Another explanation for apparent epistasis

ARISING FROM G. Hemani *et al. Nature* **508,** 249–253 (2014); doi:10.1038/nature13005

Epistasis occurs when the effect of a genetic variant on a trait is dependent on genotypes of other variants elsewhere in the genome. Hemani *et al.* recently reported the detection and replication of many instances of epistasis between pairs of variants influencing gene expression levels in humans[1]. Using whole-genome sequencing data from 450 individuals we strongly replicated many of the reported interactions but, in each case, a single third variant captured by our sequencing data could explain all of the apparent epistasis. Our results provide an alternative explanation for the apparent epistasis observed for gene expression in humans. There is a Reply to this Brief Communication Arising by Hemani, G. *et al. Nature* **514,** http://dx.doi.org/10.1038/nature13692 (2014).

Hemani *et al.*[1] identified 30 pairs of single nucleotide polymorphisms (SNPs; Table 1 in Hemani *et al.*[1]) that interacted to influence the expression of 19 different gene transcripts. These interactions were robust to adjustment for multiple testing and were replicated across two independent studies. Most of the replicated apparently interacting SNP pairs were associated with gene expression in *cis* and were located close to each other on the same chromosome (all <520 kilobases). We have previously shown that low levels of correlation due to linkage disequilibrium (LD) between variants can cause apparent allelic heterogeneity at an associated locus[2]. We therefore hypothesized that low levels of LD could explain the epistasis observed by Hemani *et al.*[1].

To address this hypothesis, we used a combination of whole-genome sequence data and whole-blood gene expression traits in 450 individuals from the InCHIANTI study[2]. Gene expression levels were measured using a similar Illumina array (Human HT-12 v3.0) as Hemani *et al.*[1] used for all of their discovery and replication analyses and we used the same analysis software (epiGPU[3]).

We first replicated the apparent interactions detected and replicated by Hemani *et al.* (11 of 17 *cis*–*cis* pairs and 3 of 11 *cis*–*trans* pairs with $P < 0.05$; Table 1). Our lower success rate of replicating the *cis*–*trans* effects is consistent with their reported smaller effect sizes. We could not analyse two of the gene expression traits because either the probe or one of the SNPs failed quality control in our study. We next identified the single most strongly associated variant for each of the 17 gene expression traits from our whole-genome sequencing analysis. For 27 out of 28 SNP pairs the individual variant most strongly associated with gene expression in our data was more strongly associated than the 8 degrees of freedom (8 d.f.) full model formed from the pair of SNPs reported in Hemani *et al.* (Table 1). For all 17 putatively interacting pairs where both SNPs occurred on the same chromosome our more strongly associated variant was moderately correlated with both of the interacting SNPs (Table 2). These correlations occurred despite very low levels of LD between the two SNPs described by Hemani *et al.*

We next re-evaluated the evidence for interaction but this time corrected for the presence of our most strongly associated variant. The inclusion of our third variant removed any evidence for interaction (Table 1). This included the removal of apparently strong interactions involving *cis* variants for *MBNL1* and *TMEM149* (also known as *IGFLR1*), the two transcripts that account for all of the *cis*–*trans* interactions. Additionally, the most strongly associated variant for *MBNL1* occurs in the probe sequence used to detect expression of the gene, raising the possibility

Table 1 | Results from running pairwise SNP interaction analyses on SNP pairs identified and replicated by Hemani *et al.*[1] and the results observed after conditioning on the most strongly associated additive *cis* variant identified in the InCHIANTI sequencing study (IncSeq)

