**Of the 28 epistasis pairs listed in the table provided by Michael Weedon, there are 17 unique INC seq snps. All 17 display large additive effects for the cis probe in InCHIANTI. 15 of these SNPs are also present in the imputed (1000 Genomes) BSGS data. All 15 also display large additive effects for the cis probe in BSGS data.**

**These SNPs are in modest to high LD with one or both of the epistasis SNPs. We expect there to be large main effects at one or both of the epistasis SNPs. We also expect there to be other SNPs that explain more additive variance in LD with the one or both of the epistasis SNPs.**

**In terms of the cis-cis effects the question is about haplotype effects. There was a possibility that our cis-cis effects were haplotype effects, and we state this as a caveat in the paper. If there is a SNP in LD with both epistasis cis SNPs then either that SNP is the true underlying additive effect with the cis-cis interaction being an artifact, or that SNP is tagging a haplotype on which there is a local interaction. The former is certainly the most parsimonious, but as far as we know there isn’t a way to test that it is the true model. It should also be noted that there is support for the latter possibility too - there is fairly convincing evidence that cis interacting epistasis does occur, e.g.**[**https://www.cell.com/AJHG/abstract/S0002-9297(11)00353-3**](https://www.cell.com/AJHG/abstract/S0002-9297(11)00353-3)**.**

**Some relevant observations**

**Question: Do the INC seq SNPs explain more variance for the probe then the pairwise combination of epistasis SNPs? In both the InCHIANTI data and BSGS the pairwise combination explain more variance in the probe expression levels than the Inc seq SNPs (table 3). It is unclear to me why they observe no variance explained after removing the additive effects of the InC SNP (see column S in the file from Michael).**

**Another important point is the power to detect the interactions given the sample size = 450. This is particularly relevant for the genotype class sizes that must have a sufficient number of individuals to (accurately) estimate the genotype class mean expression. Looking at the file sent by Michael there is no observed interaction (based on p values) for 14/28 pairs in the in InCHIANTI data (table 1). Therefore, there is no interaction effect to remove by fitting the inc seq SNP. The reason for the lack of interaction is unknown, but it is possible that it is due to same sample size of the genotype classes (see table 2).**

**If there are low (or missing) genotype classes for these pairs in InCHIANTI then removing much of the genetic variance could result in the removal of significant interaction effect. The example in figure 1 is from BSGS data. Part a shows the genotype by phenotype map for BSGS data for probe ILMN\_1786426 and SNPs rs8106959 and rs6718480. Part b shows the expression levels for the same probe after the effect of inc seq SNP 19:36234489 is removed. If, in the InCHIANTI data, the genotype class aa snp1, aa snp2 was either missing or few individuals then all genetic signal will be removed with the removal of 19:36234489 effects.**

