

# Detection and replication of epistasis influencing transcription in humans

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## Abstract

Epistasis is the phenomenon whereby one polymorphism's effect on a trait depends on other polymorphisms present in the genome. The extent to which epistasis influences complex traits<sup>1</sup> and contributes to their variation<sup>2,3</sup> is a fundamental question in evolution and human genetics. Though epistasis has been demonstrated in artificial gene manipulation studies in model organisms,<sup>4,5</sup> and some examples have been reported 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 low incidence in the genetic control of complex traits,<sup>2,3</sup> but an alternative view is that it has previously been too technically challenging 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, many instances of epistasis are found between common single nucleotide polymorphisms (SNPs). In a cohort of 846 individuals with data on 7339 gene expression levels in peripheral blood, we found 501 significant pairwise epistatic interactions between common SNPs acting on the expression levels of 238 genes ( $p < 2.91 \times 10^{-16}$ ). We tested the discovery interactions for replication in two independent data sets.<sup>11,12</sup> Three hundred and forty-five interactions had replication interaction  $p$ -values that were more extreme than the 2.5% confidence interval of the distribution under the null hypothesis of no epistasis, with 30 significant at a conservative  $p < 0.05$  Bonferroni level. There was evidence of functional enrichment for the interacting SNPs, for instance 44 of the genetic interactions are located within 2Mb of regions of known physical chromosome interactions<sup>13</sup> ( $p = 1.8 \times 10^{-10}$ ). Epistatic networks of three SNPs or more influence the expression levels of 129 genes, whereby one *cis*-acting SNP is modulated by several *trans*-acting SNPs. For example MBNL1 is influenced by an additive effect at rs13069559 which itself is masked by *trans*-SNPs on 14 different chromosomes, with nearly identical genotype-phenotype (GP) maps for each *cis-trans* interaction. This study presents the first evidence for multiple instances of epistatic genetic effects emerging from natural genetic variation in humans.

## Main text

In the genetic analysis of complex traits it is usual for SNP effects to be estimated using an additive model where they are assumed to contribute independently and cumulatively to the mean of a trait. This framework has been successful in identifying thousands of associations.<sup>14</sup> But to date, though its contribution to phenotypic variance is frequently the subject of debate,<sup>1-3</sup> there is little empirical exploration of the role that epistasis plays in the architecture of complex traits in humans.<sup>7,8</sup> Outside the prism of human association studies there is 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>15</sup> and speciation.<sup>16</sup>

Methods are now available to overcome the computational problems involved in searching for epistasis, but its detection still remains problematic due to reduced statistical power. For example increased dependence on linkage disequilibrium (LD) between causal SNPs and observed SNPs,<sup>17,18</sup> increased model complexity in fitting interaction terms,<sup>19</sup> and more extreme significance thresholds to account for increased multiple testing<sup>9</sup> all make it more difficult to detect epistasis in comparison to additive effects. Thus, when combined with small genetic effect sizes, as is expected in most complex traits of interest,<sup>14</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>20</sup> another is by analysing traits that are likely to have large effect sizes among common variants. Because our focus was to ascertain the extent to which instances of epistasis occur 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>21</sup> thus many genetic effects are relatively large, maximising the chance at detecting epistasis, should it exist.

In our discovery dataset (Brisbane Systems Genetics Study, BSGS<sup>22</sup>) of 846 individuals genotyped at 528,509 SNPs, we exhaustively tested every pair of SNPs for genetic interactions against each of 7339 expression traits in peripheral blood. After stringent filtering and multiple testing correction (5% significance threshold  $p < 2.91 \times 10^{-16}$ , Methods) we identified 501 putative genetic interactions influencing the expression levels of 238 genes (Supplementary Table ). Of the 501 discovery interactions, 434 had available data and passed filtering (Methods) in two independent replication datasets, Fehrman<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 30 were significant after applying a Bonferroni correction for multiple testing (5% significance threshold  $p < 0.0001$ , Table 1). These significant interactions exhibited remarkable similarity in GP maps between all three datasets (Figure 1).

