysis was performed again. Adiposity was analyzed as it had the largest average narrow sense heritability across sexes (0.653) (Table 1). Main effects were detected for adiposity with sex randomly assigned for 100 permutations. Among the 100 permutations, there were few instances where significant main effects were detected in either females (mean = 0.41, SD = 2.04, range= 0,17) or males

(mean = 0.79, SD = 2.06, range= 0,13) (Table S10), indicating that the sex-specific QTLs were not artifacts, and instead represent true sexual-dimorphic QTLs.

Concordant Directionality of Marginal Effects in Male and Female Mice

Given the increased number of significant main effects detected in males relative to females, we wondered whether associations in females had the same direction of effect on each trait as that detected 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 ($p < 1.27E-11$, Beta = 2.94), 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 ($p < 0.11$, Beta = 0.74) (Fig. S3). To test the 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 is that the direction of effects in the two sexes should be the same ~50% of the time, based on the equivalence of random effects. However, among two of the three traits for which significant main effects were detected in males (body weight and fat mass), the allelic direction of effect was the same in males and females significantly more frequently than by chance alone (Table 3). The enrichment in number of significant main effects with the same allelic direction of effect was significant in body weight ($p = 4.20E-8$) and fat mass ($p = 0.003$) but not in adiposity ($p = 0.22$) (Table 3). Therefore, although the significant main effects may not be statistically significant in both sexes, they nonetheless had a consistent effect direction in both male and female mice (Table S11).

Table 3. Bonferroni Significant Main Effect Compared Between Sexes

Test	Trait	N	% of Betas with the Same Sign	P-Value

Main Effect	Adiposity	58	62.86%	0.22
	Body Weight	22	100%	4.20E-08
	Fat Mass	60	82.22%	0.003

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 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 all significant main effects were detected 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 (Fig. 1A, Fig 1B) 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 male p-values increased, asymptotically approaching 50% as p-values approached 1.

To estimate the number of SNPs with heritable effects on a given trait that would be consistent with the observed enrichment of greater than 50% of loci with effects in the same direction in males and females, phenotypes were simulated based on the exact HMDP genotypes. Phenotypes were simulated with a heritability of 0.4 to replicate the heritability of the metabolic traits (Table 1). To recapitulate the independent analysis of male and female mice, all SNPs in the simulation were assigned an artificial beta value. For SNPs defined as having a “true” causal affect that was consistent in both male and female mice, these SNPs were assigned beta values in the same direction (i.e. both positive or both negative) in both the male and female simulation. To simulate a SNP with no causal affect, beta values were randomly assigned separately in the male and female analysis and were thus on average in the same direction 50% of the time. Phenotypes were simulated with a range of “causal” SNPs, from a minimum of zero causal SNPs to a maximum where half of the total number of SNPs are

estimated as causal (n=10,998). After independently simulating the phenotype data under each of these conditions 10 times, linear regression analyses were performed on each of the 10 simulations as was done for the actual trait data.

As expected, in the absence of any “causal” SNPs in the simulation, there was no enrichment of male and female concordance above the 50% expected by chance (Fig. 1C). In contrast, when at least 100 “causal” SNPs were included in the model, a clear increase in the percentage of concordant effects in males and females was detected relative to the 50% expected by chance (Fig. 1C). Comparing the results from the real phenotype data to the results from the simulated phenotypic data, the metabolic and blood traits likely include between 200 and 1,100 SNPs with a true marginal effect (Fig. 1). These data demonstrate that the marginal effects detected for many loci throughout the genome, even though they do not reach the conservative p-value threshold for significance, are not simply random noise and instead indicate that these loci are in fact contributing to trait heritability via their marginal effects.

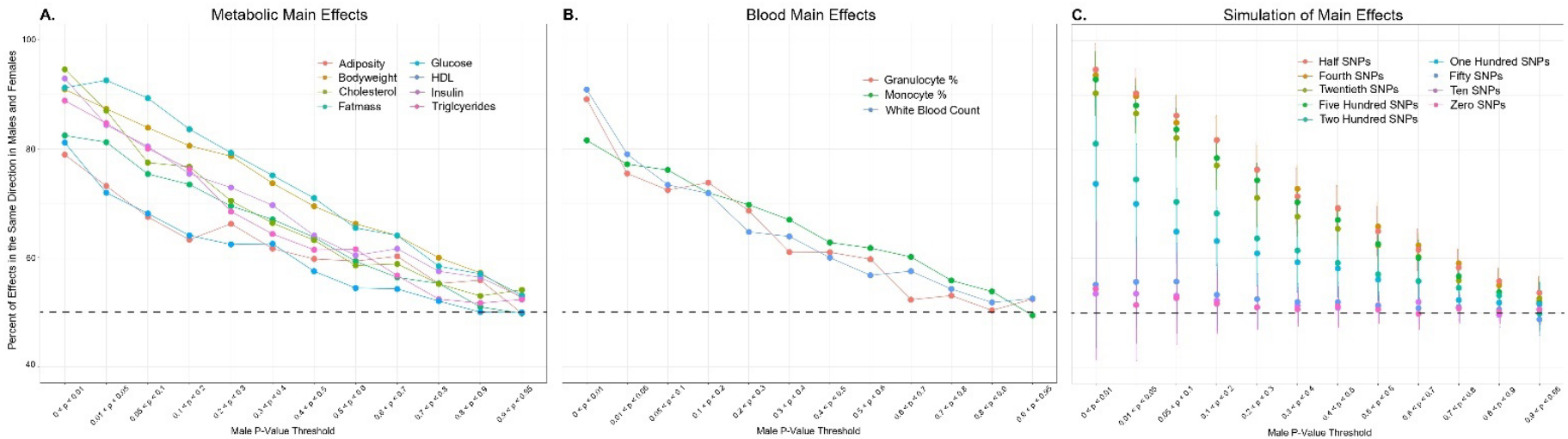


Figure 1. Detection of Widespread Main Effects in the HMDP. The percentage of all HMDP main effect loci with consistent direction of effects in the two sexes were plotted according to the male derived p-values. Main effect p-values > 0.95 were excluded due to many low frequency alleles. Directionality was determined using the sign of the effect size of each main effect loci. The phenotypes measured included (A) metabolic traits, (B) blood traits, and (C) simulated phenotypes using an increasing number of "causal" SNPs.

Phantom Detection of Loci with Interaction Effects

Given the increased power of using male/female concordance to estimate the number of contributing loci compared to a conservative Bonferroni threshold for significance, we wanted to assess whether this could be applied to SNP-SNP interactions, as the role of epistasis in complex traits remains controversial (Hill et al. 2008; Wilkins et al. 2016). Replicating the methods described for main effects, we tested whether the direction of effects were similar in males and females for all pairwise interactions analyzed, regardless of statistical significance. Concordance between males and females were measured and sorted based on the p-value for association in males. For all traits examined, the percentage of loci with effects in the same direction in males and females far exceeded the ~50% expected by chance across nearly all levels of statistical significance (Fig. 2A, Fig. 2B). As seen with the main effects, enrichment of concordant interactions relative to chance was greatest among the most significant interactions and decreased towards the 50% expected by chance as the p-values increased, asymptotically approaching 50% as p-values approached 1. To evaluate the number of genome-wide pairwise interactions consistent with these findings, we again turned to the simulations of phenotypic data. Given the estimate that at least 200 main effects contribute to the heritable regulation of each trait, we applied that model of genetic architecture to the detection of genetic interactions. For comparison, we also modeled the effects of zero main effects as a negative control, and >10,000 main effects as a likely overestimation. Importantly, the genetic architecture simulated by the model included only main effects. Thus, if given this simulated genetic architecture no interactions were detected, it would support the validity of widespread interactions as indicated by the elevated concordance of the male/female direction of effects. However, if the simulated genetic architecture, including only marginal effects, recapitulated the elevated concordance of the male/female direction of effects, it would indicate that these findings were artifacts of the widespread main effects and did not represent true interactions.

