

# Epistasis is widespread in the genetic control of transcription in humans

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## Abstract

A long standing question in evolution and human genetics is the extent to which epistasis, the phenomenon whereby one polymorphism's effect on a trait depends on other polymorphisms present in the genome, contributes to complex traits.<sup>1-3</sup> Though epistasis has been demonstrated in artificial gene manipulation studies in model organisms,<sup>4,5</sup> and some examples have been shown in other species,<sup>6</sup> few convincing examples exist for epistasis amongst natural polymorphisms in human traits.<sup>7,8</sup> Its absence from empirical findings may simply be due to its unimportance in the genetic control of complex traits,<sup>2,3</sup> but we sought to test the hypothesis that it has previously been too technically difficult to detect due to statistical power and computational issues.<sup>9</sup> Here we show that, using advanced computation techniques<sup>10</sup> and a gene expression study design, evidence for multiple instances of epistasis is found. In a cohort of 846 individuals with data on 7339 gene expression levels in whole blood, we found that after stringent correction for multiple testing there were 501 significant pairwise epistatic interactions acting on the expression levels of 223 genes. Twenty-nine interactions replicated in two independent datasets<sup>11,12</sup> following Bonferroni correction for multiple testing. Of the SNPs that did not pass this replication threshold, there was significant enrichment for interaction effects (316 of 434 above the 2.5% confidence interval for uniformly distributed  $p$ -values,  $p < 1.0 \times 10^{-16}$ ). We provide evidence of functional enrichment for the interacting SNPs, for instance 44 of the genetic interactions are located within 5Mb of regions of known intra-cellular chromosome interactions<sup>7</sup> ( $p < 1.8 \times 10^{-10}$ ). Multi-locus epistatic interactions control 129 genes, whereby one *cis*-acting single nucleotide polymorphism (SNP) is modulated by several *trans*-acting SNPs. For example MBNL1 is controlled by an additive effect at rs13069559 which itself is controlled by *trans*-SNPs on 14 different chromosomes, with nearly identical genotype-phenotype (GP) maps for each *cis-trans* interaction. This study presents the first strong evidence for multiple instances of epistatic genetic effects emerging from natural genetic variation in humans, and demonstrates its significant contribution to the variance of gene expression.

# 1 Main text

The past decade has seen a tremendous amount of activity in mapping genetic polymorphisms that underlie complex traits. Typically, SNPs are treated using an additive model where they are assumed to contribute linearly, independently, and cumulatively to the mean of a trait. This has been successful in identifying thousands of associations,<sup>13</sup> but to date there is little empirical exploration of the role that epistasis plays in the architecture of complex traits in humans,<sup>7,8</sup> though its contribution to phenotypic variance is frequently the subject of debate.<sup>1-3</sup> Outside the prism of human association studies there is widespread evidence for epistasis, not only at the molecular scale from artificially induced mutations<sup>4</sup> but also at the evolutionary scale in fitness adaptation<sup>14</sup> and speciation.<sup>15</sup> Here we demonstrate that multiple instances of statistical epistasis can be detected in human traits, and that some quantification of its relative contribution to phenotypic variance can be made.

Detection of epistasis is hampered by power issues for several reasons, including increased dependence on linkage disequilibrium (LD) between causal SNPs and observed SNPs,<sup>16,17</sup> increased model complexity in fitting interaction terms,<sup>18</sup> and more extreme significance thresholds to account for increased multiple testing.<sup>9</sup> When genetic effect sizes are small, as is expected in most complex traits of interest,<sup>13</sup> the power to detect epistasis diminishes rapidly. There are two simple ways to overcome this problem. One is by using extremely large sample sizes;<sup>19</sup> another is by analysing traits that are likely to have large effect sizes. Because our focus was to ascertain the extent to which epistasis exists amongst natural genetic variation we designed a study around the latter approach and searched for epistatic genetic effects that influence gene expression levels. Transcription levels can be measured for thousands of genes. These traits are largely heritable but on average less polygenic than high level phenotypes,<sup>20</sup> thus it is expected that many genetic effects will be relatively large, maximising the chance at detecting epistasis, should it exist.

In a dataset of 846 individuals genotyped at 528509 SNPs, we exhaustively tested every pair of SNPs for genetic interactions against each of 7339 expression traits in whole blood. After stringent filtering and multiple testing correction we had identified 501 putative genetic interactions influencing 238 gene expression levels. Of the 501 discovery interactions, data was available for 434 in two independent replication datasets, Fehrmann<sup>12</sup> and the Estonian Genomics Centre University of Tartu (EGCUT),<sup>11</sup> in which we saw convincing evidence for replication. We used the summary statistics from the replication datasets to perform a meta analysis to obtain an independent  $p$ -value for the putative interactions, and 29 were significant after applying a Bonferroni correction for multiple testing (Table ??). These significant interactions exhibited remarkable similarity in GP maps between all three datasets (Figure 2).