In addition, using the meta analysis from the replication samples only, we observed that 316 of the remaining 404 discovery SNPs had replication interaction  $p$ -values more extreme than the 2.5% confidence interval of the distribution under the null distribution of no epistatic effects ( $p \ll 1.0 \times 10^{-16}$ , Figure 2 and Supplementary Figure S1). The congruence of the epistatic networks in discovery and replication datasets is shown in Figure 3, demonstrating that these complex genetic patterns are common even across independent datasets. A further replication was attempted using the Centre for Health Discovery and Wellbeing (CHDWB) dataset,<sup>23</sup> but only 20 of the SNP pairs passed filtering because the sample size was small ( $n = 139$ ), and likely due to insufficient power we found no evidence for replication (Supplementary Figure S5). It should be noted that although it is a necessary step to establish the veracity of the signals from the discovery set, replication of epistasis is difficult in practice because

the dependence on LD between observed SNPs and causal variants is up to three orders of magnitude higher than it is for independent additive effects.<sup>17,18</sup> Therefore these results are 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 strategy for its detection. The majority of our discovery interactions comprised of one SNP that was significantly associated with the gene expression level in the discovery dataset, and one SNP that had no previous association<sup>21</sup> (439 out of 501, Methods). Only nine interactions were between SNPs that both had known main effects while 64 were between SNPs that had no known main effects. Additionally, we observed that the largest epistatic variance component for the 501 interactions was equally divided amongst additive  $\times$  additive, additive  $\times$  dominance, dominance  $\times$  additive and dominance  $\times$  dominance at the discovery stage ( $p = 0.22$  for departure from expectation). This is not surprising because the patterns of epistasis used for statistical decomposition are not designed to resemble biological function.<sup>24</sup>

Of the discovery interactions, 47 were *cis-cis* acting (both SNPs were on the same chromosome as the expression gene), 441 were *cis-trans*-acting, and 13 were *trans-trans*-acting. We observed a wide range of significant GP maps (Figure 1) 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>25</sup>) has a *cis* effect at rs13069559 which in turn is controlled by 13 *trans*-SNPs and one *cis*-SNP that each 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 (Supplementary Figure S6). Each of these interactions has evidence for replication in at least one dataset and six are significantly replicated at the Bonferroni level (Supplementary Figure S2). We see similar epistatic networks involving multiple (eight or more) *trans*-acting SNPs for other gene expression levels too, for example TMEM149 (Supplementary Figure S7), NAPRT1 (Supplementary Figure S8), TRAPPC5 (Supplementary Figure S9), and CAST (Supplementary Figure S10). We observed that from pedigree analysis these five gene expression phenotypes had non-additive variance component estimates within the 95th percentile of the 17,994 gene expression phenotypes that were analysed previously<sup>21</sup> (Supplementary Table S2, Methods).

In total the 501 interactions comprised 781 unique SNPs, which we analysed for functional enrichment (Methods). We tested the SNPs for cell-type specific overlap with transcriptionally active chromatin regions, tagged by histone-3-lysine-4,tri-methylation (H3K4me3) chromatin marks, in 34 cell types<sup>26</sup> (Supplementary Figure S4). 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 after adjusting for multiple testing). However *trans*-acting SNPs did not show any tissue specific enrichment ( $p > 0.1$  for all tissues). This difference between *cis* and *trans* SNPs suggests different roles in epistatic in-

teractions where tissue specificity is provided by the *cis* SNPs. There is also enrichment for *cis*-SNPs to be localised in regions with regulatory genomic features as measured by chromatin states<sup>27</sup> (Supplementary Figure S3).

We also demonstrate physical organisation of interacting loci within the cell, suggesting a mechanism by which biological function can lead to epistatic genetic variance. It has been shown that different chromosomal regions spatially colocalise in the cell through chromatin interactions.<sup>13</sup> We cross-referenced our epistatic SNPs with a map of chromosome interacting regions ( $n = 96,139$ ) in K562 blood cell lines<sup>28</sup> (Methods) and found that 44 epistatic interactions mapped to within 2Mb ( $p < 1.8 \times 10^{-10}$ ), (Supplementary Figure S11). Interaction of distant loci may occur through physical proximity in transcriptional factories that organise across different chromosome regions and can regulate transcription of related genes.<sup>29,30</sup>