As expected, when zero marginal effects were included in the model, there was no overrepresentation of male/female concordance for interactions relative to chance. However, when 200 main effects were included in the model, the male/female concordance rate was far greater than the 50% expected by chance, mirroring the results from the actual HMDP data (Fig. 2C). As the simulated data including only marginal effects had a similar pattern as the actual data, it is consistent with an interpretation that the interaction results were artifacts due to the widespread underlying main effects. There may nonetheless be some epistatic interactions contributing to the phenotype, as the broad sense heritability was higher than the narrow sense heritability, but we were unable to detect the signature of widespread interactions analogous to that which was detected for the marginal effects.

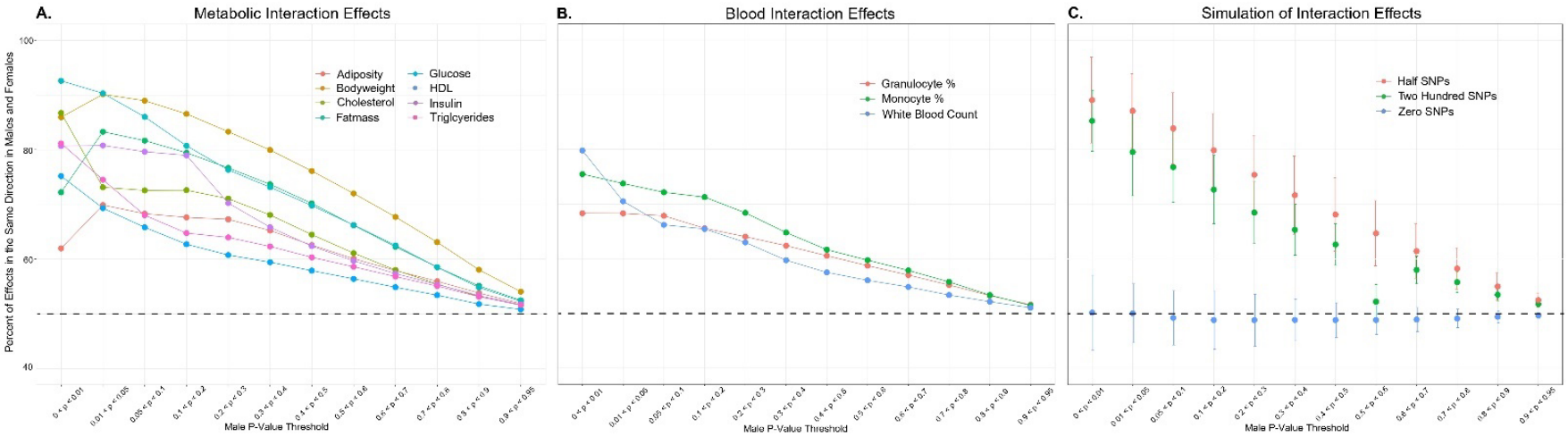


Figure 2. No Widespread Detection of Pairwise Epistasic Interactions in the HMDP. The percentage of all HMDP pairwise interacting loci with consistent direction of effects in the two sexes were plotted according to the male derived p-values. Interaction p-values > 0.95 were excluded due to many low frequency alleles. Directionality was determined using the sign of the Beta value for each interaction. The phenotypes measured included (A) metabolic traits, (B) blood traits, and (C) simulated phenotypes using an increasing number of “causal” SNPs.

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 always comprised a majority 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 (Bloom et al. 2013; Bennett et al. 2015; Bloom et al. 2015; Chen et al. 2017; Matsui et al. 2022). It is worth noting that adiposity, body weight, and fat mass, the traits for which significant main effects were identified in males, exhibited the highest levels of narrow sense heritability relative to all other traits. As main effects were detected in males only for these three traits, it is unsurprising that the narrow sense heritability was greater in males than in females for the traits adiposity and fat mass. Surprisingly, narrow sense was greater in females than in males for body weight. This indicated there could be genetic differences underlying body weight that differs by sex.

The underlying genetic differences of metabolic and blood traits were further studied through the genetic correlation of traits across sexes and between traits within a single sex. Body weight was highly correlated across sexes, but adiposity and fat mass were less strongly correlated. Interestingly, some traits were more strongly correlated with another trait within the same sex than to the same trait in the other sex. Thus, the genetic makeup of some traits may be more closely related to other phenotypically related traits in the same sex. We therefore speculate that there are substantial genetic differences regulating some traits across sexes.

This study aimed to better understand the pervasive role of sex dependent marginal effects in the underlying genetic architecture. Studies were performed using standard linear

regression analyses and simulated phenotypes to identify significant marginal associations and non-additive epistatic interactions, and many such loci were discovered (Table 2). Normalization, randomization, and simulation studies all supported the finding of many more marginal associations in males relative to females. However, the simulated phenotypes analysis also indicated that the detected epistatic interactions were likely the result of spurious signals from marginal effects mimicking an interaction, as has been previously reported (Hemani et al. 2014; Wood et al. 2014; Hemani et al. 2021). Nonetheless, the higher broad sense heritability estimates relative to narrow sense are still consistent with a non-additive contribution, albeit given the statistical difficulties in detecting these interactions, functional studies will be needed to discover interaction effects.

Many detected main effects were sex-dependent as they were detected in analyses of male mice only but were undetected in females alone or sex-combined analyses. Prior human GWAS have detected sex-dependent marginal effects for depression phenotypes (Silveira et al. 2023), apical periodontitis (Petty et al. 2023), and Behçet's disease (Jo et al. 2022). The sex-dependent nature of the significant main effects in our studies was further supported by randomizing sex in the analyses, which abolished the findings of associations, providing an important control for spurious findings in the sex-specific analyses. Nonetheless, the significant main effects in males largely had the same direction of effect in females. This concept was seen beyond the statistically significant loci, and thus numerous possible marginal effects were detected for each trait. The detection of many marginal effects likely contributing to the genetic architecture echoes previous findings of widespread genetic effects (Boyle et al. 2017), although a different statistical method was used, different traits were examined, and a different organism was studied. Together the results support the robustness of a highly polygenic model as a key feature in the genetic architecture of most, if not all, complex traits. It is expected that if

statistical power were further increased with the inclusion of additional strains and replicates, the number of detected loci may yet be higher, potentially approaching omnigenic inheritance.

