Response to: An alternative explanation for apparent epistasis

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. For *cis-cis* pairs that do replicate, Wood *et al*. report sequenced SNPs (IncSeq SNPs) in *cis* that explain a large proportion of phenotypic variation when fitted singly and additively, and that after a linear (additive) adjustment for the IncSeq SNPs in the InCHIANTI dataset there is no remaining statistically significant interaction variation explained by the original *cis-cis* SNP pairs. Wood *et al*. conclude that additive effects of ungenotyped *cis*-eSNPs created spurious interaction variation due to the confounding correlation between the pair of genotyped SNPs and the ungenotyped *cis*-eSNP. 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 claims made by Wood el al. we conducted their analysis in our discovery and replication datasets [[1](#_ENREF_1)]. The meta-analysis of these results (total n=2,973) 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*.

For 11 of the *cis*-*cis* pairs that were replicated by Wood *et al*. there is strong evidence for additional genetic variation over and above the additive variation explained by the IncSeq SNPs, from SNP association in *cis* (**Table 2**). Hence the IncSeq SNPs are not the only (causal) variants in *cis* for these transcripts and therefore the additive effect of the IncSeq SNPs may contain additive effects of additional variants in *cis*. 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 [[2](#_ENREF_2)] (**Table 3**).

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 4**).

When the following lines of investigation are combined: (i) applying their method of fitting the IncSNP in the discovery and replication cohorts fails to abrogate the statistical evidence for epistasis, (ii) the non-replication of the majority of our reported results due to small sample size in the InCHIANTI data (including no evidence presented 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*, and (v) evidence of interaction variation for pairs of SNPs that include the IncSeq SNPs 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 for gene expression, additive genetic variation explains much more of the total genetic variation than non-additive variation [[1](#_ENREF_1),[2](#_ENREF_2)].

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. 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.

3. 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 |** Details of additive SNP effects at the *cis*-locus for each probe. SNP effects are estimated using a conditional analysis, initially fitting the IncSeq SNP, followed by the additionally identified SNPs. For each probe the conditional analysis was run until no additional significant effects were identified.

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| **ILMN\_GENE** | **PROBE\_ID** | **IncSeq SNP** | **IncSeq P value** | **Conditionally associated SNPs** | **Total R2 of conditional SNPs** |  |
| ADK | ILMN\_2358626 | rs67594352 |  |  |  |  |
| ATP13A1 | ILMN\_2134224 | / | / |  |  |  |
| C21ORF57 | ILMN\_1795836 | rs11702450 |  |  |  |  |
| CSTB | ILMN\_1761797 | rs35285321 |  |  |  |  |
| CTSC | ILMN\_2242463 | rs56375235 |  |  |  |  |
| FN3KRP | ILMN\_1652333 | / | / |  |  |  |
| GAA | ILMN\_2410783 | rs4889970 |  |  |  |  |
| HNRPH1 | ILMN\_2101920 | rs10078796 |  |  |  |  |
| LAX1 | ILMN\_1769782 | rs2185079 |  |  |  |  |
| MBNL1 | ILMN\_2313158 | rs67903230 |  |  |  |  |
| MBP |  | rs470929 |  |  |  |  |
| NAPRT1 | ILMN\_1710752 | rs10093709 |  |  |  |  |
| NCL | ILMN\_2121437 | rs13019380 |  |  |  |  |
| PRMT2 | ILMN\_1675038 | rs4819255 |  |  |  |  |
| SNORD14A | ILMN\_1799381 | rs2354863 |  |  |  |  |
| TMEM149 | ILMN\_1786426 | rs28656784 |  |  |  |  |
| VASP | ILMN\_1743646 | rs4803827 |  |  |  |  |

**Table 3 |** Correlation coefficients are calculated between relative pairs in BSGS [[3](#_ENREF_3)]. 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 [[2](#_ENREF_2)]. 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 4 |** 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 |