Additionally, there was extreme enrichment for interaction effects among the discovery SNPs that did not reach the stringent significance threshold for replication (Figure ??). We observed that 316 of the remaining 405 discovery SNPs had replication interaction  $p$ -values exceeding the 2.5% confidence interval

under the null distribution of no effects ( $p \ll 1.0 \times 10^{-16}$ , Supplementary Figure ??). The congruence of the epistatic networks in discovery and replication datasets is shown in Figure 1, demonstrating that these complex genetic patterns are common even across independent populations. A further replication was attempted using the Centre for Health Discovery and Wellbeing (CHDWB) dataset,<sup>21</sup> but only 185 of the SNP pairs passed filtering because the sample size was small ( $n = 139$ ), and due to insufficient power we found no evidence for replication. Though a necessary step to establish the veracity of the signals from the discovery set, it is theoretically difficult to replicate epistasis because the dependence on LD between observed SNPs and causal variants is on average three orders of magnitude higher than it is for independent additive effects.<sup>16,17</sup> Therefore these results are very encouraging with regards to the detection and replication of epistasis.

Though seldom the focus of association studies, SNPs with known main effects are often tested for additive  $\times$  additive genetic interactions,<sup>9</sup> but our analysis shows that this is unlikely to be the most effective framework for its detection. The majority of interactions comprised of one SNP that had a previously known association and one SNP that had no previous association in the dataset<sup>20</sup> (439 out of 501). Only 9 interactions were between SNPs that both had marginal effects while 64 were between SNPs that had no known marginal effects. Additionally, we observed that the largest epistatic variance component for the 501 interactions were divided amongst additive  $\times$  additive, additive  $\times$  dominance and dominance  $\times$  dominance terms proportional to what is expected by change ( $p = 0.22$  for divergence from expectation). This is perhaps to be expected because the patterns of epistasis used for statistical decomposition are not designed to resemble biological function.<sup>22</sup>

We observed a wide range of significant GP maps (Figure 2) but the most common pattern of epistasis that we detected involved a *trans*-SNP masking the effect of an additive *cis*-SNP. For example, MBNL1 (involved in RNA modification and regulation of splicing<sup>23</sup>) has a *cis* effect at rs13069559 which in turn is controlled by 14 *trans*-SNPs that exhibit a masking pattern, such that when the *trans*-SNP is homozygous for the masking allele the decreasing allele of the *cis*-SNP no longer has an effect. Each of these interactions have evidence for replication in at least one dataset and eight have evidence for replication in both datasets (Supplementary Figure S3). We observed that nine of the 14 *trans* SNPs are located in intronic regions (proportion of SNP panel in introns = 0.05,  $p = 3.11 \times 10^{-9}$ ), suggesting a putative mechanism by which epistatic interactions may regulate transcription.

Of the 434 interactions, 66 were *cis-cis* acting (both SNPs were on the same chromosome as the expression gene), 470 were *cis-trans*-acting, and 13 were *trans-trans*-acting. In total the 434 interactions comprised 835 unique SNPs, which we analysed for functional enrichment. We tested the SNPs for cell-type specific overlap with transcriptionally active chromatin regions, tagged by histone-3-lysine-4,3-methylation (H3K4me3) chromatin marks, in 34 cell types.<sup>24</sup> There was significant enrichment for *cis*-acting SNPs in haematopoietic cell types only ( $p < 1 \times 10^{-4}$  for the three tissues with the strongest enrichment

are significant at after adjusting for multiple testing, Supplementary Figure S5). However *trans*-acting SNPs did not show any tissue specific enrichment ( $p > 0.1$  for all tissues, Supplementary Figure S5). This difference between *cis* and *trans* SNPs suggests that there is a range of molecular mechanisms by which epistasis might arise beyond tissue specific transcription. There is also strong enrichment for SNPs to be localised in enhancer regions<sup>25</sup> (Supplementary Figure S8). This enrichment is consistent for both *cis* and *trans* SNPs ( $p < 1 \times 10^{-6}$ ). In particular, there was substantial enrichment for the GATA2 binding motif within 1kb of all epistatic SNPs, a known regulator of transcription in haematopoietic cells<sup>26</sup> ( $p = 1 \times 10^{-40}$ ).

We also demonstrate another putative novel mechanism by which biological function can lead to epistatic genetic variance. We cross referenced our epistatic SNPs with a map of chromosome interacting regions ( $n = 96139$ ) in K562 blood cell lines.<sup>27</sup> Forty-nine epistatic SNP pairs mapped to within 500kb of a chromosome interaction, and 69 mapped to within 2Mb ( $p < 1.1 \times 10^{-70}$ ), (Supplementary Figure S7), but decyphering the exact cellular processes underlying this interaction requires further research.

Though we present many instances of epistasis, quantifying its relative importance to complex traits in humans remains an open question. In this study we are able to identify 249 probes with at least one significant interaction given our experiment-wide threshold. Using the same threshold ( $p < 2.91 \times 10^{-16}$ ) for the same data but searching for only additive effects returns 517 probes out of the 7339 analysed with at least one significant expression quantitative trait locus (eQTL),<sup>28</sup> therefore we argue that the number of instances of epistasis are substantial. However in terms of their contribution to complex traits a more important metric might be the proportion of the variance that the epistatic loci explain.<sup>2</sup> Ideally one would approach this question from a whole genome level<sup>29</sup> but this is intractable for non-additive variance components. Yet some inference can be made from the ascertained effects in these analyses and it is evident that additive variance is overall a larger component than epistatic variance as has been argued previously.<sup>2,3</sup> We observe that of the significant interactions detected the average additive contribution to the total detected genetic variance is 72.7%, over three times higher than the epistatic contribution of 24.1% (Figure 3).