Accounting for the highly polygenic or potentially omnigenic nature of the genetic architecture may be important for improving the modeling of trait heritability in complex traits driven by many main effects. The incorporation of many loci may improve the ability to predict disease risk via more accurate polygenic risk scores (PRS) that account for variability accounted for by sex. As argued previously, we suggest PRS can be used to efficiently model the complex traits using many main effects (Wray et al. 2018). As we detected between two hundred and ~1,000 SNPs to have a marginal effect, PRS could be constructed using the top main effects as estimated based on male/female concordance as an additional method, beyond using an arbitrary p-value cutoff. In this study, we were able to detect these polygenic effects with only 700 mice from each sex. Thus, this method maybe successful in studies of all sample sizes, including studies with less statistical power. Using the top main effects that fall within the number of variants suggested to contribute based on male/female concordance rates is an additional method that could more accurately model the contribution of marginal effects in complex traits regardless of sample size.

In conclusion, we present a simple but novel analysis applied to the HMDP to detect poly- and perhaps omnigenic marginal effects regulating several complex traits, even for those traits that lacked any statistically significant marginal effects. This indicates that many loci spanning the genome are likely 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 polygenic 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 (Bloom et al. 2013; Bloom et al. 2015; Martin et al. 2019; Veturi et al. 2019; Mathieson 2021; Matsui et al. 2022). These studies were made possible by performing both sex-stratified and sex-combined analyses. The results also indicate that there are likely multiple sex-dependent genetic effects/architecture that sex-combined analyses, even adjusted for sex, cannot detect. Therefore, 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 that play a role in heritability but are not statistically significant. With a more comprehensive understanding of the many main effects required to model genetic architecture, a clearer understanding of disease etiology will hopefully be detected for many complex traits. Continued advances towards a better understanding of the genetic architecture of complex traits and diseases promises to improve disease risk prediction, help unravel disease pathophysiology, and guide the use of personalized medications and treatments with improved precision.

Methods

Mice:

The 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/) (Bennett et al. 2015; Bogue et al. 2020). 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. S4) and in a sex-combined analysis. 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 (Seecharan et al. 2003) and four additional strains were removed due to missing phenotype or genotype data (Table S12). 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 S12) (Bennett et al. 2015). 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 S12).

Genotype analysis:

The HMDP strains were previously genotyped using the Mouse Diversity Genotyping Array (Bogue et al. 2020). The number of genotyped SNPs in females was 200,885 and in males was 199,910 (Table S13) (Bennett et al. 2015). 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 (Purcell et al. 2007) (Table S13). Haplotype blocks were calculated in PLINK from the remaining SNPs using a max block size of 15,000 kb (Purcell et al. 2007). 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 (Table S3). 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 (Table S13). The total number of haplotype blocks and SNPs for each trait were used to calculate the Bonferroni significance

thresholds for female metabolism main effects (3.44E-6), male metabolism main effects (2.89E-6), female blood main effects (2.87E-6), and male blood main effects (2.91E-6). When determining significant marginal associations within a haplotype block, only the SNP with the most significant p-value within the block was used. Similarly, when estimating the direction of effect for pairwise interactions between haplotype blocks or SNPs outside of the haplotype blocks, only the most significant SNP-SNP interactions within a block were considered. The thresholds for significance applied to main effects varied between this analysis and that of Bennett et al. (Bennett et al. 2015), 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 (Bennett et al. 2015).

In the sex-combined analyses, SNPs were removed that were not present in both male and female genotype data (1,773) or had a differing minor allele (3,922). After removing these SNPs, LD pruning was performed as previously described. The sex-combined metabolic and blood traits had 22,284 SNPs remaining resulting in 12,229 haplotype blocks with 5,129 SNPs located outside of the blocks (Table S13). The total number of haplotype blocks and SNPs outside of the blocks were used to calculate the Bonferroni significant threshold for combined main effects (2.87E-6).

Statistical Analysis:

Trait heritability was estimated using two approaches, “narrow sense heritability” and “broad sense heritability” (Yang et al. 2010). Narrow sense heritability, which refers to heritability due to additive genetic variance, was calculated using the Genome-wide Complex Trait Analysis (GCTA) software (Yang et al. 2011). 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 (Yang et al. 2011). Broad

sense heritability, which includes additive effects as well as dominance and non-additive effects (Yang et al. 2011), was calculated using the R package “heritability” and was estimated via reproducibility of trait measures in individuals of the same strain (Kruijer 2015).

The genetic correlation for trait data between and within sexes was estimated using GRM and GREML in the Genome-wide Complex Trait Analysis (GCTA) program (Yang et al. 2011). GRM estimates the genetic relatedness from SNPs and GREML estimates the variance explained by the SNPs in the GRM (Yang et al. 2011). The reml-bivar command was used to compare the variance explained by the SNPs in the GRM for each trait across sexes. It also compared across traits within the same sex, comparing two traits at a time.

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 (Lippert et al. 2011). 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 (Lippert et al. 2011). 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 (Lippert et al. 2011). The enrichment of associations identified at p-value < 0.05 were calculated using chi-square tests.

The normality of all phenotypes was measured using the Shapiro-Wilk’s method via the R package “stats” (version 3.6.2). All phenotypes were rank normalized using the R package “RNOmni” (version 1.0.0). Trait data was also Box-Cox normalized using the R package “bestNormalize” (version 1.8.3) (Peterson 2021). Outliers were removed using two methods:

removing individual mice outside of 3 standard deviations away from the mean and strain averages outside of 3 standard deviations away from the mean.

GCTA was also used to simulate the phenotypic data (Yang et al. 2011). The simulated phenotypes were calculated with a heritability of 0.4, similar to the heritability of the metabolic trait data for males and females (Table 1).

Each simulation was performed separately for males and females to mimic the sex-specific analyses performed with the real HMDP data. Within the genotype data, we modeled SNPs considered to be either causal or had no effect. Beta values were assigned to both categories of SNPs. Causal SNPs were assigned a beta value that was identical in both size and direction in the independent male and female simulations. SNPs modeled to have no effect were randomly assigned a normally distributed beta value in each of the male and female simulations, with no link between these separate analyses as to the direction or magnitude of the effect for a given SNP. To mirror the effect sizes detected in the real trait data, the SNPs considered to have no effect were assigned an average beta value of ± 0.2, while the SNPs considered to have a causal effect were assigned an average beta value of ± 3.0. The number of causal SNPs that were simulated ranged from zero to half of all SNPs and included the following sets of causal SNPs: half, one-fourth, one-eighth, one twentieth, two hundred, one hundred, fifty, ten, and zero. For the simulations where zero SNPs were considered causal, no beta values were included in the simulation so that they were again randomly assigned in each of the male and female simulations. Interactions were tested using simulated data based on either half, two hundred, or zero SNPs considered as causal for a marginal effect, with no true interactions modeled in the simulated data. Each phenotype simulation and analysis of main effects and interactions was repeated 10 times.

Sex-stratified main effect analyses were also analyzed with sex randomized using 100 permutations. In each permutation, the number of males and the number of females were consistent in each analysis as there were more female mice than males. Randomization was performed in R using the transform command in base R (version 3.6.2).