		SNP pairs from Hemani *et al.* Table 1			Two SNPs from Hemani *et al.*		Adjusted for IncSeq variant	
Cis/trans	Gene (chr)	SNP1 (chr)	SNP2 (chr)	IncSeq variant*	8 d.f. full model *P*	Interaction *P*	8 d.f. full model *P*	Interaction *P*
Cis	*ADK* (10)	rs2395095 (10)	rs10824092 (10)	10:75928933	3.2×10^{-19}	9.1×10^{-04}	0.99	0.86
Cis	*ATP13A1* (19)	rs4284750 (19)	rs873870 (19)	19:19756073	2.1×10^{-05}	7.9×10^{-03}	0.87	0.64
Cis	*C21ORF57* (21)	rs9978658 (21)	rs11701361 (21)	21:47703649	3.8×10^{-05}	7.2×10^{-03}	0.02	0.43
Cis	*CSTB* (21)	rs9979356 (21)	rs3761385 (21)	21:45201832	6.2×10^{-07}	8.3×10^{-07}	0.98	0.99
Cis	*CTSC* (11)	rs7930237 (11)	rs556895 (11)	11:88015717	3.5×10^{-15}	5.0×10^{-06}	7.0×10^{-08}	0.04
Cis	*FN3KRP* (17)	rs898095 (17)	rs9892064 (17)	17:80678628	2.8×10^{-11}	2.9×10^{-12}	0.07	0.43
Cis	*GAA* (17)	rs11150847 (17)	rs12602462 (17)	17:78096086	0.09	0.15	0.22	0.34
Cis	*HNRPH1* (5)	rs6894268 (5)	rs4700810 (5)	5:178978883	0.08	0.53	0.36	0.45
Cis	*LAX1* (1)	rs1891432 (1)	rs10900520 (1)	1:203747772	8.3×10^{-08}	1.6×10^{-04}	0.27	0.52
Cis	*MBNL1* (3)	rs16864367 (3)	rs13079208 (3)	3:152182577	1.1×10^{-07}	2.7×10^{-06}	0.41	0.16
Trans	*MBNL1* (3)	rs7710738 (5)	rs13069559 (3)	3:152182577	3.1×10^{-05}	2.3×10^{-02}	0.05	0.02
Trans	*MBNL1* (3)	rs2030926 (6)	rs13069559 (3)	3:152182577	2.2×10^{-05}	3.2×10^{-02}	0.19	0.21
Trans	*MBNL1* (3)	rs2614467 (14)	rs13069559 (3)	3:152182577	3.7×10^{-04}	0.24	0.47	0.55
Trans	*MBNL1* (3)	rs218671 (17)	rs13069559 (3)	3:152182577	1.4×10^{-03}	0.90	0.38	0.79
Trans	*MBNL1* (3)	rs11981513 (7)	rs13069559 (3)	3:152182577	1.6×10^{-05}	1.6×10^{-02}	0.11	0.10
Cis	*MBP* (18)	rs8092433 (18)	rs4890876 (18)	18:74723459	1.2×10^{-02}	0.05	0.67	0.28
Cis	*NAPRT1* (8)	rs2123758 (8)	rs3889129 (8)	8:144684215	6.8×10^{-34}	6.2×10^{-06}	0.40	0.84
Cis	*NCL* (2)	rs7563453 (2)	rs4973397 (2)	2:232320581	0.09	0.10	0.85	0.71
Cis	*PRMT2* (21)	rs2839372 (21)	rs11701058 (21)	21:47887791	2.6×10^{-15}	2.6×10^{-04}	0.52	0.30
Cis	*SNORD14A* (11)	rs2634462 (11)	rs6486334 (11)	11:17230389	1.7×10^{-05}	0.37	0.41	0.17
Cis	*TMEM149* (19)	rs807491 (19)	rs7254601 (19)	19:36234489	3.0×10^{-31}	2.9×10^{-06}	0.46	0.41
Trans	*TMEM149* (19)	rs8106959 (19)	rs6926382 (6)	19:36234489	3.2×10^{-43}	0.23	0.17	0.53
Trans	*TMEM149* (19)	rs8106959 (19)	rs914940 (1)	19:36234489	3.7×10^{-42}	0.62	0.39	0.71
Trans	*TMEM149* (19)	rs8106959 (19)	rs2351458 (4)	19:36234489	3.5×10^{-42}	0.30	0.53	0.46
Trans	*TMEM149* (19)	rs8106959 (19)	rs6718480 (2)	19:36234489	6.1×10^{-42}	0.44	0.57	0.69
Trans	*TMEM149* (19)	rs8106959 (19)	rs1843357 (8)	19:36234489	4.0×10^{-41}	0.44	0.91	0.73
Trans	*TMEM149* (19)	rs8106959 (19)	rs9509428 (13)	19:36234489	3.3×10^{-42}	0.09	0.69	0.39
Cis	*VASP* (19)	rs1264226 (19)	rs2276470 (19)	19:46033382	0.12	0.81	0.71	0.56

Data was available for 28 of the 30 interactions reported by Hemani *et al.*[1]. Both the full model and interaction associations for the Hemani *et al.* SNPs are completely removed on adjustment for the additive effect of our single most associated variant.

* IncSeq variant is the most strongly associated additive variant with probe levels in *cis* (± 1Mb probe start site).

