Response to: An alternative explanation for apparent epistasis

Hemani *et al*.

We thank Wood *et al*. for their interesting observations but we do not believe that their overall conclusions are consistent with the results presented. First, although we replicate our results in large, independent samples, they do not replicate 19/30 of our reported interactions (Table 1 in [[1](#_ENREF_1)]) 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. Despite having insufficient data to draw conclusions on the *cis-trans* effects, Wood *et al.* claim that this alternative explanation implies that there remains ‘no compelling evidence for widespread epistasis in humans’. They provide no plausible mechanism whereby the *cis*-acting IncSeq SNP could explain *cis-trans* interactions.

Second, applying their method in our discovery and replication datasets [[1](#_ENREF_1)] fails to abrogate the statistical evidence for epistasis. Specifically, the meta-analysis of these results shows that interaction effects remain for 24/26 epistasis pairs after correcting for effects of the IncSeq SNP (**Table 1**). For the remaining two pairs 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*.

Third, Wood *et al*. ignore the possibility that the IncSeq SNP is either one of the epistatic causal loci, or in higher LD with the causal loci than the genotyped epistatic SNP and assume that a direct comparison of the interaction *p*-value before and after linear adjustment of the IncSeq SNP provides evidence for their alternative explanation. This is analogous to fine mapping and removing effects of one of the fine mapped loci in the epistatic SNP pair before re-testing the original pair.

Fourth, 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**).

Fifth, there is evidence of interaction variation for pairs of SNPs that include the IncSeq SNPs themselves. Due to lower minor allele frequencies of the IncSeq SNPs many of the pairwise genotype classes are missing, meaning epistatic effects cannot be tested between well-imputed IncSeq SNP and genotyped SNPs in our discovery data. However, in 3/4 pairs for which epistatic effects can be tested there is evidence for interaction variation between the imputed IncSeq SNP and the SNP from the original pair that was in least LD with it (**Table 3**).

Finally, 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. Of the remaining 26, 24 had interaction *p* values < than 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 |

**Table 3 |** Epistatic effects between the IncSeq SNP and the genotyped SNP with the lowest LD in BSGS data. IncSeq SNPs were imputed (imputation quality score > 0.99) against the 1000 Genomes reference panel [ref]. There were only 4 pairs that had sufficient data (all 9 genotype classes and a minimum genotype class size of 5 individuals) existing between the IncSeq SNP and corresponding original epistasis SNP with the lowest LD with the IncSeq SNP (denoted with \*). Of these one is CSTB that shows no interaction effect. The remaining three have strongly significant effects, and explain more genetic variance than the original interactions in two cases.

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|  |  |  |  |  | Original analysis (SNP1 and SNP2) Hemani et al. | | | | Analysis between IncSeq SNP and \* original SNP | | | |
| Gene | Probe | Original epistatic SNP1 | Original epistatic SNP2 | IncSeq SNP rs id | 4df P value | 8df P value | 8df R2 | 4df R2 | 4df P value | 8df P value | 8df R2 | 4df R2 |
| CSTB | ILMN\_1761797 | rs9979356\* | rs3761385 | rs35285321 | 12.0 | 17.2 | 0.1 | 0.07 | 0.8 | 25.5 | 0.14 | 0.01 |
| HNRPH1 | ILMN\_2101920 | rs6894268\* | rs4700810 | rs10078796 | 15.4 | 17.1 | 0.1 | 0.08 | 9.6 | 30.8 | 0.16 | 0.06 |
| MBP | ILMN\_2398939 | rs8092433\* | rs4890876 | rs470929 | 5.4 | 16.9 | 0.1 | 0.03 | 6.5 | 37.1 | 0.19 | 0.04 |
| VASP | ILMN\_1743646 | rs1264226\* | rs2276470 | rs4803827 | 5.1 | 15.6 | 0.1 | 0.03 | 7.9 | 81.9 | 0.32 | 0.05 |