[AUTHOR: When you receive the PDF proofs, please check that the display items are as follows (doi:10.1038/nature13692): Figs 0 (black & white); 0 (colour); Tables: 2; Boxes: None. Please check all figures (and tables, if any) very carefully as they have been re-labelled, re-sized and adjusted to Nature’s style. Please check the title and the first paragraph with care, as they may have been re-written to aid accessibility for non-specialist readers. Please check email address and Competing Interests statement. Please check the symbols for addresses with care, and ensure that each address has a zip/postcode. Where a reference citation could be misread as an index, it has been set on the line, not as a superscript. Please also note any queries embedded in the reference list. Genetic material is set in italics, but gene products are set upright. Single-letter variables are set in italics (but not their subscripts unless these are also variables). We do not use italics for emphasis. Please check that italicization is correct throughout. Please ensure that any error bars in the figures are defined in the figure legends.]

Hemani *et al*. reply

**replying to** A. R. Wood *et al. Nature* **5xx,** http://dx.doi.org/10.1038/nature13692 (2014).

We thank Wood *et al.*1 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 ref. 2), Wood *et al.*1 do not replicate in the InCHIANTI data set (*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 data sets2 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.*1 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.*2 The remaining results may remain significant owing to imperfect imputation of the IncSeq SNP (although 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 owing to violations of normality assumptions.

For 11 of the *cis*–*cis* pairs that were replicated by Wood *et al.*1 there is evidence for additional *cis*-genetic variation to that explained by the IncSeq SNPs3. 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 methods4 (Table 2).

Finally, we note that we did not report that epistasis was widespread and pointed out that for gene expression additive genetic variation explains much more of the total genetic variation than non-additive variation2,4.

**Table 1** Meta-analysis of results from discovery and replication cohorts

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***Cis/trans*** | **Gene (chr)** | **SNP1 (chr)** | **SNP2 (chr)** | **IncSeq**  **SNP from imputed data** | **Interaction log(*P* value) (three studies)** | **Interaction –log(*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 |

The analysis followed that of Wood *et al.*1. 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.*2 and the results combined using Fisher’s method (see Hemani *et al.*2) using results from all three data sets or just the two replication data sets. 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 *r*2 > 0.98 in the Brisbane Systems Genetics Study (BSGS). Of the remaining 26, 24 had interaction *P* values < 0.05/26 = 1.9 × 103.

**Table 2** Correlation coefficients are calculated between relative pairs in BSGS5

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ILMN\_GENE** | **PROBE\_ID** | **PP** | **PO** | **DZ** | **SIB** | **MZ** | ***h*2** | ***d*2** |
| *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 |

PP, parent–parent; PO, parent–offspring; DZ, dizygotic twins; SIB, sibling pairs not including DZ and MZ twins; MA, monozygotic twins. Estimates of additive (*h*2) and non-additive (*d*2) variance components estimated from pedigree data4. All probes are within the top 90th percentile of *h*2 estimates and the 95th percentile of *d*2 (from 17,994 probes).

*TMEM149* and *C21ORF57* are also known as *IGFLR1* and *YBEY*, respectively**[Author: HUGO symbols OK? - yes]**.

Gibran Hemani1,2, Konstantin Shakhbazov1,2, Harm-Jan Westra3, Tonu Esko4,5,6, Anjali K. Henders7, Allan F. McRae1,2, Jian Yang1, Greg Gibson8, Nicholas G. Martin7, Andres Metspalu4, Lude Franke3, Grant W. Montgomery7\*, Peter M. Visscher1,2 & Joseph E. Powell1,2

1Queensland Brain Institute, University of Queensland, Brisbane, Queensland 4072, Australia.

email: g.hemani@uq.edu.au

2University of Queensland Diamantina Institute, University of Queensland, Princess Alexandra Hospital, Brisbane, Queensland 4072, Australia.

3Department of Genetics, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands.

4Estonian Genome Center, University of Tartu, Tartu, 51010, Estonia.

5Medical and Population Genetics, Broad Institute, Cambridge, Massachusetts 02142, USA.

6Divisions of Endocrinology, Children's Hospital, Boston, Massachusetts 02115, USA.

7Queensland Institute of Medical Research, Brisbane, Queensland 4006, Australia.

8School of Biology and Centre for Integrative Genomics, Georgia Institute of Technology, Atlanta, Georgia 30332, USA.

<jrn>1. Wood, A. R. *et al.* Another explanation for apparent epistasis. Nature 5xx**,** http://dx.doi.org/10.1038/nature13691 (2014). [Medline](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=22563384&dopt=Abstract) [CrossRef](http://dx.doi.org/10.1371/journal.pone.0035430)</jrn>

<jrn>2. Hemani, G. *et al.* Detection and replication of epistasis influencing transcription in humans. Nature 508**,** 249–253 (2014). [Medline](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=24572353&dopt=Abstract) [CrossRef](http://dx.doi.org/10.1038/nature13005)</jrn>

<jrn>3. Westra, H. J. *et al.* Systematic identification of *trans* eQTLs as putative drivers of known disease associations. Nature Genet. 45**,** 1238–1243 (2013). [Medline](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=24013639&dopt=Abstract) [CrossRef](http://dx.doi.org/10.1038/ng.2756)</jrn>

<jrn>4. Powell, J. E. *et al.* Congruence of additive and non-additive effects on gene expression estimated from pedigree and SNP data. PLoS Genet. 9**,** e1003502 (2013). [Medline](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=23696747&dopt=Abstract)</jrn>

<jrn>5. Powell, J. E. *et al.* The Brisbane Systems Genetics Study: genetical genomics meets complex trait genetics. PLoS ONE 7**,** e35430 (2012). [Medline](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=22563384&dopt=Abstract) [CrossRef](http://dx.doi.org/10.1371/journal.pone.0035430)</jrn>