Response to: An alternative explanation for apparent epistasis

Hemani *et al*.

Though we replicate our results in large, independent samples, Wood *et al*. do not replicate 19/30 of our reported interactions (Table 1 in [[1](#_ENREF_1)]) in the relatively small 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 make any conclusion 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’.

To test these claims we conducted their analysis in our discovery and replication datasets [[1](#_ENREF_1)]. The meta-analysis of these results shows that interaction effects remain for 24/26 epistasis pairs after correcting for effects of the IncSNP (**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*.

Wood *et al*. assume that a direct comparison of the interaction *p*-value before and after linear adjustment of the IncSNP provides evidence for an alternative explanation. For a single SNP-trait association, this is analogous to adjusting the trait for a newly identified SNP in LD with the associated SNP and then concluding that the original SNP is not associated with the trait after all. The linear adjustment in both examples soaks up variation, which may include interaction variation in the former

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 IncSNPs [[2](#_ENREF_2)]. Hence the IncSNPs are not the only (causal) variants in *cis* and therefore the additive effect of the IncSNPs 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 orthogonal to SNP based methods [[3](#_ENREF_3)] (**Table 2**).

Due to lower minor allele frequencies of the IncSNPs many of the pairwise genotype classes are missing, meaning epistatic effects cannot be tested between well-imputed IncSNP 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 IncSNP and the SNP from the original pair that was in least LD with it (**Table 3**).

When the following lines of investigation are combined: (i) applying their method fails to abrogate the statistical evidence for epistasis, (ii) the non-replication of the majority of our reported results (including no evidence for any of the *cis-trans* interactions), (iii) absence of a plausible mechanism whereby the *cis*-acting IncSNP could explain *cis-trans* interactions, (iv) evidence of additional additive variation in *cis*, (v) evidence of interaction variance from orthogonal pedigree approach and (vi) evidence of interaction variation for pairs of SNPs that include the IncSNPs themselves, we do not believe that the conclusions drawn by Wood *et al*. are supported by the results presented. Finally, we did not report that epistasis was ‘widespread’ and in fact pointed out that 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.). Two IncSeq SNPs were either not in the 1000 Genomes reference panel or did not pass imputation quality control. 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**  **Variant from imputed data** | **Interaction**  **P value** |
| *cis* | *ADK* (10) | rs2395095 (10) | rs10824092 (10) | rs67594352 | 5.7e-4 |
| *cis* | *ATP13A1* (19) | rs4284750 (19) | rs873870 (19) | NA | NA |
| *cis* | *C21ORF57* (21) | rs9978658 (21) | rs11701361 (21) | rs11702450 | 2.4e-7 |
| *cis* | *CSTB* (21) | rs9979356 (21) | rs3761385 (21) | rs35285321 | 2.3e-2 |
| *cis* | *CTSC* (11) | rs7930237 (11) | rs556895 (11) | rs56375235 | 2.97e-11 |
| *cis* | *FN3KRP* (17) | rs898095 (17) | rs9892064 (17) | NA | NA |
| *cis* | *GAA* (17) | rs11150847 (17) | rs12602462 (17) | rs4889970 | 1.4e-12 |
| *cis* | *HNRPH1* (5) | rs6894268 (5) | rs4700810 (5) | rs10078796 | 1.5e-11 |
| *cis* | *LAX1* (1) | rs1891432 (1) | rs10900520 (1) | rs2185079 | 1.0e-1 |
| *cis* | *MBLN1* (3) | rs16864367 (3) | rs13079208 (3) | rs67903230 | 6.5e-5 |
| *trans* | *MBLN1* (3) | rs7710738 (5) | rs13069559 (3) | rs67903230 | 3.8e-4 |
| *trans* | *MBLN1* (3) | rs2030926 (6) | rs13069559 (3) | rs67903230 | 4.9e-6 |
| *trans* | *MBLN1* (3) | rs2614467 (14) | rs13069559 (3) | rs67903230 | 7.7e-4 |
| *trans* | *MBLN1* (3) | rs218671 (17) | rs13069559 (3) | rs67903230 | 1.4e-5 |
| *trans* | *MBLN1* (3) | rs11981513 (7) | rs13069559 (3) | rs67903230 | 3.3e-7 |
| *cis* | *MBP* (18) | rs8092433 (18) | rs4890876 (18) | rs470929 | 8.3e-5 |
| *cis* | *NAPRT1* (8) | rs2123758 (8) | rs3889129 (8) | rs10093709 | 8.4e-4 |
| *cis* | *NCL* (2) | rs7563453 (2) | rs4973397 (2) | rs13019380 | 3.3e-4 |
| *cis* | *PRMT2* (21) | rs2839372 (21) | rs11701058 (21) | rs4819255 | 1.6e-16 |
| *cis* | *SNORD14A* (11) | rs2634462 (11) | rs6486334 (11) | rs2354863 | 1.1e-5 |
| *cis* | *TMEM149* (19) | rs807491 (19) | rs7254601 (19) | rs28656784 | 1.5e-4 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs6926382 (6) | rs28656784 | 7.3e-4 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs914940 (1) | rs28656784 | 3.4e-4 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs2351458 (4) | rs28656784 | 1.7e-5 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs6718480 (2) | rs28656784 | 1.4e-5 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs1843357 (8) | rs28656784 | 4.6e-4 |
| *trans* | *TMEM149* (19) | rs8106959 (19) | rs9509428 (13) | rs28656784 | 8.8e-4 |
| *cis* | *VASP* (19) | rs1264226 (19) | rs2276470 (19) | rs4803827 | 3.9e-5 |

**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 |