From an experiment-wide perspective, the proportion of the total phenotypic variance for all 7339 probes explained by additive effects significant at the  $p < 2.91 \times 10^{-16}$  threshold, as reported in an independent study on the same dataset that focused on additive effects only,<sup>28</sup> is 1.9%. In contrast, the epistatic signals detected in this study explains 0.27%, approximately seven times lower than the additive variance. There are two caveats to this comparison, firstly the power of a 1 *d.f.* test exceeds that of an 8 *d.f.* test. Secondly the non-additive variance at causal variants is expected to be grossly underestimated by observed SNPs in comparison to estimates for additive variance,<sup>16,17</sup> due to differences in the rate of decay estimated variance between observed and causal SNPs as LD decreases. Therefore this is likely to be a lower bound on the estimate of the relative contribution of epistasis.

Overall, we have demonstrated that it is possible to identify and replicate epistasis in complex traits amongst common human variants. The functional analysis of the significant epistatic loci suggests that there are a large number of possible mechanisms that can lead to non-additive genetic variation, thus it is not surprising that instances of epistasis should be widespread. Further research into such genetic effects may provide a useful portal to understanding molecular mechanisms with greater clarity. With data and computational techniques now widely available the search for epistasis in larger datasets for traits of broader interest is warranted.

## 1.1 Methods Summary

We searched for pairwise epistasis exhaustively in the Brisbane Systems Genetics Study (BSGS) dataset,<sup>28</sup> which comprises 846 individuals who are genotyped at 528509 autosomal SNPs and who have gene expression levels measured in whole blood samples for 7339 probes representing 6158 RefSeq genes. Recent hardware and software<sup>10</sup> advances made it possible to perform the  $1.03 \times 10^{15}$  statistical tests to complete this analysis. We used permutation analysis<sup>30</sup> to calculate an experiment-wide significance threshold of  $2.91 \times 10^{-16}$  at the 5% family-wise error rate (FWER). SNP pairs were modelled for full genetic effects, including marginal additive and dominance at both SNPs plus four interaction terms. Though we could have used a less complex model to improve statistical efficiency, we deemed it important to be agnostic about the type of epistasis that might exist, and therefore chose not to over-parameterise the test.<sup>17,18</sup> Because there are many large marginal effects present in these data it was necessary to perform several filtering steps to exclude SNP pairs that were significant due to marginal effects alone. All SNP pairs with LD  $r^2 > 0.1$  were removed, and were required to have at least five data points in all nine genotype classes. If multiple SNP pairs were present on the same chromosomes for a particular expression trait then only the sentinel SNP pair was retained. Finally, a nested test contrasting the full genetic model against the marginal additive and dominance model was performed for each remaining SNP pair (Methods), resulting in 501 significant interactions after Bonferroni correction for multiple testing of the filtered SNPs. The significant SNP pairs were carried forward for replication in two independent datasets that used the same expression assays for analysing transcription in whole blood, the Fehrmann dataset<sup>12</sup> ( $n = 1240$ ) and the Estonian Genome Centre University of the University of Tartu (EGCUT) dataset<sup>11</sup> ( $n = 891$ ). Of these, 434 passed filtering in both replication datasets.

## 2 Figures

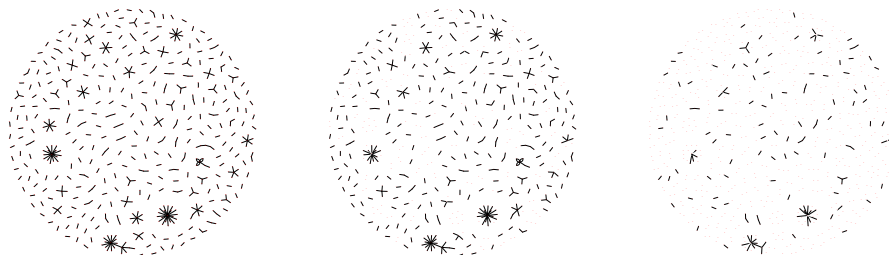
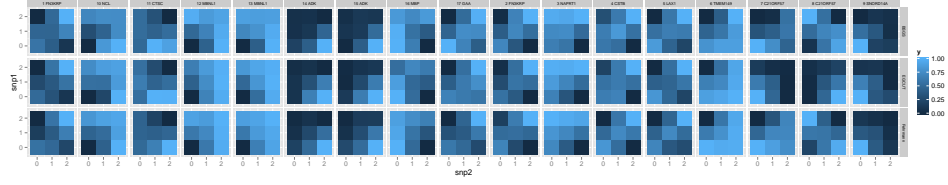


Figure 1: **Replication of epistatic networks in two cohorts** Panel A represents all 434 genetic interactions (edges) discovered in the discovery set. Panel B shows those interactions that were present at the 5% FDR level in at least one replication dataset, and panel C shows which interactions were significant in both replication datasets. Red nodes represent probes and black nodes represent SNPs. The congruence between datasets is indicative of the consistent epistatic instances in independent populations.