**Table 1** | selected columns from the file sent by Michael Weedon. The highlighted blue and yellow columns are the interaction p-values (from epiGPU) for the pair of epistatic snps before and after the additive effect of the incSeq SNP is removed. The rows highlighted orange are those that showed no initial interaction (based on p-values) in the InCHIANTI data.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Probe** | **SNP1** | **SNP1 B37 Pos** | **SNP2** | **SNP2 B37 Pos** | **incSeq** | **Interaction P** | **Interaction P** |
| ILMN\_2358626 | rs2395095 | 10:76446305 | rs10824092 | 10:75929517 | 10:75928933 | 9.1E-04 | **0.86** |
| ILMN\_2134224 | rs4284750 | 19:19810050 | rs873870 | 19:19738554 | 19:19756073 | 7.9E-03 | **0.64** |
| ILMN\_1795836 | rs9978658 | 21:48027084 | rs11701361 | 21:47764477 | 21:47703649 | 7.2E-03 | **0.43** |
| ILMN\_1761797 | rs9979356 | 21:45230974 | rs3761385 | 21:45198355 | 21:45201832 | 8.3E-07 | **0.99** |
| ILMN\_2242463 | rs7930237 | 11:88117962 | rs556895 | 11:88077479 | 11:88015717 | 5.0E-06 | **0.04** |
| ILMN\_1652333 | rs898095 | 17:80890638 | rs9892064 | 17:80827903 | 17:80678628 | 2.9E-12 | **0.43** |
| ILMN\_2410783 | rs11150847 | 17:78153130 | rs12602462 | 17:78146016 | 17:78096086 | 0.15 | **0.34** |
| ILMN\_2101920 | rs6894268 | 5:179032488 | rs4700810 | 5:178991794 | 5:178978883 | 0.53 | **0.45** |
| ILMN\_1769782 | rs1891432 | 1:203877662 | rs10900520 | 1:203780591 | 1:203747772 | 1.6E-04 | **0.52** |
| ILMN\_2313158 | rs11981513 | 7:94648239 | rs13069559 | 3:152187431 | 3:152182577 | 1.6E-02 | **0.10** |
| ILMN\_2313158 | rs16864367 | 3:152234166 | rs13079208 | 3:152116652 | 3:152182577 | 2.7E-06 | **0.16** |
| ILMN\_2313158 | rs2030926 | 6:114067127 | rs13069559 | 3:152187431 | 3:152182577 | 3.2E-02 | **0.21** |
| ILMN\_2313158 | rs218671 | 17:6604708 | rs13069559 | 3:152187431 | 3:152182577 | 0.90 | **0.79** |
| ILMN\_2313158 | rs2614467 | 14:99770138 | rs13069559 | 3:152187431 | 3:152182577 | 0.24 | **0.55** |
| ILMN\_2313158 | rs7710738 | 5:22101322 | rs13069559 | 3:152187431 | 3:152182577 | 2.3E-02 | **0.02** |
| ILMN\_2398939 | rs8092433 | 18:74747424 | rs4890876 | 18:74732087 | 18:74723459 | 0.05 | **0.28** |
| ILMN\_1710752 | rs2123758 | 8:144663661 | rs3889129 | 8:144613680 | 8:144684215 | 6.2E-06 | **0.84** |
| ILMN\_2121437 | rs7563453 | 2:232301670 | rs4973397 | 2:232291471 | 2:232320581 | 0.10 | **0.71** |
| ILMN\_1675038 | rs2839372 | 21:48063862 | rs11701058 | 21:47776382 | 21:47887791 | 2.6E-04 | **0.30** |
| ILMN\_1799381 | rs2634462 | 11:17339127 | rs6486334 | 11:17015557 | 11:17230389 | 0.37 | **0.17** |
| ILMN\_1786426 | rs807491 | 19:36268923 | rs7254601 | 19:36147315 | 19:36234489 | 2.9E-06 | **0.41** |
| ILMN\_1786426 | rs8106959 | 19:36219525 | rs1843357 | 8:13822381 | 19:36234489 | 0.44 | **0.73** |
| ILMN\_1786426 | rs8106959 | 19:36219525 | rs2351458 | 4:113317583 | 19:36234489 | 0.30 | **0.46** |
| ILMN\_1786426 | rs8106959 | 19:36219525 | rs6718480 | 2:233879066 | 19:36234489 | 0.44 | **0.69** |
| ILMN\_1786426 | rs8106959 | 19:36219525 | rs6926382 | 6:161683974 | 19:36234489 | 0.23 | **0.53** |
| ILMN\_1786426 | rs8106959 | 19:36219525 | rs914940 | 1:242889492 | 19:36234489 | 0.62 | **0.71** |
| ILMN\_1786426 | rs8106959 | 19:36219525 | rs9509428 | 13:21473952 | 19:36234489 | 0.09 | **0.39** |
| ILMN\_1743646 | rs1264226 | 19:46063167 | rs2276470 | 19:45974668 | 19:46033382 | 0.81 | **0.56** |