Table 2 | Linkage disequilibrium measures between SNP pairs identified by Hemani *et al.*[1] and the most strongly associated *cis* variant identified in the InCHIANTI sequencing study

		SNP pairs from Hemani *et al.* Table 1			Linkage disequilibrium between variants		
Cis/trans	Gene (chr)	SNP1 (chr)	SNP2 (chr)	IncSeq variant*	SNP1 – SNP2 r^2/D'	SNP1 – IncSeq r^2/D'	SNP2 – IncSeq r^2/D'
Cis	*ADK* (10)	rs2395095 (10)	rs10824092 (10)	10:75928933	0/0.01	0.39/0.81	0.1/1
Cis	*ATP13A1* (19)	rs4284750 (19)	rs873870 (19)	19:19756073	0.01/0.11	0.07/0.9	0.04/0.82
Cis	*C21ORF57* (21)	rs9978658 (21)	rs11701361 (21)	21:47703649	0.02/0.19	0.02/0.2	0.02/0.21
Cis	*CSTB* (21)	rs9979356 (21)	rs3761385 (21)	21:45201832	0.04/0.23	0.05/0.25	0.14/0.38
Cis	*CTSC* (11)	rs7930237 (11)	rs556895 (11)	11:88015717	0/0.07	0.22/0.9	0.11/0.94
Cis	*FN3KRP* (17)	rs898095 (17)	rs9892064 (17)	17:80678628	0/0.04	0.01/0.12	0.05/0.27
Cis	*GAA* (17)	rs11150847 (17)	rs12602462 (17)	17:78096086	0.01/0	0.3/1	0.11/0.94
Cis	*HNRPH1* (5)	rs6894268 (5)	rs4700810 (5)	5:178978883	0.02/0.23	0.05/0.42	0.3/0.63
Cis	*LAX1* (1)	rs1891432 (1)	rs10900520 (1)	1:203747772	0.03/0.23	0.21/0.51	0.05/0.29
Cis	*MBNL1* (3)	rs16864367 (3)	rs13079208 (3)	3:152182577	0.08/0.42	0.13/0.62	0.06/1
Trans	*MBNL1* (3)	rs7710738 (5)	rs13069559 (3)	3:152182577	NA	NA	0.44/1
Trans	*MBNL1* (3)	rs2030926 (6)	rs13069559 (3)	3:152182577	NA	NA	0.44/1
Trans	*MBNL1* (3)	rs2614467 (14)	rs13069559 (3)	3:152182577	NA	NA	0.44/1
Trans	*MBNL1* (3)	rs218671 (17)	rs13069559 (3)	3:152182577	NA	NA	0.44/1
Trans	*MBNL1* (3)	rs11981513 (7)	rs13069559 (3)	3:152182577	NA	NA	0.44/1
Cis	*MBP* (18)	rs8092433 (18)	rs4890876 (18)	18:74723459	0.04/0.22	0.11/0.43	0.21/0.62
Cis	*NAPRT1* (8)	rs2123758 (8)	rs3889129 (8)	8:144684215	0.03/0.17	0.4/0.96	0.06/0.68
Cis	*NCL* (2)	rs7563453 (2)	rs4973397 (2)	2:232320581	0.04/0.25	0.29/0.83	0.16/0.76
Cis	*PRMT2* (21)	rs2839372 (21)	rs11701058 (21)	21:47887791	0.07/0.28	0.01/0.11	0.33/0.95
Cis	*SNORD14A* (11)	rs2634462 (11)	rs6486334 (11)	11:17230389	0/0	0.07/0.62	0.04/0.59
Cis	*TMEM149* (19)	rs807491 (19)	rs7254601 (19)	19:36234489	0/0.11	0.11/0.93	0.51/0.9
Trans	*TMEM149* (19)	rs8106959 (19)	rs6926382 (6)	19:36234489	NA	0.84/0.99	NA
Trans	*TMEM149* (19)	rs8106959 (19)	rs914940 (1)	19:36234489	NA	0.84/0.99	NA
Trans	*TMEM149* (19)	rs8106959 (19)	rs2351458 (4)	19:36234489	NA	0.84/0.99	NA
Trans	*TMEM149* (19)	rs8106959 (19)	rs6718480 (2)	19:36234489	NA	0.84/0.99	NA
Trans	*TMEM149* (19)	rs8106959 (19)	rs1843357 (8)	19:36234489	NA	0.84/0.99	NA
Trans	*TMEM149* (19)	rs8106959 (19)	rs9509428 (13)	19:36234489	NA	0.84/0.99	NA
Cis	*VASP* (19)	rs1264226 (19)	rs2276470 (19)	19:46033382	0.01/0.12	0.05/0.47	0.1/0.57

NA, not applicable because the SNPs are on different chromosomes.
* IncSeq variant is the most strongly associated additive variant with probe levels in *cis* (± 1Mb probe start site).