Data Access

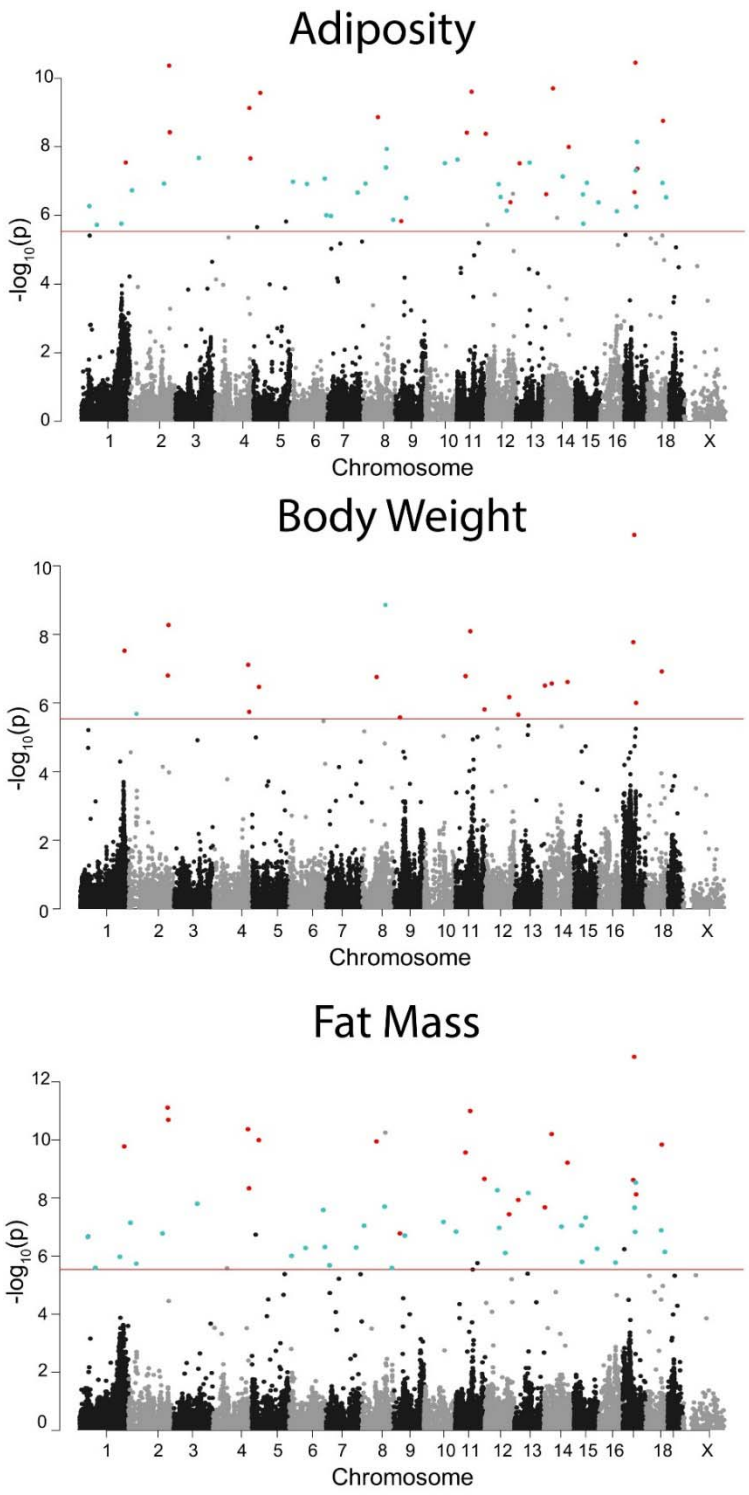
All genetic and phenotypic data for the HMDP was obtained from the Mouse Phenome Database (https://phenome.jax.org/).

Competing Interest Statement

The authors have no competing interests to disclose.

Acknowledgement

This work made use of the High-Performance Computing Resource in the Core Facility for Advanced Research Computing at Case Western Reserve University. We acknowledge Dr. Carl Kadie for his assistance with analyses in FaST-LMM. This research was funded by grants DK119305, NLM010098, and 5T32HL007567-35.


Adiposity
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Chromosome
Body Weight
-log10(p)
Chromosome
Fat Mass
-log10(p)
Chromosome

Figure S1

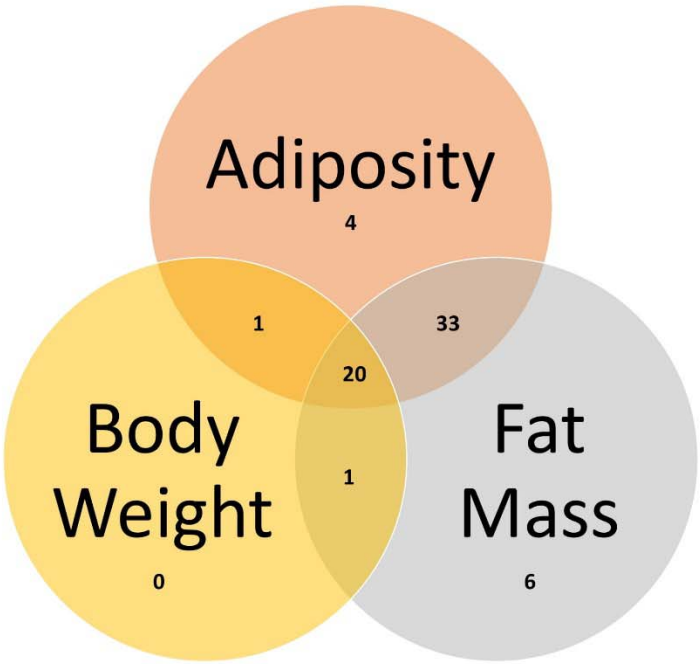
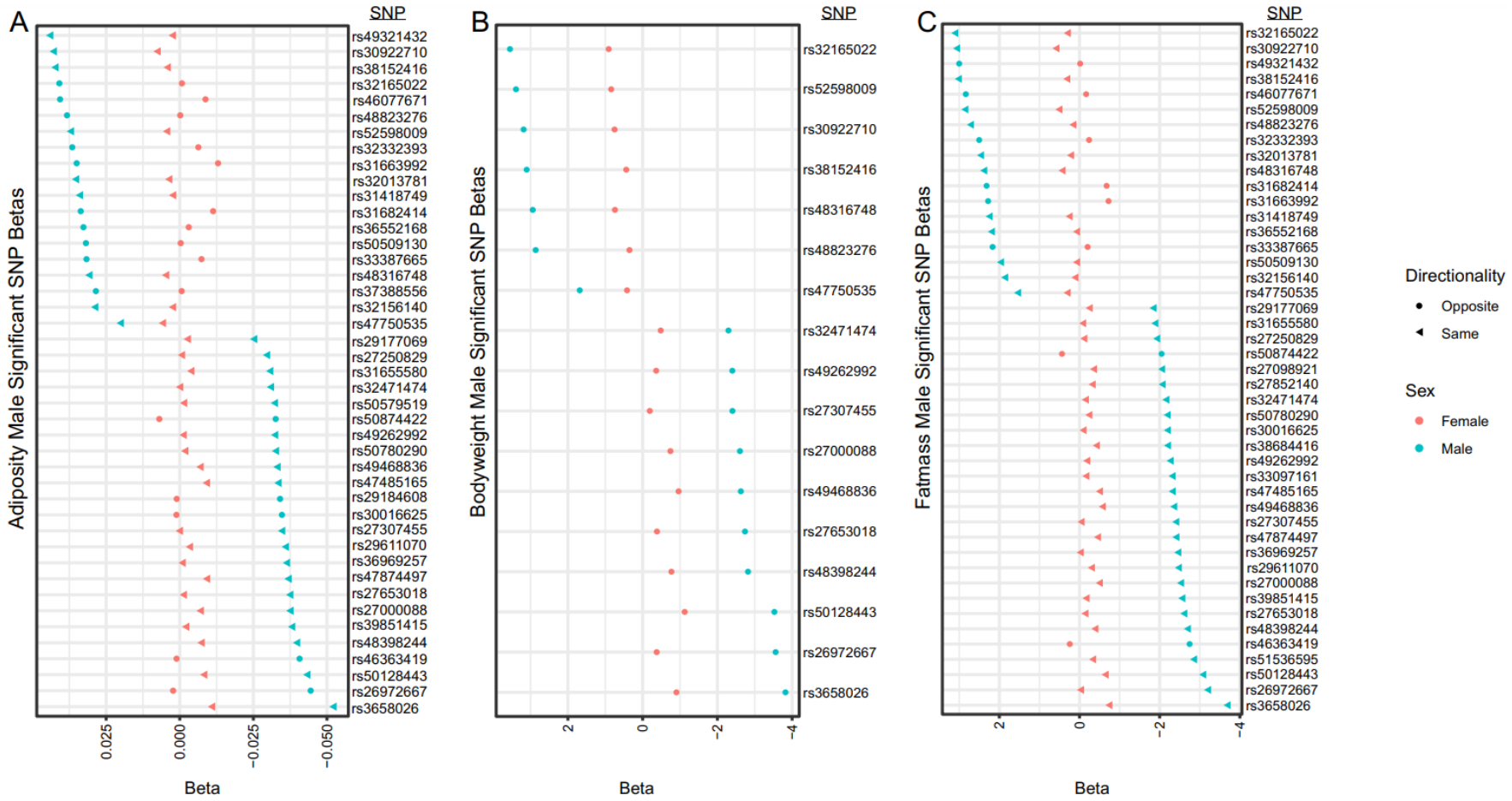


