**Re: An alternative explanation for apparent epistasis**

Wood et al claim that the interaction effects that we detected and replicated in Northern Europeans could be removed by the inclusion of a single fine-mapped cis-acting variant in a relatively underpowered sample of 450 individuals of Italian origin. This paints an overly simplistic interpretation of the genetic architecture for the transcription levels that we reported. We will discuss the cis-cis effects and cis-trans effects separately as the same genetic mechanisms do not operate on both.

**Cis-trans effects**

In order to detect most genetic interactions, sample size must be sufficiently large to have adequate representation in each genotype class. For almost all cases in the InChianti data, this criterion was not met for cis-trans effects. As such the original interactions were not replicated, and strong conclusions about these effects cannot be made from the InChianti data alone.

Of the three that did replicate in InChianti (p < 0.05), one remained significant (at the same p-value) even after adjusting for the InChianti variant, and the other two explained almost all the same variance through interaction terms as they did prior to adjusting for the InChianti variant.

An obvious explanation for cis-trans interaction effects being captured by a second cis-acting variant is not forthcoming, but the haplotype model described by Wood et al neither captures all the epistatic variance, nor offers a plausible mechanism by which cis-trans interaction terms might manifest due to a single cis-acting locus. In addition, a single additive variant model is not consistent with our observation that those gene expression probes that were influenced by multiple interactions exhibited non-additive genetic variance as estimated in family studies (table 4).

**Cis-cis effects**

Haplotype effects, like those postulated by Wood et al, are known to be confounding factors in cis-cis interactions and we stated this in the original manuscript. It is not surprising that a single variant can tag two interacting variants, and this observation is uninformative as to the true underlying model. Convincing evidence for cis-cis interactions does exist where the experimental design is appropriate (Lappalainen 2011), and abundant evidence exists for multiple cis-acting variants at a single locus, including for those gene expression levels in these data.

- As reported in Westra et al, and as replicated in BSGS, conditional analysis demonstrates that there are multiple additive effects working in cis in addition to the InChianti variants (table 7), so although a single cis-InChianti variant is a simple explanation, it is not consistent with the empirically deduced genetic architecture.

If we predict the genotypes at the InCHIANTI SNP using the epistasis SNP genotypes we find a) no signal for increased prediction beyond cis LD for the cis-trans pairs. No signal for increased prediction beyond LD with a cis epistasis SNP for all but three probes (table 5). I am currently working haplotype testing for the cis-cis ones.

When we are able to calculate them (4 insistences) we find evidence of epistatic interactions between the InCHANTI seq SNP and the ‘other’ epistasis snp for 3 probes.

Finally, we make an important final remark that Wood et al misrepresented the original manuscript, stating that our claim was that epistasis is widespread in humans. We must emphasise that no such claim was made. On the contrary we inferred that additive variance was substantially more abundant than non-additive variance. Our initial claim was that instances of epistasis could be robustly detected, and following further analysis it is evident that the data supports this conclusion.