**Table 2** | The expected genotype class sizes in InCHIANTI data. Note, this is assuming that the allele frequencies are the same as in BSGS and that in the InCHIANTI data every probe is significantly expressed in 100% of the 450 individuals. We required that all 9 genotype classes were present and that the minimum genotype class size for discovery and replication was >5. The purpose of this to accurately estimate the genotype class mean expression level.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| probe | snp1 | snp2 | aa1aa2 | aA1aa2 | AA1aa2 | aa1aA2 | aA1aA2 | AA1aA2 | aa1AA2 | aA1AA2 | AA1AA2 |
| ILMN\_2358626 | rs2395095 | rs10824092 | 6 | 12 | 35 | 12 | 23 | 69 | 14 | 28 | 84 |
| ILMN\_2134224 | rs4284750 | rs873870 | 11 | 22 | 46 | 21 | 43 | 92 | 13 | 27 | 57 |
| ILMN\_1795836 | rs9978658 | rs11701361 | 3 | 7 | 9 | 7 | 13 | 19 | 37 | 74 | 105 |
| ILMN\_1761797 | rs9979356 | rs3761385 | 2 | 4 | 26 | 4 | 8 | 53 | 10 | 21 | 130 |
| ILMN\_2242463 | rs7930237 | rs556895 | 7 | 14 | 77 | 14 | 28 | 154 | 5 | 10 | 56 |
| ILMN\_1652333 | rs898095 | rs9892064 | 20 | 39 | 21 | 39 | 79 | 41 | 37 | 73 | 38 |
| ILMN\_2410783 | rs11150847 | rs12602462 | 14 | 28 | 21 | 28 | 55 | 43 | 33 | 66 | 51 |
| ILMN\_2101920 | rs6894268 | rs4700810 | 6 | 12 | 31 | 12 | 24 | 62 | 16 | 33 | 83 |
| ILMN\_1769782 | rs1891432 | rs10900520 | 5 | 10 | 13 | 10 | 19 | 26 | 34 | 68 | 92 |
| ILMN\_2313158 | rs11981513 | rs13069559 | 3 | 6 | 3 | 6 | 11 | 6 | 78 | 156 | 81 |
| ILMN\_2313158 | rs16864367 | rs13079208 | 8 | 15 | 61 | 15 | 31 | 122 | 8 | 15 | 62 |
| ILMN\_2313158 | rs2030926 | rs13069559 | 3 | 6 | 3 | 6 | 11 | 6 | 78 | 156 | 81 |
| ILMN\_2313158 | rs218671 | rs13069559 | 2 | 4 | 4 | 4 | 8 | 8 | 58 | 116 | 104 |
| ILMN\_2313158 | rs2614467 | rs13069559 | 2 | 5 | 4 | 4 | 9 | 7 | 62 | 124 | 99 |
| ILMN\_2313158 | rs7710738 | rs13069559 | 2 | 5 | 4 | 4 | 9 | 7 | 62 | 123 | 100 |
| ILMN\_2398939 | rs8092433 | rs4890876 | 18 | 36 | 26 | 36 | 72 | 52 | 29 | 59 | 42 |
| ILMN\_1710752 | rs2123758 | rs3889129 | 7 | 14 | 27 | 14 | 28 | 53 | 21 | 41 | 78 |
| ILMN\_2121437 | rs7563453 | rs4973397 | 2 | 3 | 14 | 3 | 7 | 29 | 17 | 33 | 146 |
| ILMN\_1675038 | rs2839372 | rs11701058 | 1 | 2 | 11 | 2 | 4 | 22 | 16 | 33 | 163 |
| ILMN\_1799381 | rs2634462 | rs6486334 | 4 | 7 | 28 | 7 | 14 | 55 | 14 | 27 | 108 |
| ILMN\_1786426 | rs807491 | rs7254601 | 4 | 7 | 9 | 7 | 14 | 18 | 41 | 82 | 99 |
| ILMN\_1786426 | rs8106959 | rs1843357 | 2 | 4 | 40 | 4 | 7 | 81 | 5 | 11 | 125 |
| ILMN\_1786426 | rs8106959 | rs2351458 | 2 | 4 | 51 | 4 | 9 | 102 | 5 | 9 | 108 |
| ILMN\_1786426 | rs8106959 | rs6718480 | 2 | 5 | 54 | 5 | 10 | 109 | 5 | 9 | 103 |
| ILMN\_1786426 | rs8106959 | rs6926382 | 3 | 6 | 74 | 6 | 13 | 148 | 4 | 7 | 80 |
| ILMN\_1786426 | rs8106959 | rs914940 | 3 | 5 | 58 | 5 | 10 | 115 | 4 | 9 | 99 |
| ILMN\_1786426 | rs8106959 | rs9509428 | 3 | 7 | 76 | 7 | 13 | 153 | 3 | 7 | 78 |
| ILMN\_1743646 | rs1264226 | rs2276470 | 16 | 32 | 33 | 31 | 63 | 66 | 22 | 44 | 46 |