Figure S2

Figure S3


A
Adiposity Male Significant SNP Betas
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rs31663992
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rs48398244
rs46363419
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rs26972667
rs3658026
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0.000
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Beta
B
Bodyweight Male Significant SNP Betas
SNP
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rs52598009
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rs38152416
rs48316748
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rs47750535
rs32471474
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rs27307455
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rs27653018
rs48398244
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rs26972667
rs3658026
2
0
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−4
Beta
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Fatmass Male Significant SNP Betas
SNP
rs32165022
rs30922710
rs49321432
rs38152416
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Beta
Directionality
Opposite
Same
Sex
Female
Male

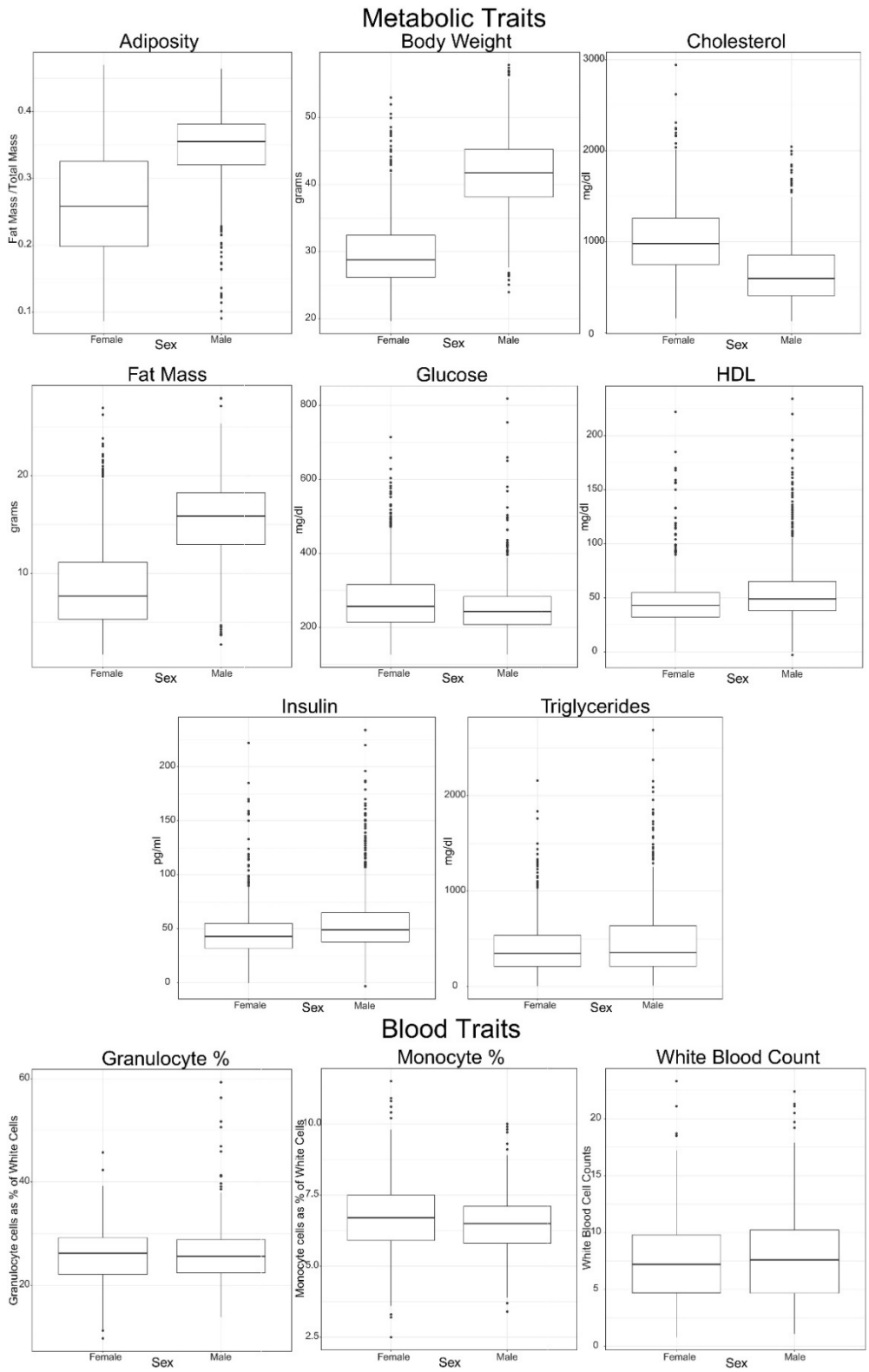
Metabolic Traits
Adiposity
Fat Mass /Total Mass
Body Weight
grams
Cholesterol
mg/dl
Fat Mass
grams
Glucose
mg/dl
HDL
mg/dl
Insulin
pg/ml
Triglycerides
mg/dl
Female
Sex
Male
Blood Traits
Granulocyte %
Granulocyte cells as % of White Cells
Monocyte %
Monocyte cells as % of White Cells
White Blood Count
White Blood Cell Counts

Figure S4

Figure S1. Overlap Between Main Effect Associations Identified for Adiposity, Body Weight, and Fat Mass in Male Mice. Manhattan plots for adiposity, body weight, and fat mass are shown. The red line indicates the significance threshold of 2.89E-6. A red dot indicates a significant association for all 3 traits. A blue dot indicates a significant association for 2 of the 3 traits. The loci for all significant associations can be found in Table S3.