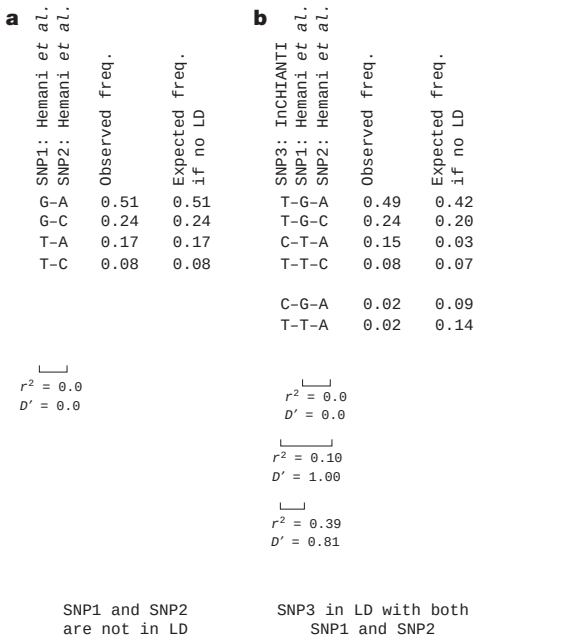

Figure 1 | Haplotype and linkage disequilibrium structure. **a**, **b**, Haplotype and LD structure are shown at the *ADK* locus of two proposed epistatic SNPs from Hemani *et al.*[1] (**a**) and when adding a third SNP captured by sequencing in 450 Italian individuals (**b**). The two "epistatic" SNPs form all four of the possible haplotypes. When adding the third SNP no new haplotypes are formed at >2.4% frequency. Haplotypes were estimated using Haploview[8].

of a technical explanation for the *cis–trans* interactions. Our results mean that the apparent epistasis reported by Hemani *et al.* is more likely to be due to moderate levels of LD between each of the two SNPs and a single causal allele rather than genuine epistasis.

Hemani *et al.* attempted to remove interaction effects driven by low levels of correlation with additive variants by removing pairs of SNPs with pairwise $r^2 < 0.1$ and $D'^2 < 0.1$ (Table 2). However, it is possible to have substantial multi-locus LD but no pairwise LD[4]. Fig. 1 provides an example of the haplotype structure for the *ADK* locus, where there is no LD between the two interacting SNPs, but the most associated variant from our study has moderate LD with both of the SNPs.

In summary, using whole-genome sequencing and independent data, we have provided an alternative explanation for the findings of Hemani *et al.*[1] and conclude that there remain few robust examples of epistasis in humans.

Methods

Gene expression profiles were captured using an Illumina HumanHT-12 v3.0 BeadChip array[2]. Whole-genome sequencing was performed at the Beijing Genomics Institute (Shenzhen, China) using the Illumina HiSeq 2000 (median read depth 7×). Reads were processed using GATK[5] before genotype recovery and refinement through within-sample imputation using BEAGLE[6]. Analysis of the 8 d.f. model and interaction term was performed using epiGPU[3]. To determine whether the observed interactions were driven by unaccounted for additive variants, we obtained the most strongly associated variant in *cis* (1 megabase ± probe start site) using MACH2QTL[7], generated a phenotype of residuals for each expression trait by regressing out the variant, and then repeated the epiGPU analysis using the adjusted trait.

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Author Contributions A.R.W., T.M.F. and M.N.W. designed the study. A.R.W., M.A.T. and M.N.W. performed the bioinformatics analyses. M.A.N., D.G.H., S.B., A.B.S., D.M. and L.F. provided the Inchianti study and expression data. A.R.W., T.M.F. and M.N.W. wrote the manuscript. All authors commented on the manuscript.

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Hemani *et al.* reply

REPLYING TO A. R. Wood *et al. Nature* **514,** http://dx.doi.org/10.1038/nature13691 (2014)

We thank Wood *et al.* for their interesting observations in the accompanying Comment[1], 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.

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 $< 0.05/26 = 1.9 \times 10^{-3}$.

Table 2 | Correlation coefficients are calculated between relative pairs in BSGS[5]

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 data[4]. 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.

Applying their method in our discovery and replication data sets[2] 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 SNPs[3]. 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[4] (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 variation[2,4].

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