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 238 gene expression traits with at least one significant interaction given our experiment-wide threshold. How does this compare to the number of traits influenced by additive effects? The BSGS dataset has been previously analysed for additive effects at all expression traits,<sup>22</sup> and if we take all the additive eQTLs that were significant at the epistatic threshold of  $p < 2.91 \times 10^{-16}$  we find that 453 gene expression levels out of the 7339 analysed had at least one significant expression quantitative trait locus (eQTL). Therefore it can be argued that the number of instances of detectable 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 perspective<sup>31</sup> but this is intractable for non-additive variance components. Nevertheless, 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> Taking the additive effects detected in Powell *et al* (2012) at the  $p < 2.91 \times 10^{-16}$  threshold, we calculate that on average they explain 1.73% of the phenotypic variance of each of the 7339 probes. By contrast, the epistatic variance from the interacting SNPs detected in this study on average explain 0.25% of phenotypic variance, approximately seven times lower than the additive variance. There are several caveats to this comparison. Firstly, the ratio of additive to epistatic variance may differ at different effect sizes, and our estimate is determined by the threshold used. Secondly, the power of a 1 *d.f.* test exceeds that of an 8 *d.f.* test. And thirdly, the non-additive variance at causal variants is expected to be underestimated by observed SNPs in comparison to estimates for additive variance, due to differences in the rate of decay of the estimate of the genetic variance of the causal SNPs as LD decreases with the observed SNPs.

Overall, we have demonstrated that it is possible to identify and replicate epistasis in complex traits amongst common human variants, despite the relative contribution of pairwise epistasis to phenotypic variation being small. The

bioinformatic analysis of the significant epistatic loci suggests that there are a large number of possible mechanisms that can lead to non-additive genetic variation. Further research into such epistatic effects may provide a useful framework to understanding molecular mechanisms and complex trait variation in greater detail. With computational techniques and data now widely available the search for epistasis in larger datasets for traits of broader interest is warranted.

## Methods Summary

We searched for pairwise epistasis exhaustively in the BSGS discovery dataset,<sup>22</sup> which comprises 846 individuals who are genotyped at 528,509 autosomal SNPs. Each individual had gene expression levels measured in peripheral blood at 47,323 probes. Only the probes that passed quality control and had significant expression in  $\geq 90\%$  of individuals were used in the analysis (7,339 probes representing 6,158 RefSeq genes). Recent hardware and software<sup>10</sup> advances that use graphics processing units (GPUs) made it possible to perform the  $1.03 \times 10^{15}$  statistical tests to complete this analysis. We used permutation analysis<sup>32</sup> to calculate an experiment-wide significance threshold of  $T_e = 2.91 \times 10^{-16}$  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>18,19</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  $r^2 > 0.1$  and  $D'^2 > 0.1$  were removed to minimise the possibility of haplotype effects. All SNP pairs 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 peripheral blood, the Fehrman 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. A meta analysis on the interaction  $p$ -values from each replication dataset was performed to provide an overall replication statistic for each putative interaction.

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## Tables

Table 1: Epistatic interactions significant at the Bonferroni level in two replication sets