Figure S2. Venn Diagram of the Overlap between Main Effect Associations Identified for Adiposity, Body Weight, and Fat Mass. The numbers of unique and overlapping associations for adiposity, body weight, and fat mass are shown for male mice in the HMDP.

Figure S3. Beta Directionality of Male Significant SNPs by Sex. The beta values of the significant main effects in males were plotted for both male and female sex-stratified analyses. Pink dots represent female betas and blue dots represent male betas. Circles represent SNPs with the same direction of effect and triangles represent SNPs with different direction of effects across sexes. The dark line a beta of zero.

Figure S4. Metabolic and Blood Trait Values by Sex. The average value of each trait was calculated and plotted as a box plot. The line corresponds to the average value, the box corresponds to the 95% confidence intervals, and outliers are indicated by individual dots.

Supplemental Table 1. Genetic Correlation Between Metabolic Traits in the Hybrid Mouse Diversity Panel

Males

	Body Weight	Cholesterol	Fat Mass	Glucose	HDL	Insulin	Triglycerides	Granulocyte %	Monocyte %	White Blood Count
Adiposity	0.18	-0.02	0.19	0.18	0.31	-0.06	-0.14	-0.18	0.25	-0.56
Body Weight		0.48	0.86	0.19	-0.03	0.63	0.60	0.39	0.52	-0.46
Cholesterol			0.43	0.68	-0.27	0.17	0.88	0.33	0.32	-0.08
Fat Mass				0.47	0.02	0.43	0.48	0.06	0.43	-0.54
Glucose					-0.23	0.17	0.56	0.11	0.28	-0.50
HDL						-0.03	-0.69	-0.20	-0.07	0.05
Insulin							0.34	0.15	0.15	-0.60
Triglycerides								0.48	0.47	-0.12
Granulocyte %									0.83	-0.41
Monocyte %										-0.48
White Blood Count										

Females

Adiposity	0.18	-0.08	0.10	-0.26	0.14	-0.04	0.13	0.05	0.22	-0.38
Body Weight		0.12	0.55	0.20	0.27	0.76	0.52	0.18	0.13	-0.20
Cholesterol			0.21	0.52	0.03	0.19	0.65	0.26	0.23	-0.07
Fat Mass				0.32	0.25	0.83	0.57	0.11	0.21	-0.35
Glucose					-0.46	0.33	0.65	0.19	0.04	-0.41
HDL						0.00	-0.42	0.13	0.08	0.20
Insulin							0.50	0.15	0.08	-0.52
Triglycerides								0.29	0.15	-0.23
Granulocyte									0.66	-0.76

%										
Monocyte %										-0.29
White Blood Count										

Supplemental Table 2. Genetic Correlation of Traits Between Males and Females in the Hybrid Mouse Diversity Panel

Trait	Genetic Correlation
Adiposity	0.55
Body Weight	0.88
Cholesterol	0.41
Fat Mass	0.58
Glucose	0.86
HDL	0.17
Insulin	0.75
Triglycerides	0.58
Granulocyte %	0.59
Monocyte %	0.70
White Blood Count	0.35

Supplemental Table 3. Bonferroni Significant Main Effects

Large file located online at: https://docs.google.com/spreadsheets/d/1cH1vJ2RW1vVUu-VbFehzgho89XzNBcxw/edit?usp=sharing&ouid=107182236362856136813&rtpof=true&sd=true

Supplemental Table 4. Detection of Enrichment of Main and Interaction Effects, P-value < 0.05

	Trait	Female Main Effects	Male Main Effects	Combined Main Effects
Expected Metabolic		***727***	***865***	***664***
Metabolic	Adiposity	1,214*	988*	1,459*
	Body Weight	1,333*	1,258*	821*
	Cholesterol	862*	1,111*	870*
	Fat Mass	1,319*	1,006*	847*
	Glucose	1,239*	1,034*	1,004*
	HDL	633[&]	1,047*	919*
	Insulin	1,522*	1,166*	925*
	Triglycerides	1,116*	1,052*	951*
Expected Blood		***871***	***859***	***727***
Blood	Granulocyte %	1,093*	900*	1,206*
	Monocyte %	1,134*	954*	1,221*
	White Blood Count	1,070*	1,040*	1,166*

***Significantly More Detected than Expected by Chance ($p < 0.05$)
[&]Significantly Fewer Detected than Expected by Chance ($p < 0.05$)

Supplemental Table 5. Replicated Detection of Marginal Effects Using Less Stringent Threshold in Female Mice

Trait	SNP	P-Value
Adiposity	JAX00692679	7.02E-06
	JAX00692959	9.17E-06
	JAX00691999	9.20E-06
	JAX00692715	1.22E-05
	JAX00692466	1.28E-05
HDL	JAX00135190	8.12E-06

Supplemental Table 6. Number of Overlapping Significant Main Effects in Male Mice

Trait	Number of Total Main Effect SNP	Number of Unique Main Effect SNPs	Number of Overlapping Main Effect SNPs
Adiposity	58	4	54
Body Weight	22	0	22
Fat Mass	60	6	54

Traits	Number of Overlapping Effects
Adiposity - Body Weight - Fat Mass	20
Only Adiposity - Body Weight	1
Only Adiposity - Fat Mass	33
Only Body Weight - Fat Mass	1

Supplemental Table 7. Comparing Parks et al Significant Hits With HMDP Data

Trait	Sex	Chr.	Peak SNP	Position (Mb)	P-Value	Nearby Significant Loci (within 5 Mb)	R'
HOMA-IR	Male	1	rs32316569	175768939	5.22E-07	JAX00277704	0.046
Insulin	Male	1	rs31614030	173308737	4.66E-06	NA	NA
Insulin	Female	4	rs27896920	71287837	4.46E-06	NA	NA
Glucose	Male	7	rs3680765	49397123	1.52E-07	NA	NA
Plasma Triglycerides	Male	7	rs31371723	76638409	4.64E-07	NA	NA
Plasma Triglycerides	Female	7	rs33903345	65789990	7.94E-12	NA	NA
Mesenteric Fat/Bodyweight	Male	7	rs32000744	67559538	2.71E-06	NA	NA
Mesenteric Fat/Bodyweight	Female	7	rs32000744	67559538	8.73E-07	NA	NA
HOMA-IR	Male	9	rs36804270	104875641	1.84E-06	NA	NA
Glucose	Female	11	rs27001755	112944793	3.33E-07	NA	NA
Insulin	Male	15	rs32269281	21154862	2.85E-06	NA	NA
Mesenteric Fat/Bodyweight	Female	15	rs32263766	91948444	8.53E-07	JAX00410644	0.02
Mesenteric Fat/Bodyweight	Female	15	rs37298722	28527523	3.88E-06	NA	NA
Plasma Triglycerides	Female	17	rs33060525	35595846	4.18E-06	NA	NA
Mesenteric Fat/Bodyweight	Female	17	rs33115366	47452733	4.14E-06	JAX00076238	0.067