**Figure 2: Replication of genotype-phenotype (GP) maps in two cohorts**  
The GP maps for each epistatic interaction that is significant at the Bonferroni level in both replication datasets are shown. Each tile represents the expression level for that two-locus genotype class. Phenotypes are for gene transcript levels, where the gene symbol is labelled in the panel columns. Panel rows are labelled for the dataset. Phenotypes for each interaction within each cohort are scaled to be between 0 and 1. There is a general trend of the GP maps replicating across all three datasets.

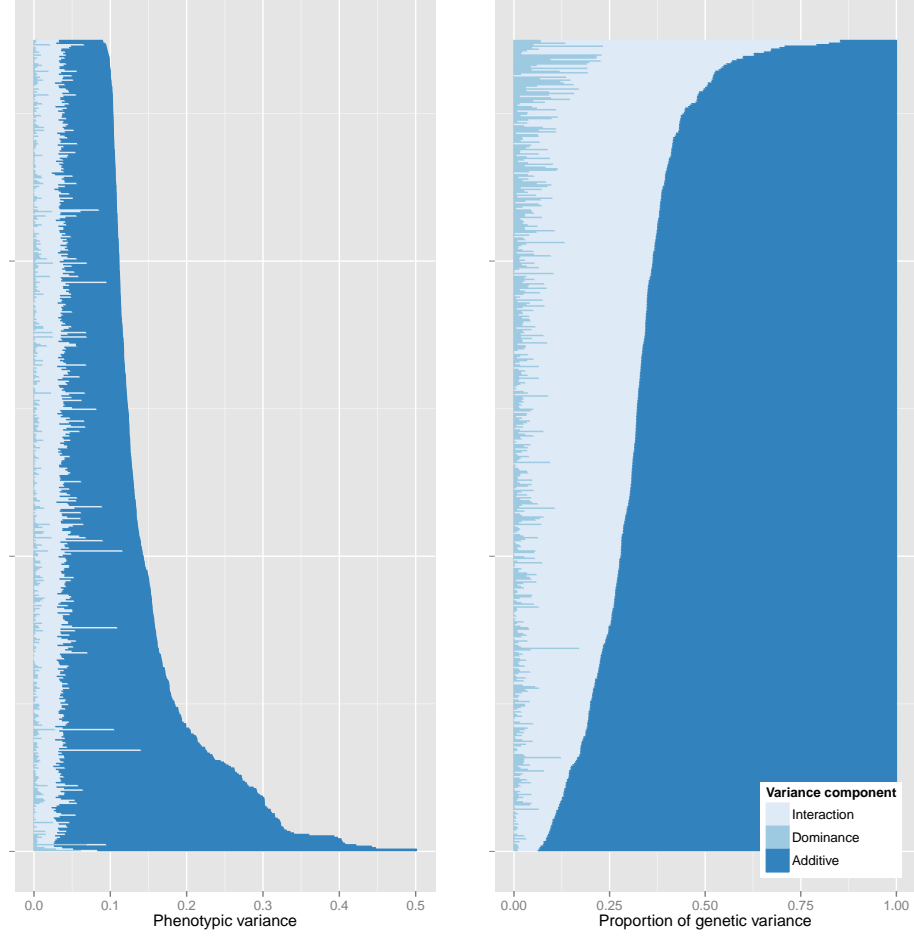


Figure 3: **Comparison of genetic variances** Panel A shows the proportion of phenotypic variation ( $x$ -axis) that is captured by additive, dominant and interaction components for all 529 significant interactions, ordered by total phenotypic variance explained by the SNP pair along the  $y$ -axis. Panel B shows the proportion of the genetic variance ( $x$ -axis) that is attributable to the three variance components, ordered by the proportion that is additive.



### 3 Supplementary Figures

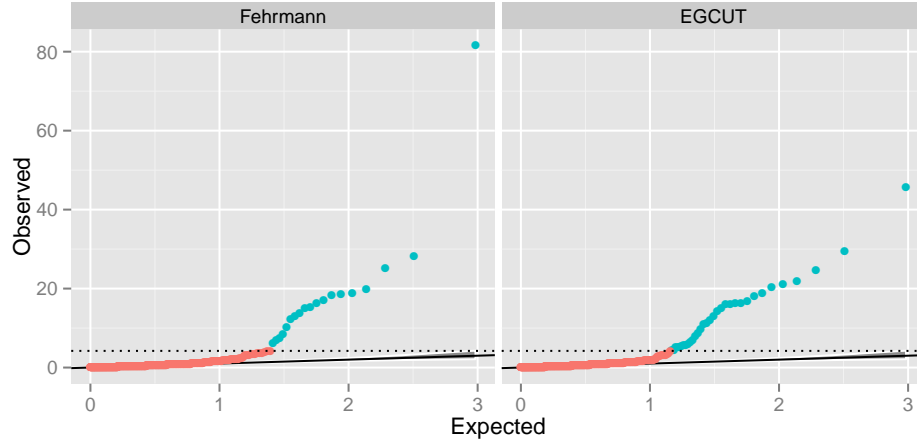


Figure S1: **Q-Q plots of interaction  $p$ -values in two independent datasets** Of the 434 discovery interactions, interaction  $p$ -values (4 *d.f.* test) were obtained for 480 SNP pairs that passed filtering in the two replication datasets. The  $p$ -values plotted along the  $y$ -axis, and the expected  $p$ -values are plotted along the  $x$ -axis. Green points represent  $p$ -values that surpass Bonferroni correction.