**Table 3 |** proportion of variance explained by the epistasis and inc seq SNPs. For the InCHIANTI data this is calculated by; R2/(1-R) ~ df \* chisq(df) / N. with df=1 or 8 depending on the model and chisq calculated from the reported p-values.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| probe | var% bsgs snp1 InCHIANTI | var% bsgs snp2 InCHIANTI | | var% inc snp InCHIANTI | var% epiGPU 8DF InCHIANTI | var%inc snp in bsgs | var% epiGPU 8DF bsgs |
| ILMN\_2358626 | 12.34 | 2.88 | | 24.77 | 62.8 | 15.366 | 48.866 |
| ILMN\_2134224 | 2.91 | 1.24 | | 19.37 | 36.27 | / | 46.83 |
| ILMN\_1795836 | 3.5 | 0.04 | | 18.19 | 35.32 | 36.634 | 47.301 |
| ILMN\_1761797 | 0.96 | 1.49 | | 15.43 | 41.2 | 21.206 | 48.456 |
| ILMN\_2242463 | 9.12 | 0.82 | | 10.09 | 57.81 | 5.465 | 51.311 |
| ILMN\_1652333 | 0.92 | 0.11 | | 26.56 | 51.45 | / | 50.719 |
| ILMN\_2410783 | 0.15 | 0.96 | | 5.9 | 17.92 | 13.667 | 46.902 |
| ILMN\_2101920 | 1.73 | 0.62 | | 3.09 | 18.42 | 19.71 | 48.268 |
| ILMN\_1769782 | 3.38 | 1.15 | | 11.6 | 43.64 | 44.233 | 59.606 |
| ILMN\_2313158 | 0.46 | 3.78 | | 12.66 | 36.2 | 19.968 | 58.403 |
| ILMN\_2313158 | 1.87 | 0.43 | | 12.66 | 43.31 | 19.968 | 58.566 |
| ILMN\_2313158 | 0.16 | 3.78 | | 12.66 | 36.2 | 19.968 | 58.288 |
| ILMN\_2313158 | 0 | 3.78 | | 12.66 | 31.41 | 19.968 | 59.029 |
| ILMN\_2313158 | 0.19 | 3.78 | | 12.66 | 31.41 | 19.968 | 58.011 |
| ILMN\_2313158 | 0.02 | 3.78 | | 12.66 | 35.65 | 19.968 | 58.87 |
| ILMN\_2398939 | 0.65 | 1.55 | | 7.76 | 23.76 | 20.232 | 48.044 |
| ILMN\_1710752 | 20.29 | 1.1 | | 30.56 | 73.8 | 37.386 | 66.063 |
| ILMN\_2121437 | 0.8 | 0.11 | | 3.23 | 17.92 | 6.923 | 46.786 |
| ILMN\_1675038 | 0.02 | 11.3 | | 18.62 | 58 | 5.299 | 47.712 |
| ILMN\_1799381 | 3.92 | 1.87 | | 30.62 | 36.64 | 25.123 | 47.244 |
| ILMN\_1786426 | 4.7 | 17.46 | | 34 | 72.34 | 47.119 | 74.265 |
| ILMN\_1786426 | 29.16 | 0.01 | | 34 | 77.1 | 47.119 | 73.819 |
| ILMN\_1786426 | 29.16 | 0.92 | | 34 | 77.52 | 47.119 | 74.342 |
| ILMN\_1786426 | 29.16 | | 0.32 | 34 | 77.42 | 47.119 | 74.488 |
| ILMN\_1786426 | 29.16 | | 0.45 | 34 | 77.91 | 47.119 | 73.864 |
| ILMN\_1786426 | 29.16 | | 0 | 34 | 77.51 | 47.119 | 73.997 |
| ILMN\_1786426 | 29.16 | | 0.17 | 34 | 77.52 | 47.119 | 74.979 |
| ILMN\_1743646 | 0.42 | | 1.84 | 13.31 | 17 | 49.341 | 56.315 |

Figure 1 | expression levels (z-axis) of ILMN\_1786426 before (a) and after (b) removing the effects of inc seq SNP 19:36234489.

a) b)