Supplemental Table 8. Test of Normality for Metabolic and Blood Traits

Trait	Female	Male
Adiposity	2.45E-08	<2.2E-16
Body Weight	4.18E-16	0.013
Cholesterol	7.33E-10	<2.2E-16
Fat Mass	<2.2E-16	7.30E-06
Glucose	<2.2E-16	<2.2E-16
HDL	<2.2E-16	<2.2E-16
Insulin	<2.2E-16	<2.2E-16
Triglycerides	<2.2E-16	<2.2E-16
Granulocyte %	0.22	3.89E-15
Monocyte %	0.016	0.11
White Blood Count	2.76E-09	8.77E-11

Supplemental Table 9. Main Effects of Trait Data Normalized or With Outliers Removed

Rank Based Normalized of Main Effects

Trait	Female Bonferroni Significant Effects	Lambda	Male Bonferroni Significant Effects	Lambda	Combined Sex Bonferroni Significant Effects	Lambda
Adiposity	0	1.09	58	0.99	1 (1)	0.96
Body Weight	0	1.21	22	1.07	3 (3)	0.58
Cholesterol	0	1.05	0	1.73	0	0.90
Fat Mass	0	1.13	60	1.53	0	0.76
Glucose	0	1.12	0	1.85	0	1.10
HDL	0	1.04	0	1.74	0	0.98
Insulin	0	1.19	0	1.76	0	0.93
Triglycerides	0	1.10	0	1.92	0	0.92
Granulocyte %	0	1.16	0	1.17	1	1.05
Monocyte %	0	1.17	0	1.07	0	1.13
White Blood Count	0	0.90	0	1.08	0	1.04

Box-Cox Based Normalized of Main Effects

Trait	Female Bonferroni Significant Effects	Lambda	Male Bonferroni Significant Effects	Lambda	Combined Sex Bonferroni Significant Effects	Lambda
Adiposity	0	1.14	63 (58)	0.96	1 (1)	0.94
Body Weight	0	1.10	22 (22)	1.07	5 (5)	0.54
Cholesterol	0	1.05	1 (0)	1.04	0	0.91
Fat Mass	0	1.16	54 (54)	0.98	3 (3)	0.68

Glucose	0	1.13	0	1.15	0	1.1
HDL	0	1.15	2 (0)	1.11	0	0.95
Insulin	0	1.19	0	1.07	0	1.03
Triglycerides	0	1.00	0	1.04	0	0.88
Granulocyte %	0	1.12	0	1.16	0	1.02
Monocyte %	0	1.07	0	1.17	0	1.13
White Blood Count	0	1.06	0	0.94	0	1.04

Main Effects Excluding Individual Outliers

Trait	**Female Bonferroni Significant Effects**	**Lambda**	**Male Bonferroni Significant Effects**	**Lambda**	**Combined Sex Bonferroni Significant Effects**	**Lambda**
Adiposity	NA	NA	35 (35)	0.96	NA	NA
Body Weight	0	1.84	24 (22)	1.65	NA	NA
Cholesterol	0	1.06	NA	NA	0	0.87
Fat Mass	0	1.87	55 (53)	1.57	NA	NA
Glucose	0	1.76	0	1.18	0	1.08
HDL	0	.16	0	1.09	0	1
Insulin	0	1.88	0	1.78	0	1
Triglycerides	1	1.62	0	1.83	0	0.95
Granulocyte %	0	1.17	0	1.11	0	1.03
Monocyte %	NA	NA	0	1.08	0	1.12
White Blood Count	0	0.91	0	1.06	0	1.06

Main Effects Excluding Strain Outliers

Trait	Female Bonferroni Significant Effects	Lambda	Male Bonferroni Significant Effects	Lambda	Combined Sex Bonferroni Significant Effects	Lambda
Adiposity	NA	NA	NA	NA	NA	NA
Body Weight	0	1.84	NA	NA	NA	NA
Cholesterol	NA	NA	NA	NA	NA	NA
Fat Mass	NA	NA	NA	NA	NA	NA
Glucose	NA	NA	NA	NA	NA	NA
HDL	NA	NA	NA	NA	NA	NA
Insulin	NA	NA	0	1.14	0	0.89
Triglycerides	NA	NA	NA	NA	0	0.92
Granulocyte %	0	1.17	0	1.14	NA	NA
Monocyte %	NA	NA	NA	NA	NA	NA
White Blood Count	NA	NA	NA	NA	NA	NA

*Parentheses notates the number of significant main effects replicated in the unadjusted analysis

Supplemental Table 10. Randomized Sex Main Effects in Adiposity

	Female	Male
Permutation 1	2	0
Permutation 2	0	4
Permutation 3	0	0
Permutation 4	0	1
Permutation 5	17	0
Permutation 6	0	0
Permutation 7	2	2
Permutation 8	0	3
Permutation 9	11	4
Permutation 10	1	3
Permutation 11	0	0
Permutation 12	0	2
Permutation 13	0	0
Permutation 14	0	0
Permutation 15	0	0
Permutation 16	0	0
Permutation 17	0	1
Permutation 18	0	2
Permutation 19	0	0
Permutation 20	0	0
Permutation 21	0	10
Permutation 22	0	0
Permutation 23	0	1
Permutation 24	0	0
Permutation 25	0	0
Permutation 26	0	0
Permutation 27	0	0
Permutation 28	0	10
Permutation 29	0	0
Permutation 30	0	0
Permutation 31	0	0
Permutation 32	0	0
Permutation 33	1	0
Permutation 34	0	0
Permutation 35	0	0
Permutation 36	0	0
Permutation 37	0	0

Permutation 38	0	0
Permutation 39	0	0
Permutation 40	0	0
Permutation 41	0	0
Permutation 42	0	0
Permutation 43	0	0
Permutation 44	0	0
Permutation 45	0	0
Permutation 46	0	1
Permutation 47	1	0
Permutation 48	0	1
Permutation 49	0	0
Permutation 50	0	0
Permutation 51	0	0
Permutation 52	0	0
Permutation 53	1	0
Permutation 54	0	0
Permutation 55	0	0
Permutation 56	0	0
Permutation 57	0	0
Permutation 58	0	2
Permutation 59	0	0
Permutation 60	0	2
Permutation 61	0	0
Permutation 62	0	0
Permutation 63	0	0
Permutation 64	0	0
Permutation 65	0	0
Permutation 66	1	1
Permutation 67	0	0
Permutation 68	0	1
Permutation 69	0	2
Permutation 70	0	0
Permutation 71	0	0
Permutation 72	0	1
Permutation 73	0	0
Permutation 74	0	1
Permutation 75	1	0
Permutation 76	0	1
Permutation 77	0	0

