An alternative explanation for apparent epistasis

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Opening paragraph

Epistasis occurs when the effect of a genetic variant on a trait is dependent on the presence or absence of another variant elsewhere in the genome. It has proven hard to find examples of such “gene-gene” interactions in humans. Recently, the first evidence for widespread epistasis affecting human traits was described. Hemani *et al* detected and replicated many instances of interactions between pairs of variants influencing gene expression levels[1]. Here we sought further replication but used whole genome sequence data to capture more completely the variation around the putatively interacting variants. Using 450 unrelated individuals from the InCHIANTI study we strongly replicated many of the reported pairwise interaction effects. However, in each case, a third variant captured by our sequencing data could explain all of the apparent epistasis. This third variant was often moderately correlated with each of the two putatively interacting variants, despite very low levels of correlation between the original pair. Our results provide an alternative explanation for the apparent epistasis observed for gene expression traits in humans.

Main Text

Epistasis, often referred to as “gene-gene” interaction, has been very hard to detect in humans. Hemani *et al.* recently described examples of apparent epistasis influencing gene expression in humans[1]. Thirty pairs of single nucleotide polymorphisms (SNPs; Table 1 in Hemani *et al.*) interacted to influence the expression of 19 different gene transcripts. These interactions were robust to adjustment for multiple testing and were replicated across two independent studies.

Most of the replicated apparently interacting SNP pairs were associated with gene expression in *cis* and were located close to each other on the same chromosome (all < 520kb). We have previously shown that low levels of correlation due to linkage disequilibrium (LD) between variants can cause apparent allelic heterogeneity at an associated locus[2]. We

therefore hypothesised that low levels of linkage disequilibrium could explain the epistasis observed by Hemani *et al.*[1]

To address this hypothesis we used a combination of whole genome sequence data and whole blood gene expression traits in 450 individuals from the InCHIANTI study. Gene expression levels were measured using a very similar Illumina array (Human HT-12 v3.0) as Hemani *et al.* used for all of their discovery and replication analyses. We used the same analysis software (epiGPU[3]) as Hemani *et al*.[1]

We first replicated the apparent interactions detected and replicated by Hemani *et al* (11 of 17 *cis-cis* pairs and 3 of 11 *cis-trans* pairs with $P<0.05$; **Table 1**). Our lower success rate of replicating the *cis-trans* effects is consistent with their reported smaller effect sizes. We could not analyse two of the gene expression traits because either the probe or one of the SNPs failed quality control in our study. We next identified the most strongly associated individual variant for each of the 19 gene expression traits from our whole genome sequencing analysis (**Table 1**). For 27 out of 28 SNP pairs the individual variant most strongly associated with gene expression in our data was more strongly associated than the 8df full model formed from the pair of SNPs reported in Hemani *et al* (the full model includes all possible additive, dominance and interaction effects between the two SNPs). For all seventeen putatively interacting pairs where both SNPs occurred on the same chromosome our more strongly associated variant was moderately correlated with both of the interacting SNPs (**Table 1**). These correlations occurred despite very low levels of LD between the two SNPs described by Hemani *et al.* (**Table 2**).

We next re-evaluated the evidence for interaction but this time corrected for the presence of our most strongly associated variant. For the examples where both SNPs occurred on the same chromosome, the inclusion of our third variant removed any evidence for interaction (**Table 1**). This included the removal of apparently strong interactions involving *cis* variants for *MBLN1* and *TMEM149*, the two transcripts that account for all of the *cis-trans* interactions. Our results mean that the apparent epistasis reported by Hemani *et al.* is more likely to be due to moderate levels of LD between each of the two SNPs and a single causal allele, rather than epistasis.

Hemani *et al.* attempted to remove haplotype effects by removing pairs of SNPs with pairwise $r^2<0.1$ and D' squared <0.1, but they did not account for multi-locus LD of which there is a substantial amount (**Table 2**). It is possible to have substantial multi-locus LD but no pairwise LD[4]. **Figure 1** provides an example of the haplotype structure for the *ADK* locus, where there is no LD between the two interacting SNPs, but the most associated variant from our study has moderate LD with both of the SNPs.

