Response to: An alternative explanation for apparent epistasis

Hemani *et al*.

We thank Wood *et al*. for their interesting observations and although their proposed mechanism does not explain all our reported results, we acknowledge that alternative mechanisms could be behind the observation of epistatic signals. Although we replicate our results in large, independent samples, 19/30 of our reported interactions (Table 1 in [[1](#_ENREF_1)]), Wood *et al*. do not replicate in the InCHIANTI dataset (N=450) at a type-I error rate of 0.05/30=0.002, including none of our reported *cis-trans* interactions. Having insufficient data to replicate the discovery interactions makes it problematic to draw firm conclusions on the reported *cis-trans* effects.

Applying their method in our discovery and replication datasets [[1](#_ENREF_1)] does not completely abrogate the statistical evidence for epistasis. Specifically, the meta-analysis of these results shows that weaker interaction effects remain for 24/26 epistasis pairs after correcting for effects of the IncSeq SNP (**Table 1**). For the remaining two pairs (at CSTB and LAX1) we cannot rule out a haplotype effect such as postulated by Wood *et al*. and this may indeed be a more parsimonious explanation for these two pairs. Haplotype effects are known to be confounding factors in *cis-cis* interactions, as stated in Hemani *et al*. The remaining results may remain significant due to imperfect imputation of the IncSeq SNP (though imputation *r*2 is high), and we acknowledge that the presence of imperfectly tagged *cis* SNPs with large additive effects could lead to inflation of the F-statistic for epistatic interactions due to violations of normality assumptions.

For 11 of the *cis*-*cis* pairs that were replicated by Wood *et al*. there is evidence for additional *cis*-genetic variation to that explained by the IncSeq SNPs [[2](#_ENREF_2)]. Hence the IncSeq SNPs are not the only (causal) variants in *cis* and therefore the additive effect of the IncSeq SNPs may contain additive effects of additional variants. Furthermore, these probes are within the 95th percentile of non-additive genetic variation estimated using a pedigree-based method that is completely orthogonal to SNP based methods [[3](#_ENREF_3)] (**Table 2**).

Finally, we note that we did not report that epistasis was ‘widespread’ and in fact pointed out that for gene expression additive genetic variation explains much more of the total genetic variation than non-additive variation [[1](#_ENREF_1),[3](#_ENREF_3)].

1. Hemani G, Shakhbazov K, Westra H, Esko T, Henders AK, et al. (2014) Detection and replication of epistasis influencing transcription in humans. Nature In Press.

2. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, et al. (2013) Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 45: 1238-U1195.

3. Powell JE, Henders AK, McRae AF, Kim J, Hemani G, et al. (2013) Congruence of Additive and Non-Additive Effects on Gene Expression Estimated from Pedigree and SNP Data. PLoS Genet 9.

4. Powell JE, Henders AK, McRae AF, Caracella A, Smith S, et al. (2012) The Brisbane Systems Genetics Study: genetical genomics meets complex trait genetics. PLoS One 7: e35430.

**Table 1 |** Meta-analysis of results from discovery and replication cohorts. The analysis followed that of Wood *et al*. In each cohort the effect of the imputed IncSeq SNP was regressed against the probe levels and the residuals used as an adjusted phenotype. Interaction effects were estimated following Hemani et al. and the results combined using Fisher’s method (see Hemani et al.) using results from all three datasets or just the two replication datasets. Two IncSeq SNPs were either not in the 1000 Genomes reference panel or did not pass imputation quality control. Remaining imputed IncSeq SNPs had imputation accuracy *R2* > 0.98 in the Brisbane Systems Genetics Study (BSGS). Of the remaining 26, 24 had interaction *p* values < 0.05/26 = 1.9e-3.