Permutation 78	0	0
Permutation 79	0	0
Permutation 80	0	0
Permutation 81	0	0
Permutation 82	0	0
Permutation 83	0	0
Permutation 84	2	0
Permutation 85	0	0
Permutation 86	0	13
Permutation 87	0	0
Permutation 88	0	0
Permutation 89	0	0
Permutation 90	0	0
Permutation 91	0	0
Permutation 92	0	0
Permutation 93	0	4
Permutation 94	0	2
Permutation 95	0	0
Permutation 96	0	0
Permutation 97	0	1
Permutation 98	0	0
Permutation 99	0	3
Permutation 100	1	0

Supplemental Table 11. Average Value and Direction of Betas for All Tested Main Effects

Trait	Sex	Positive Beta	Negative Beta	Average Beta
Adiposity	Male	35.29%	65.71%	0.04
	Female	25.71%	74.29%	0.004
Body Weight	Male	38.89%	61.11%	2.91
	Female	36.04%	63.96%	0.56
Fat mass	Male	40.00%	60.00%	2.45
	Female	31.11%	68.89%	0.30

Supplemental Table 12. Strains Used and Excluded from Metabolism and Blood Trait Analyses

	Metabolism Traits*		Blood Traits**	
Strains	**Male n**	**Female n**	**Male n**	**Female n**
129X1/SvJ	6	14	6	14
A/J	12	8	12	8
AKR/J	24	21	24	21
AXB10/PgnJ	4	2	4	2
AXB12/PgnJ	3	9	3	9
AXB13/PgnJ	10	12	9	11
AXB15/PgnJ	4	6	3	6
AXB19/PgnJ	13	6	13	6
AXB19a/PgnJ	14	12	14	12
AXB19b/PgnJ	4	4	4	4
AXB2/PgnJ	4	8	4	7
AXB4/PgnJ	3	0[&]	3	0[&]
AXB5/PgnJ	10	11	10	10
AXB6/PgnJ	3	3	3	3
AXB8/PgnJ	0[&]	2	0[&]	2
BALB/cByJ	12	4	12	4
BALB/cJ	16	18	16	17
BTBR	8	13	8	13
BUB/BnJ	10	19	10	19
BXA1/PgnJ	0[&]	1	0[&]	1
BXA11/PgnJ	7	7	7	7
BXA14/PgnJ	4	4	4	4
BXA16/PgnJ	4	3	4	3
BXA2/PgnJ	14	11	14	11
BXA24/PgnJ	18	13	18	13
BXA25/PgnJ	1	1	1	1
BXA4/PgnJ	4	4	4	4

BXA7/PgnJ	11	6	8	4
BXA8/PgnJ	10	9	10	9
BXD1/TyJ	1	5	1	5
BXD11/TyJ	2	4	2	4
BXD12/TyJ	2	0[&]	2	0[&]
BXD13/TyJ	4	3	4	3
BXD14/TyJ	4	3	4	3
BXD18/TyJ	0[&]	3	0[&]	3
BXD2/TyJ	4	1	4	1
BXD20/TyJ	4	6	4	6
BXD21/TyJ	11	7	11	6
BXD22/TyJ	2	1	2	1
BXD24/TyJ-Cep290<rd16>/J	0[&&]	0[&&]	0[&&]	0[&&]
BXD28/TyJ	2	0[&]	2	0[&]
BXD31/TyJ	3	2	0[&]	2
BXD32/TyJ	4	7	4	7
BXD34/TyJ	2	1	2	1
BXD36/TyJ	3	5	2	2
BXD38/TyJ	2	2	2	2
BXD40/TyJ	7	5	7	4
BXD43/RwwJ	5	4	5	4
BXD44/RwwJ	12	8	12	8
BXD48/RwwJ	8	18	8	18
BXD5/TyJ	3	6	2	6
BXD50/RwwJ	9	11	8	11
BXD55/RwwJ	9	17	9	17
BXD56/RwwJ	2	6	2	6
BXD6/TyJ	2	4	0[&]	1
BXD61/RwwJ	14	5	12	5
BXD64/RwwJ	4	9	4	9

BXD66/RwwJ	7	3	7	3
BXD68/RwwJ	1	0[&]	1	0[&]
BXD69/RwwJ	3	12	3	12
BXD70/RwwJ	9	17	9	17
BXD71/RwwJ	2	4	2	4
BXD73/RwwJ	2	10	2	10
BXD75/RwwJ	12	10	12	8
BXD79/RwwJ	1	0[&]	1	0[&]
BXD84/RwwJ	8	6	8	6
BXD86/RwwJ	1	0[&]	1	0[&]
BXD9/TyJ	1	6	1	3
BXH19/TyJ	0[&]	5	0[&]	5
BXH22/KccJ	2	5	2	5
BXH4/TyJ	1	0[&]	1	0[&]
BXH6/TyJ	3	6	3	6
BXH8/TyJ	4	5	4	5
BXH9/TyJ	3	0[&]	3	0[&]
C3H/HeJ	12	13	12	13
C57BL/6J	15	16	15	16
C57BLKS/J	8	6	8	6
C57L/J	13	8	11	8
C58/J	5	4	4	4
CBA/J	8	5	7	5
CE/J	1	3	1	3
CXB1/ByJ	6	8	6	8
CXB11/HiAJ	1	1	1	1
CXB12/HiAJ	10	5	10	5
CXB13/HiAJ	8	9	8	9
CXB2/ByJ	5	3	5	3
CXB3/ByJ	17	7	17	7

CXB4/ByJ	4	0[&]	4	0[&]
CXB6/ByJ	3	4	3	4
CXB7/ByJ	7	6	7	6
CXB8/HiAJ	2	5	0[&]	1
DBA/2J	4	10	4	10
FVB/NJ	12	13	9	12
I/LnJ	10	6	10	6
KK/HlJ	3	5	3	5
LG/J	0[&]	5	0[&]	5
LP/J	2	5	2	5
MA/MyJ	4	4	4	4
NOD/ShiLtJ	5	6	5	6
NON/ShiLtJ	6	3	6	3
NZB/BlNJ	6	6	6	6
NZW/LacJ	11	12	11	12
PL/J	12	8	12	8
RIIIS/J	8	4	8	4
SEA/GnJ	11	7	11	7
SJL/J	10	7	10	7
SM/J	6	12	6	12
SWR/J	11	15	11	15

*Traits include Adiposity, Body Weight, Cholesterol, Fat Mass, Glucose, HDL, Insulin, and Triglycerides

**Traits include Granulocyte %, Monocyte %, and White Blood Count

[&]Removed due to missing phenotype data

[&&]Removed as strains model vision phenotypes

Supplemental Table 13. SNP Pruning and Haplotype Block Detection in the HMDP.

	Original SNPs	**LD Pruned**	**Haplotype Blocks**	**External SNPs**
Female Metabolic	200,885	23,205	12,221	2,314
Male Metabolic	199,910	21,995	11,285	5,907
Female Blood	200,885	23,518	12,193	5,221
Male Blood	199,910	22,115	11,437	5,752
Combined Metabolic and Blood	195,190	22,284	12,229	5,219

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