In summary, using whole genome sequencing and independent data, we have provided an alternative explanation for the findings of Hemani *et al.*[1] and conclude that there remains no compelling evidence for widespread epistasis in humans.

Methods (100 words)

We selected 450 individuals from the InCHIANTI study[2]. Gene expression profiles were captured using Illumina's HumanHT-12 v3.0 BeadChip array as previously described[2]. Whole-genome sequencing was performed at the Beijing Genomics Institute (Shenzhen, China) using the Illumina HiSeq 2000 (median read depth 7X). Reads were processed using the Genome Analysis Toolkit (GATK)[5] pipeline prior to genotype recovery and refinement through within-sample imputation using BEAGLE[6]. Analysis of the 8df model and interaction term was performed using epiGPU[3]. To determine whether the 30 replicated interactions observed by Hemani *et al*[1] were driven by unaccounted variants, we obtained the most strongly sequenced variant in *cis* (1Mb ± probe start site) using MACH2QTL[7] and generated a phenotype of residuals for each expression trait by regressing out the variant. We repeated the epiGPU analysis on the same SNP pairs using the adjusted trait.

Table 1: Results from running pairwise SNP interaction analyses on SNP pairs identified and replicated by Hemani *et al*. and the results observed after conditioning on the most strongly associated *cis* variant identified in the InCHIANTI sequencing study. Data was available for 28 of the 30 interactions reported by Hemani *et al*. Both the full model and interaction associations for the Hemani *et al.* SNPs are completely removed on adjustment for the additive effect of our single most associated variant.