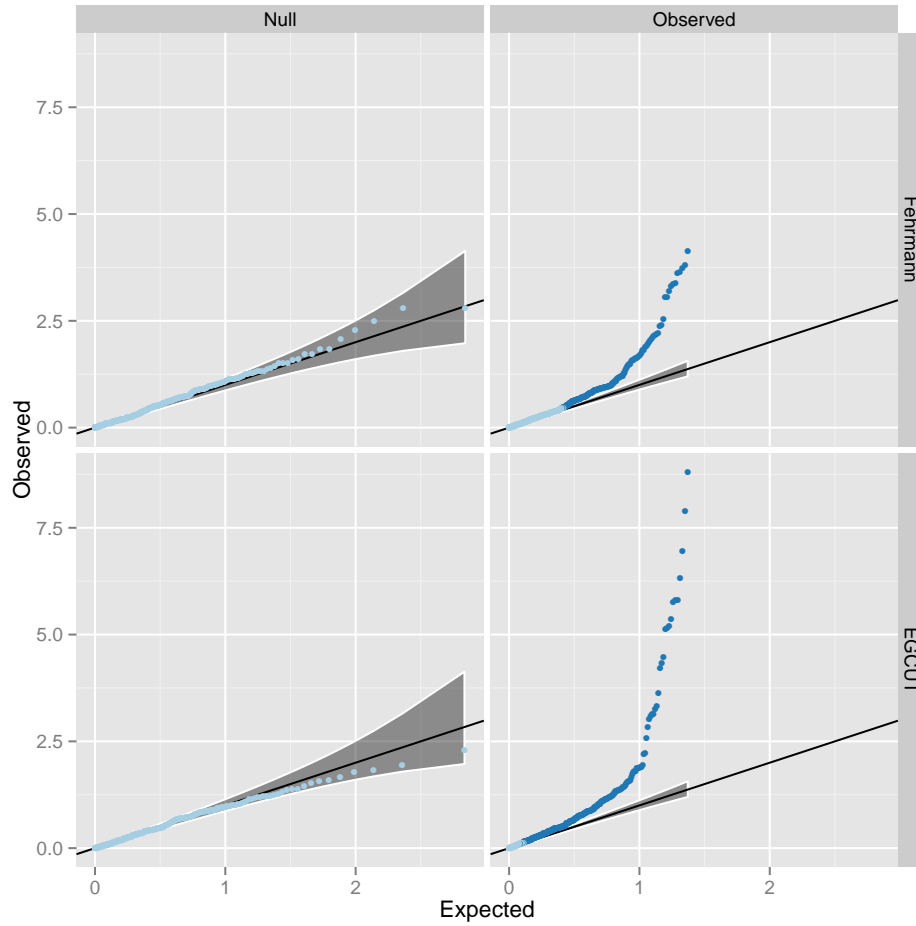


Figure S2: **Q-Q plots of replicated interaction  $p$ -values excluding the 20 most extreme  $p$ -values** The left panel (Null) shows the replication  $p$ -values from 434 randomly drawn SNP pairs in the Fehrman (top row) and EGCUT (bottom row) datasets. The right panel (Observed) shows the interaction  $p$ -values from the 460 least significant pairs that pass filtering in the two replication datasets (*i.e.* excluding the 20 most extreme  $p$ -values for clarity). Dark blue points represent  $p$ -values that surpass the 5% FDR level.