	Gene (chr.)	SNP 1 (chr.)	SNP 2 (chr.)	BSGS <sup>2</sup>	Fehrmann <sup>3</sup>	EGCUT <sup>3</sup>	Meta <sup>4</sup>
1	ADK (10)	rs2395095 (10)	rs10824092 (10)	6.69 <sup>1</sup>	18.33 <sup>1</sup>	21.21 <sup>1</sup>	39.82 <sup>1</sup>
2	ATP13A1 (19)	rs4284750 (19)	rs873870 (19)	5.30	12.18	3.25	14.23
3	C21ORF57 (21)	rs9978658 (21)	rs11701361 (21)	9.42	6.08	16.36	21.67
4	CSTB (21)	rs9979356 (21)	rs3761385 (21)	11.99	25.20	16.72	42.27
5	CTSC (11)	rs7930237 (11)	rs556895 (11)	7.16	18.76	15.06	33.53
6	FN3KRP (17)	rs898095 (17)	rs9892064 (17)	16.16	28.24	29.39	59.95
7	GAA (17)	rs11150847 (17)	rs12602462 (17)	13.91	19.98	12.99	32.60
8	HNRPH1 (5)	rs6894268 (5)	rs4700810 (5)	15.38	8.55	3.01	10.37
9	LAX1 (1)	rs1891432 (1)	rs10900520 (1)	19.16	18.60	11.22	29.24
10	MBNL1 (3)	rs16864367 (3)	rs13079208 (3)	13.49	16.25	24.74	41.56
11	MBNL1 (3)	rs7710738 (5)	rs13069559 (3)	7.92	2.55	7.89	9.28
12	MBNL1 (3)	rs2030926 (6)	rs13069559 (3)	7.10	0.91	5.80	5.53
13	MBNL1 (3)	rs2614467 (14)	rs13069559 (3)	5.74	4.13	2.22	5.30
14	MBNL1 (3)	rs218671 (17)	rs13069559 (3)	7.63	0.62	5.82	5.23
15	MBNL1 (3)	rs11981513 (7)	rs13069559 (3)	7.71	0.43	5.36	4.58
16	MBP (18)	rs8092433 (18)	rs4890876 (18)	5.40	7.06	21.91	28.73
17	NAPRT1 (8)	rs2123758 (8)	rs3889129 (8)	8.45	15.12	16.08	30.77
18	NCL (2)	rs7563453 (2)	rs4973397 (2)	7.31	7.51	6.33	12.70
19	PRMT2 (21)	rs2839372 (21)	rs11701058 (21)	4.81	0.69	4.47	4.06
20	RPL13 (16)	rs352935 (16)	rs2965817 (16)	4.98	3.79	14.41	17.24
21	SNORD14A (11)	rs2634462 (11)	rs6486334 (11)	7.31	13.11	10.96	23.22
22	TMEM149 (19)	rs807491 (19)	rs7254601 (19)	12.16	81.55	45.78	145.78
23	TMEM149 (19)	rs8106959 (19)	rs6926382 (6)	5.80	3.06	8.80	10.72
24	TMEM149 (19)	rs8106959 (19)	rs914940 (1)	6.22	3.36	6.96	9.20
25	TMEM149 (19)	rs8106959 (19)	rs2351458 (4)	7.30	0.04	9.61	8.00
26	TMEM149 (19)	rs8106959 (19)	rs6718480 (2)	8.55	3.31	5.15	7.36
27	TMEM149 (19)	rs8106959 (19)	rs1843357 (8)	6.21	3.72	3.33	6.00
28	TMEM149 (19)	rs8106959 (19)	rs9509428 (13)	9.44	0.10	5.75	4.47
29	TRA2A (7)	rs7776572 (7)	rs11770192 (7)	8.23	3.19	1.89	4.09
30	VASP (19)	rs1264226 (19)	rs2276470 (19)	5.09	0.94	5.14	4.95

<sup>1</sup> –  $\log_{10}$   $p$ -values for 4  $d.f.$  interaction tests

<sup>2</sup> Discovery dataset

<sup>3</sup> Independent replication dataset

<sup>4</sup> Meta analysis of interaction terms between replication datasets only



## Figures

Figure 1: **Replication of GP maps in two independent populations**

The GP maps for each epistatic interaction that is significant at the Bonferroni level in both replication datasets are shown. Each GP map consists of nine tiles where each tile represents the expression level for that two-locus genotype class. Phenotypes are for gene transcript levels (dark coloured tiles = high expression, light coloured tiles = low expression). Columns of GP maps are for each independent dataset. Rows of GP maps are for each of 30 significantly replicated interactions at the Bonferroni level, corresponding to the rows in Table 1. There is a clear trend of the GP maps replicating across all three datasets.

Figure 2: **Q-Q plots of interaction  $p$ -values from replication datasets**

The top panel shows all 434 discovery SNPs that were tested for interactions. Observed  $p$ -values ( $y$ -axis,  $-\log_{10}$  scale) are plotted against the expected  $p$ -values ( $x$ -axis,  $-\log_{10}$  scale). The multiple testing correction threshold for significance following Bonferroni correction is denoted by a dotted line. The bottom panel shows the same data as the top panel but excluding the 30 interactions that were significant at the Bonferroni level in the replication datasets. The shaded grey area represents the 5% confidence interval for the expected distribution of  $p$ -values. Dark blue points represent  $p$ -values that exceed the confidence interval, light blue are within the confidence interval.

Figure 3: **Discovery and replication of epistatic networks**

All 434 putative genetic interactions (edges) with data common to discovery and replication sets is shown, where black nodes represent SNPs and red nodes represent traits (gene expression probes). Three hundred and forty-five interactions had  $p$ -values exceeding the 2.5% confidence interval following meta analysis of the replication data. The remaining 89 interactions that did not replicate are depicted in grey. It is evident that a large proportion of the complex networks identified in the discovery set also exist in independent populations.

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