		Hemani *et al.* SNP Pairs Table 1			Two SNPs from Hemani *et al*		Adjusted for IncSeq variant	
cis/ trans	**Gene (chr)**	**SNP1 (chr)**	**SNP2 (chr)**	**IncSeq Variant[§]**	**8DF Full Model P**	**Interaction P**	**8DF Full Model P**	**Interaction P**
cis	*ADK* (10)	rs2395095 (10)	rs10824092 (10)	10:75928933	3.2E-19	9.1E-04	0.99	0.86
cis	*ATP13A1* (19)	rs4284750 (19)	rs873870 (19)	19:19756073	2.1E-05	7.9E-03	0.87	0.64
cis	*C21ORF57* (21)	rs9978658 (21)	rs11701361 (21)	21:47703649	3.8E-05	7.2E-03	0.02	0.43
cis	*CSTB* (21)	rs9979356 (21)	rs3761385 (21)	21:45201832	6.2E-07	8.3E-07	0.98	0.99
cis	*CTSC* (11)	rs7930237 (11)	rs556895 (11)	11:88015717	3.5E-15	5.0E-06	7.0E-08*	0.04
cis	*FN3KRP* (17)	rs898095 (17)	rs9892064 (17)	17:80678628	2.8E-11	2.9E-12	0.07	0.43
cis	*GAA* (17)	rs11150847 (17)	rs12602462 (17)	17:78096086	0.09	0.15	0.22	0.34
cis	*HNRPH1* (5)	rs6894268 (5)	rs4700810 (5)	5:178978883	0.08	0.53	0.36	0.45
cis	*LAX1* (1)	rs1891432 (1)	rs10900520 (1)	1:203747772	8.3E-08	1.6E-04	0.27	0.52
cis	*MBLN1* (3)	rs16864367 (3)	rs13079208 (3)	3:152182577	1.1E-07	2.7E-06	0.41	0.16
trans	*MBLN1* (3)	rs7710738 (5)	rs13069559 (3)	3:152182577	3.1E-05	2.3E-02	0.05	0.02
trans	*MBLN1* (3)	rs2030926 (6)	rs13069559 (3)	3:152182577	2.2E-05	3.2E-02	0.19	0.21
trans	*MBLN1* (3)	rs2614467 (14)	rs13069559 (3)	3:152182577	3.7E-04	0.24	0.47	0.55
trans	*MBLN1* (3)	rs218671 (17)	rs13069559 (3)	3:152182577	1.4E-03	0.90	0.38	0.79
trans	*MBLN1* (3)	rs11981513 (7)	rs13069559 (3)	3:152182577	1.6E-05	1.6E-02	0.11	0.10
cis	*MBP* (18)	rs8092433 (18)	rs4890876 (18)	18:74723459	1.2E-02	0.05	0.67	0.28
cis	*NAPRT1* (8)	rs2123758 (8)	rs3889129 (8)	8:144684215	6.8E-34	6.2E-06	0.40	0.84
cis	*NCL* (2)	rs7563453 (2)	rs4973397 (2)	2:232320581	0.09	0.10	0.85	0.71
cis	*PRMT2* (21)	rs2839372 (21)	rs11701058 (21)	21:47887791	2.6E-15	2.6E-04	0.52	0.30
cis	*SNORD14A* (11)	rs2634462 (11)	rs6486334 (11)	11:17230389	1.7E-05	0.37	0.41	0.17
cis	*TMEM149* (19)	rs807491 (19)	rs7254601 (19)	19:36234489	3.0E-31	2.9E-06	0.46	0.41
trans	*TMEM149* (19)	rs8106959 (19)	rs6926382 (6)	19:36234489	3.2E-43	0.23	0.17	0.53
trans	*TMEM149* (19)	rs8106959 (19)	rs914940 (1)	19:36234489	3.7E-42	0.62	0.39	0.71
trans	*TMEM149* (19)	rs8106959 (19)	rs2351458 (4)	19:36234489	3.5E-42	0.30	0.53	0.46
trans	*TMEM149* (19)	rs8106959 (19)	rs6718480 (2)	19:36234489	6.1E-42	0.44	0.57	0.69
trans	*TMEM149* (19)	rs8106959 (19)	rs1843357 (8)	19:36234489	4.0E-41	0.44	0.91	0.73
trans	*TMEM149* (19)	rs8106959 (19)	rs9509428 (13)	19:36234489	3.3E-42	0.09	0.69	0.39
cis	*VASP* (19)	rs1264226 (19)	rs2276470 (19)	19:46033382	0.12	0.81	0.71	0.56

[§] IncSeq variant: most strongly associated variant with probe levels in *cis* (±1Mb probe start site)