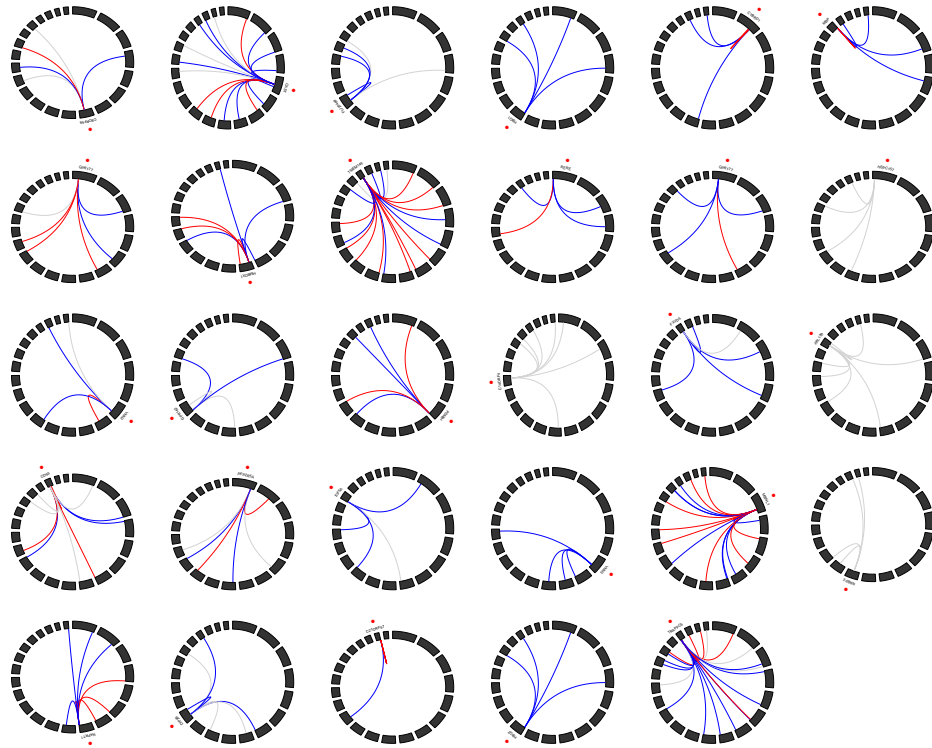


Figure S3: **Gene expression traits with four or more genetic interactions** Circle plots represent the genomic positions for SNPs (linking lines) and expression probes (red points). Chromosomes are represented by black blocks and ordered from 1 to 22 clockwise, starting from the top. Grey lines represent no evidence for replication, blue lines denote replication in at least one dataset, and red lines denote replication in two datasets. Most interactions are characterised as being *cis-trans* to the expression probe.

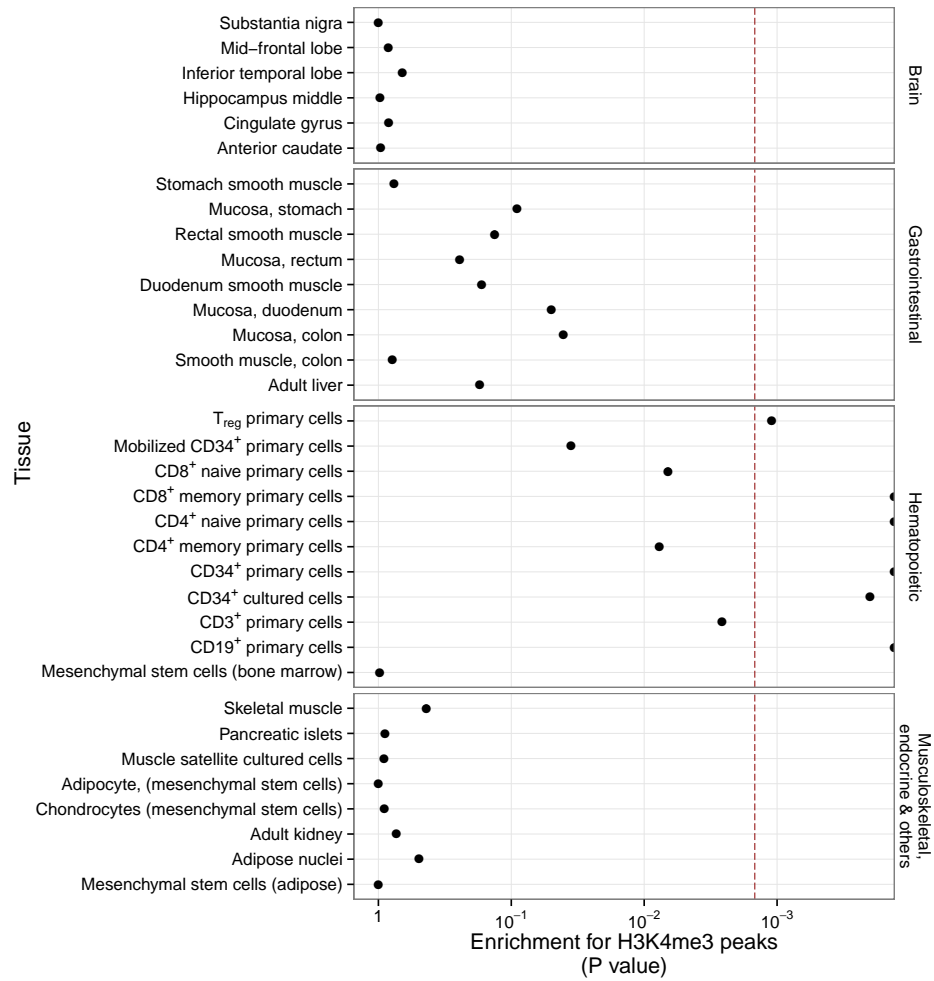
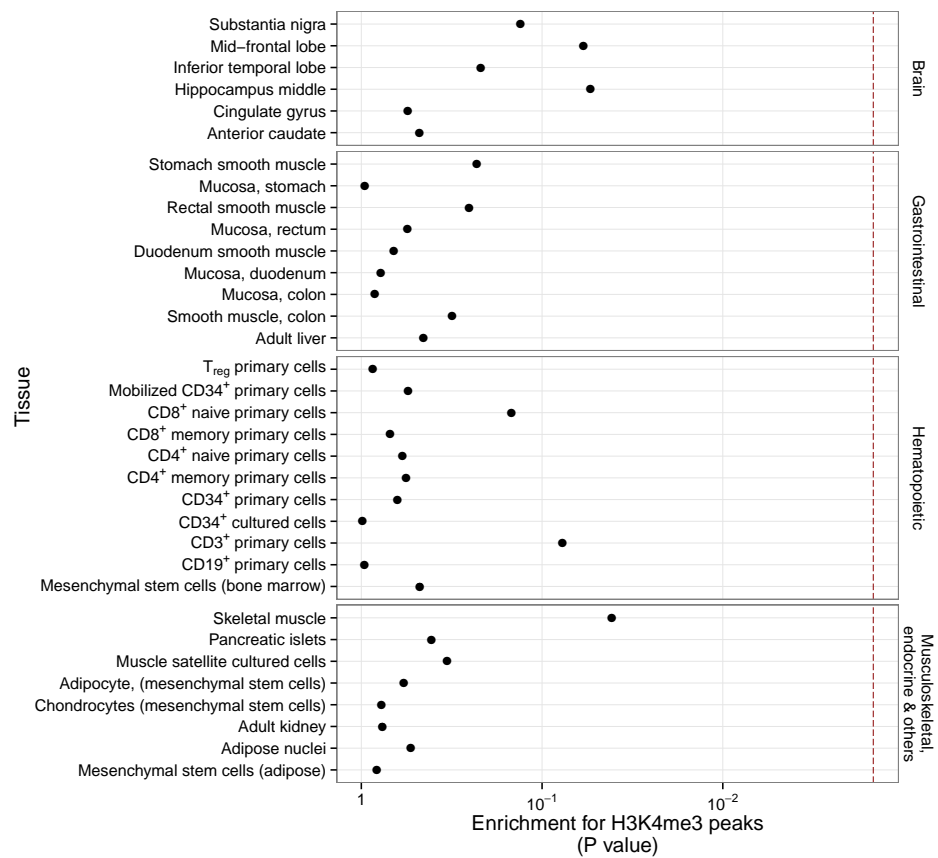