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| ***cis/***  ***trans*** | **Gene (chr)** | **SNP1 (chr)** | **SNP2 (chr)** | **IncSeq**  **SNP from imputed data** | **Interaction**  **-log10 P value (three studies)** | **Interaction**  **–log10 P value (two studies)** |
| *cis* | *ADK* (10) | rs2395095 (10) | rs10824092 (10) | rs67594352 | 3.25 | 2.9 |
| *cis* | *ATP13A1* (19) | rs4284750 (19) | rs873870 (19) | NA | NA | NA |
| *cis* | *C21ORF57* (21) | rs9978658 (21) | rs11701361 (21) | rs11702450 | 6.62 | 5.57 |
| *cis* | *CSTB* (21) | rs9979356 (21) | rs3761385 (21) | rs35285321 | 1.64 | 1.63 |
| *cis* | *CTSC* (11) | rs7930237 (11) | rs556895 (11) | rs56375235 | 10.53 | 7.88 |
| *cis* | *FN3KRP* (17) | rs898095 (17) | rs9892064 (17) | NA | NA | NA |
| *cis* | *GAA* (17) | rs11150847 (17) | rs12602462 (17) | rs4889970 | 11.85 | 8.29 |
| *cis* | *HNRPH1* (5) | rs6894268 (5) | rs4700810 (5) | rs10078796 | 10.82 | 4.91 |
| *cis* | *LAX1* (1) | rs1891432 (1) | rs10900520 (1) | rs2185079 | 1.01 | 1 |
| *cis* | *MBLN1* (3) | rs16864367 (3) | rs13079208 (3) | rs67903230 | 4.19 | 3.23 |
| *trans* | *MBLN1* (3) | rs7710738 (5) | rs13069559 (3) | rs67903230 | 3.42 | 2.97 |
| *trans* | *MBLN1* (3) | rs2030926 (6) | rs13069559 (3) | rs67903230 | 5.31 | 3.96 |
| *trans* | *MBLN1* (3) | rs2614467 (14) | rs13069559 (3) | rs67903230 | 3.12 | 2.88 |
| *trans* | *MBLN1* (3) | rs218671 (17) | rs13069559 (3) | rs67903230 | 4.85 | 2.84 |
| *trans* | *MBLN1* (3) | rs11981513 (7) | rs13069559 (3) | rs67903230 | 6.49 | 5.75 |
| *cis* | *MBP* (18) | rs8092433 (18) | rs4890876 (18) | rs470929 | 4.08 | 3.27 |
| *cis* | *NAPRT1* (8) | rs2123758 (8) | rs3889129 (8) | rs10093709 | 4.07 | 2.95 |
| *cis* | *NCL* (2) | rs7563453 (2) | rs4973397 (2) | rs13019380 | 3.48 | 3.24 |
| *cis* | *PRMT2* (21) | rs2839372 (21) | rs11701058 (21) | rs4819255 | 15.80 | 12.16 |
| *cis* | *SNORD14A* (11) | rs2634462 (11) | rs6486334 (11) | rs2354863 | 5.01 | 3.66 |
| *cis* | *TMEM149* (19) | rs807491 (19) | rs7254601 (19) | rs28656784 | 4.82 | 3.57 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs6926382 (6) | rs28656784 | 3.14 | 2.91 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs914940 (1) | rs28656784 | 3.47 | 3.12 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs2351458 (4) | rs28656784 | 4.77 | 4.01 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs6718480 (2) | rs28656784 | 4.86 | 3.69 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs1843357 (8) | rs28656784 | 3.34 | 3.14 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs9509428 (13) | rs28656784 | 3.06 | 2.73 |
| *cis* | *VASP* (19) | rs1264226 (19) | rs2276470 (19) | rs4803827 | 4.41 | 3.27 |

**Table 2 |** Correlation coefficients are calculated between relative pairs in BSGS [[4](#_ENREF_4)]. PP = parent-parent, PO = parent-offspring, DZ = dizygotic twins, SIB = Sibling pairs not including DZ and MZ twins, MA = monozygotic twins. Estimates of additive (*h2*) and non-additive (*d2*) variance components estimated from pedigree data [[3](#_ENREF_3)]. All probes are within the top 90th percentile of *h2* estimates and the 95th percentile of *d2* (from 17,994 probes).

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| **ILMN\_GENE** | **PROBE\_ID** | **PP** | **PO** | **DZ** | **SIB** | **MZ** | ***h2*** | ***d2*** |
| ADK | ILMN\_2358626 | 0.01 | 0.14 | 0.12 | 0.09 | 0.38 | 0.41 | 0.12 |
| ATP13A1 | ILMN\_2134224 | -0.02 | 0.16 | 0.14 | 0.20 | 0.61 | 0.67 | 0.16 |
| C21ORF57 | ILMN\_1795836 | -0.02 | 0.15 | 0.17 | 0.23 | 0.47 | 0.51 | 0.08 |
| CSTB | ILMN\_1761797 | -0.06 | 0.16 | 0.15 | 0.17 | 0.30 | 0.25 | 0.04 |
| CTSC | ILMN\_2242463 | 0.12 | 0.14 | 0.20 | 0.16 | 0.37 | 0.27 | 0.08 |
| FN3KRP | ILMN\_1652333 | -0.07 | 0.17 | 0.14 | 0.21 | 0.43 | 0.31 | 0.11 |
| GAA | ILMN\_2410783 | -0.05 | 0.16 | 0.14 | 0.13 | 0.39 | 0.39 | 0.06 |
| HNRPH1 | ILMN\_2101920 | 0.01 | 0.15 | 0.12 | 0.13 | 0.24 | 0.17 | 0.05 |
| LAX1 | ILMN\_1769782 | -0.06 | 0.14 | 0.17 | 0.19 | 0.36 | 0.27 | 0.04 |
| MBNL1 | ILMN\_2313158 | 0.02 | 0.18 | 0.16 | 0.18 | 0.42 | 0.18 | 0.11 |
| NAPRT1 | ILMN\_1710752 | -0.06 | 0.19 | 0.21 | 0.28 | 0.51 | 0.37 | 0.14 |
| NCL | ILMN\_2121437 | -0.02 | 0.14 | 0.18 | 0.14 | 0.40 | 0.31 | 0.08 |
| PRMT2 | ILMN\_1675038 | -0.04 | 0.20 | 0.19 | 0.18 | 0.40 | 0.34 | 0.06 |
| SNORD14A | ILMN\_1799381 | 0.03 | 0.17 | 0.14 | 0.13 | 0.52 | 0.43 | 0.14 |
| TMEM149 | ILMN\_1786426 | 0.06 | 0.27 | 0.23 | 0.17 | 0.49 | 0.41 | 0.09 |
| VASP | ILMN\_1743646 | 0.00 | 0.14 | 0.27 | 0.18 | 0.52 | 0.38 | 0.13 |