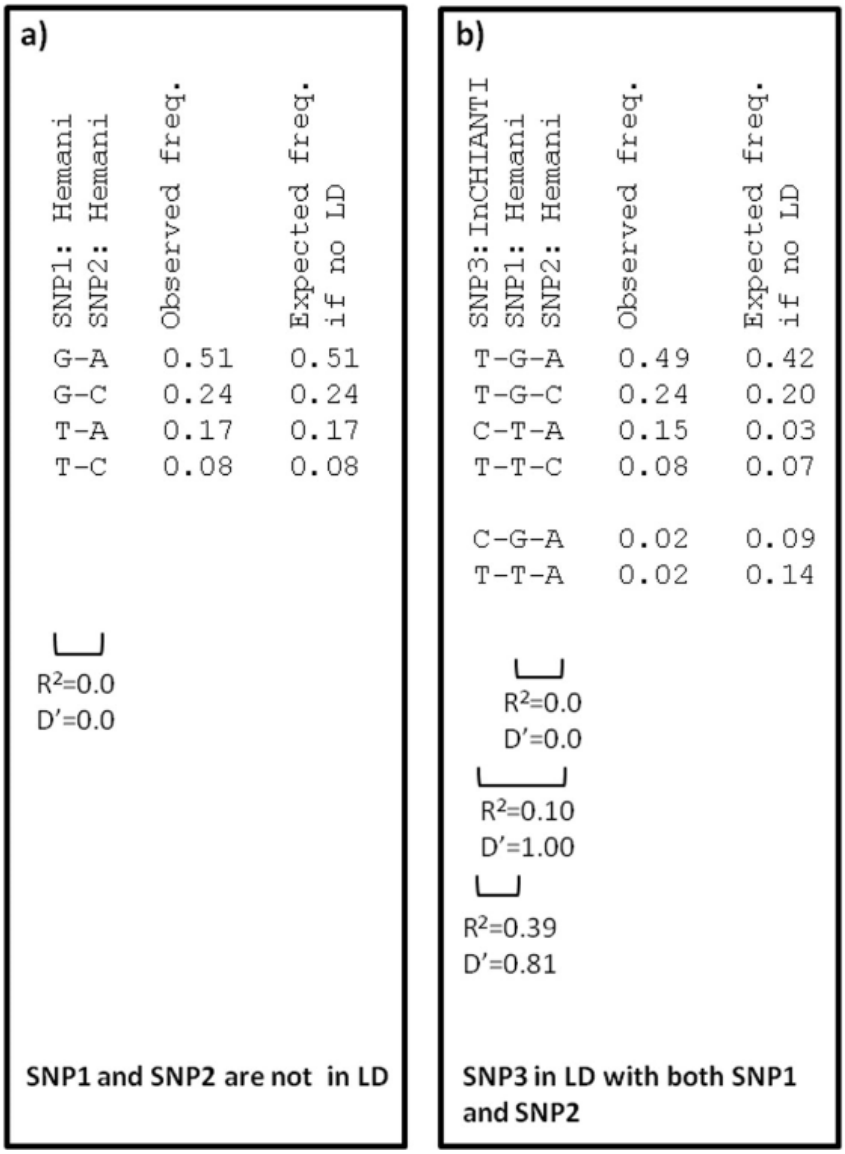
[*] Full 8 D.F Model remains significant (7E-08) but not significantly different from model with all 3 SNPs together in an additive model (P=0.17) suggestive of a second independent additive effect at this locus.

Table 2: Linkage Disequilibrium measures between SNP pairs identified by Hemani *et al.*[1] and the most strongly associated *cis* variant identified in the InCHIANTI sequencing study.