Figure S4: **Tissue specific enrichment of SNPs in transcriptionally active regions** The locations of transcriptional activity can be predicted by chromatin marks, assayed by H3K4me3. Here we show that there is



**Figure S5: Tissue specific enrichment of SNPs in transcriptionally active regions** The locations of transcriptional activity can be predicted by chromatin marks, assayed by H3K4me3. Here we show that there is

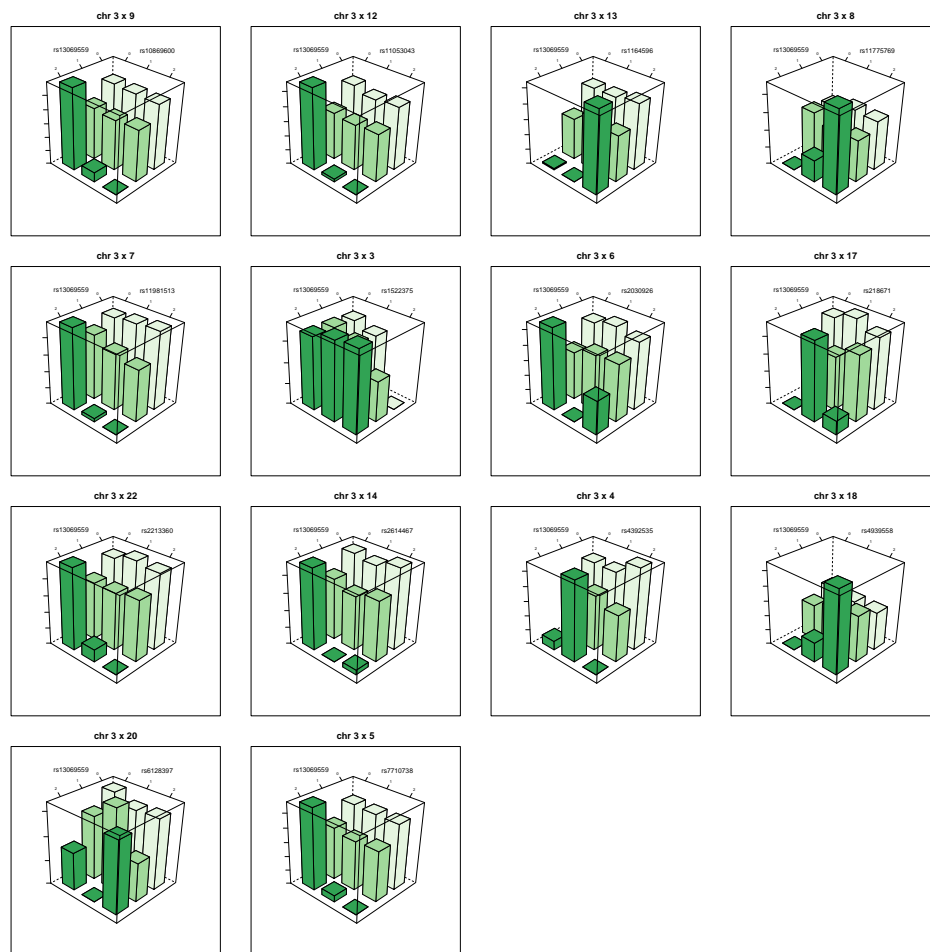
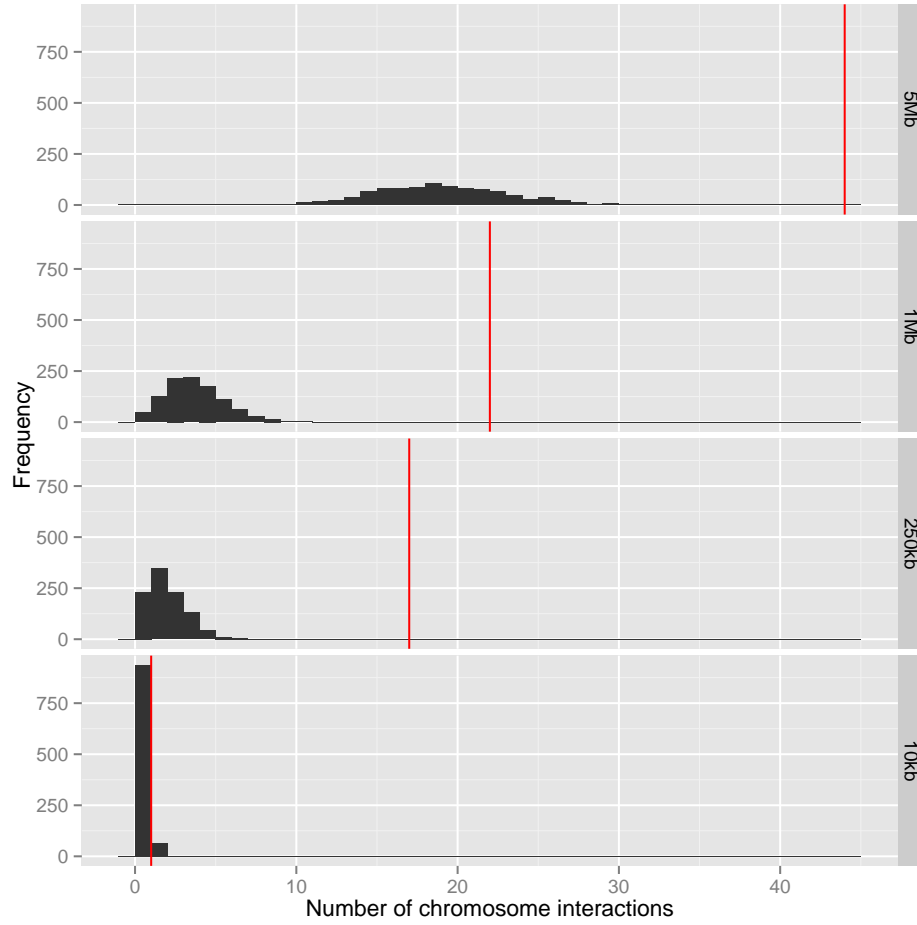