		Hemani *et al.* SNP Pairs Table 1			Linkage Disequilibrium Between Variants		
cis/ trans	Gene (chr)	SNP1 (chr)	SNP2 (chr)	IncSeq Variant[§]	SNP1 - SNP2 r2 / D'	SNP1 - IncSeq r2 / D'	SNP2 - IncSeq r2 / D'
cis	*ADK* (10)	rs2395095 (10)	rs10824092 (10)	10:75928933	0 / 0.01	0.39 / 0.81	0.1 / 1
cis	*ATP13A1* (19)	rs4284750 (19)	rs873870 (19)	19:19756073	0.01 / 0.11	0.07 / 0.9	0.04 / 0.82
cis	*C21ORF57* (21)	rs9978658 (21)	rs11701361 (21)	21:47703649	0.02 / 0.19	0.02 / 0.2	0.02 / 0.21
cis	*CSTB* (21)	rs9979356 (21)	rs3761385 (21)	21:45201832	0.04 / 0.23	0.05 / 0.25	0.14 / 0.38
cis	*CTSC* (11)	rs7930237 (11)	rs556895 (11)	11:88015717	0 / 0.07	0.22 / 0.9	0.11 / 0.94
cis	*FN3KRP* (17)	rs898095 (17)	rs9892064 (17)	17:80678628	0 / 0.04	0.01 / 0.12	0.05 / 0.27
cis	*GAA* (17)	rs11150847 (17)	rs12602462 (17)	17:78096086	0.01 / 0	0.3 / 1	0.11 / 0.94
cis	*HNRPH1* (5)	rs6894268 (5)	rs4700810 (5)	5:178978883	0.02 / 0.23	0.05 / 0.42	0.3 / 0.63
cis	*LAX1* (1)	rs1891432 (1)	rs10900520 (1)	1:203747772	0.03 / 0.23	0.21 / 0.51	0.05 / 0.29
cis	*MBLN1* (3)	rs16864367 (3)	rs13079208 (3)	3:152182577	0.08 / 0.42	0.13 / 0.62	0.06 / 1
trans	*MBLN1* (3)	rs7710738 (5)	rs13069559 (3)	3:152182577	0 / 0.12	0 / 0.18	0.44 / 1
trans	*MBLN1* (3)	rs2030926 (6)	rs13069559 (3)	3:152182577	0 / 0.12	0.01 / 0.38	0.44 / 1
trans	*MBLN1* (3)	rs2614467 (14)	rs13069559 (3)	3:152182577	0 / 0.05	0 / 0.1	0.44 / 1
trans	*MBLN1* (3)	rs218671 (17)	rs13069559 (3)	3:152182577	0 / 0.14	0 / 0.13	0.44 / 1
trans	*MBLN1* (3)	rs11981513 (7)	rs13069559 (3)	3:152182577	0.01 / 0.2	0 / 0.2	0.44 / 1
cis	*MBP* (18)	rs8092433 (18)	rs4890876 (18)	18:74723459	0.04 / 0.22	0.11 / 0.43	0.21 / 0.62
cis	*NAPRT1* (8)	rs2123758 (8)	rs3889129 (8)	8:144684215	0.03 / 0.17	0.4 / 0.96	0.06 / 0.68
cis	*NCL* (2)	rs7563453 (2)	rs4973397 (2)	2:232320581	0.04 / 0.25	0.29 / 0.83	0.16 / 0.76
cis	*PRMT2* (21)	rs2839372 (21)	rs11701058 (21)	21:47887791	0.07 / 0.28	0.01 / 0.11	0.33 / 0.95
cis	*SNORD14A* (11)	rs2634462 (11)	rs6486334 (11)	11:17230389	0 / 0	0.07 / 0.62	0.04 / 0.59
cis	*TMEM149* (19)	rs807491 (19)	rs7254601 (19)	19:36234489	0 / 0.11	0.11 / 0.93	0.51 / 0.9
trans	*TMEM149* (19)	rs8106959 (19)	rs6926382 (6)	19:36234489	0 / 0.09	0.84 / 0.99	0 / 0.04
trans	*TMEM149* (19)	rs8106959 (19)	rs914940 (1)	19:36234489	0 / 0	0.84 / 0.99	0 / 0.04
trans	*TMEM149* (19)	rs8106959 (19)	rs2351458 (4)	19:36234489	0 / 0.09	0.84 / 0.99	0.01 / 0.13
trans	*TMEM149* (19)	rs8106959 (19)	rs6718480 (2)	19:36234489	0 / 0.06	0.84 / 0.99	0 / 0.04
trans	*TMEM149* (19)	rs8106959 (19)	rs1843357 (8)	19:36234489	0 / 0.08	0.84 / 0.99	0 / 0.03
trans	*TMEM149* (19)	rs8106959 (19)	rs9509428 (13)	19:36234489	0 / 0.06	0.84 / 0.99	0 / 0.1
cis	*VASP* (19)	rs1264226 (19)	rs2276470 (19)	19:46033382	0.01 / 0.12	0.05 / 0.47	0.1 / 0.57

[§] IncSeq variant: most strongly associated variant with probe levels in *cis* (±1Mb probe start site)

Figure 1: Haplotype and linkage disequilibrium structure at the ADK locus of a) two proposed epistatic SNPs from Hemani *et al.* and b) when adding a third SNP captured by sequencing in 450 Italian individuals. The two "epistatic" SNPs form all four of the possible haplotypes. When adding the third SNP no new haplotypes are formed >2.4% frequency. Haplotypes estimated using Haploview[8].



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