Figure S6: **Genotype-phenotype maps for 14 interactions controlling MBNL1** Each bar represents the mean phenotypic value for individuals in that genotype class. The rs13069559 SNP typically has a *cis*-additive decreasing effect on the expression of MBNL1, but in many of these interactions the *cis* effect is masked when the *trans* SNP is homozygous.



**Figure S7: Number of overlaps between chromosome interactions and epistatic interactions** Interacting chromosome regions may be a possible mechanism underlying epistatic interactions. The number of epistatic interactions within 20kb, 500kb, 2Mb and 10Mb of known chromosome interacting regions are shown by red vertical lines. The histograms represent the null distribution based on random sampling of 10000 datasets for each window size.

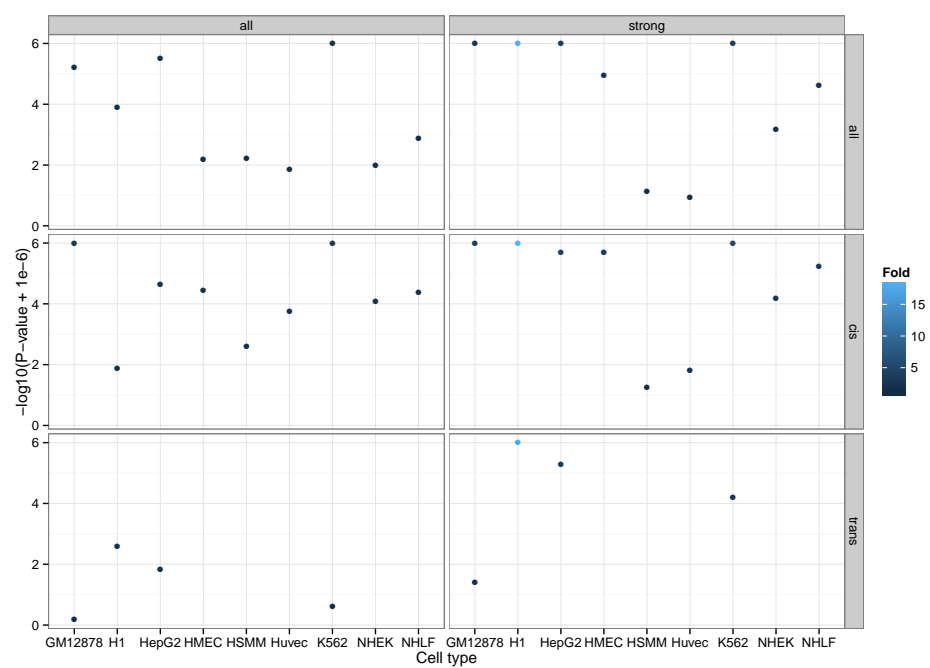


Figure S8: **There is enrichment for enhancer sequences for *cis* and *trans* SNPs**



## 4 References

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