**LD Summary information from BSGS and InCHIANTI**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **InCHIANTI DATA** | | | | **BSGS Data** | | | |
| **GENE** | **Probe** | **SNP1** | **SNP2** | **INCSEQ\_VAR** | **SNP1...SNP2.r2** | **SNP1...INCSEQ.r2** | **SNP2..INCSEQ.r2** | **rs\_id** | **r2\_snp1\_snp2** | **r2\_snp1\_inc\_snp** | **r2\_snp2\_inc\_snp** |
| ADK | ILMN\_2358626 | rs2395095 | rs10824092 | 10:75928933 | 0.00 | 0.42 | 0.11 | rs67594352 | 0.02 | 0.21 | 0.12 |
| ATP13A1 | ILMN\_2134224 | rs4284750 | rs873870 | 19:19756073 | 0.01 | 0.08 | 0.07 | chr19:19756073:D | 0.01 | 0.20 | 0.08 |
| C21ORF57 | ILMN\_1795836 | rs9978658 | rs11701361 | 21:47703649 | 0.02 | 0.02 | 0.02 | rs11702450 | 0.03 | 0.02 | 0.05 |
| CSTB | ILMN\_1761797 | rs9979356 | rs3761385 | 21:45201832 | 0.04 | 0.04 | 0.16 | rs35285321 | 0.04 | 0.06 | 0.23 |
| CTSC | ILMN\_2242463 | rs7930237 | rs556895 | 11:88015717 | 0.00 | 0.24 | 0.13 | rs56375235 | 0.01 | 0.15 | 0.08 |
| FN3KRP | ILMN\_1652333 | rs898095 | rs9892064 | 17:80678628 | 0.00 | 0.01 | 0.06 | NA | 0.06 | NA | NA |
| GAA | ILMN\_2410783 | rs11150847 | rs12602462 | 17:78096086 | 0.00 | 0.33 | 0.10 | rs4889970 | 0.00 | 0.28 | 0.21 |
| HNRPH1 | ILMN\_2101920 | rs6894268 | rs4700810 | 5:178978883 | 0.02 | 0.05 | 0.30 | rs10078796 | 0.00 | 0.02 | 0.24 |
| LAX1 | ILMN\_1769782 | rs1891432 | rs10900520 | 1:203747772 | 0.03 | 0.21 | 0.05 | rs2185079 | 0.07 | 0.16 | 0.04 |
| MBLN1 | ILMN\_2313158 | rs11981513 | rs13069559 | 3:152182577 | 0.01 | 0.00 | 0.46 | rs67903230 | 0.00 | 0.00 | 0.63 |
| MBLN1 | ILMN\_2313158 | rs16864367 | rs13079208 | 3:152182577 | 0.08 | 0.17 | 0.07 | rs67903230 | 0.05 | 0.21 | 0.08 |
| MBLN1 | ILMN\_2313158 | rs2030926 | rs13069559 | 3:152182577 | 0.00 | 0.02 | 0.46 | rs67903230 | 0.00 | 0.00 | 0.63 |
| MBLN1 | ILMN\_2313158 | rs218671 | rs13069559 | 3:152182577 | 0.00 | 0.00 | 0.46 | rs67903230 | 0.00 | 0.00 | 0.63 |
| MBLN1 | ILMN\_2313158 | rs2614467 | rs13069559 | 3:152182577 | 0.00 | 0.00 | 0.46 | rs67903230 | 0.00 | 0.00 | 0.63 |
| MBLN1 | ILMN\_2313158 | rs7710738 | rs13069559 | 3:152182577 | 0.00 | 0.00 | 0.46 | rs67903230 | 0.01 | 0.01 | 0.63 |
| MBP | ILMN\_2398939 | rs8092433 | rs4890876 | 18:74723459 | 0.03 | 0.11 | 0.21 | rs470929 | 0.03 | 0.16 | 0.26 |
| NAPRT1 | ILMN\_1710752 | rs2123758 | rs3889129 | 8:144684215 | 0.03 | 0.43 | 0.07 | rs10093709 | 0.05 | 0.29 | 0.03 |
| NCL | ILMN\_2121437 | rs7563453 | rs4973397 | 2:232320581 | 0.05 | 0.33 | 0.18 | rs13019380 | 0.03 | 0.17 | 0.16 |
| PRMT2 | ILMN\_1675038 | rs2839372 | rs11701058 | 21:47887791 | 0.06 | 0.01 | 0.32 | rs4819255 | 0.09 | 0.04 | 0.27 |
| SNORD14A | ILMN\_1799381 | rs2634462 | rs6486334 | 11:17230389 | 0.00 | 0.07 | 0.06 | rs2354863 | 0.01 | 0.14 | 0.13 |
| TMEM149 | ILMN\_1786426 | rs807491 | rs7254601 | 19:36234489 | 0.00 | 0.10 | 0.52 | rs28656784 | 0.00 | 0.10 | 0.46 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs1843357 | 19:36234489 | 0.00 | 0.85 | 0.00 | rs28656784 | 0.00 | 0.68 | 0.00 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs2351458 | 19:36234489 | 0.00 | 0.85 | 0.01 | rs28656784 | 0.00 | 0.68 | 0.00 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs6718480 | 19:36234489 | 0.00 | 0.85 | 0.00 | rs28656784 | 0.00 | 0.68 | 0.00 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs6926382 | 19:36234489 | 0.00 | 0.85 | 0.00 | rs28656784 | 0.00 | 0.68 | 0.00 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs914940 | 19:36234489 | 0.00 | 0.85 | 0.00 | rs28656784 | 0.00 | 0.68 | 0.00 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs9509428 | 19:36234489 | 0.00 | 0.85 | 0.00 | rs28656784 | 0.00 | 0.68 | 0.00 |
| VASP | ILMN\_1743646 | rs1264226 | rs2276470 | 19:46033382 | 0.01 | 0.05 | 0.10 | rs4803827 | 0.02 | 0.07 | 0.16 |

**Table 1 |** summary of the LD information from the InCHIANTI and BSGS datasets.

**Table 2 |** Summary of the allele frequencies in InCHIANTI and BSGS data

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | InCHIANTI | | | BSGS | | | |
| GENE | Probe | SNP1 | SNP2 | INCSEQ\_VAR | a\_freq | A\_freq | rs\_id | snp1\_maf | snp2\_maf | inc\_snp\_maf |
| ADK | ILMN\_2358626 | rs2395095 | rs10824092 | 10:75928933 | 0.83 | 0.17 | rs67594352 | 0.29 | 0.39 | 0.14 |
| ATP13A1 | ILMN\_2134224 | rs4284750 | rs873870 | 19:19756073 | 0.96 | 0.04 | chr19:19756073:D | 0.32 | 0.47 | 0.09 |
| C21ORF57 | ILMN\_1795836 | rs9978658 | rs11701361 | 21:47703649 | 0.57 | 0.43 | rs11702450 | 0.37 | 0.23 | 0.38 |
| CSTB | ILMN\_1761797 | rs9979356 | rs3761385 | 21:45201832 | 0.69 | 0.31 | rs35285321 | 0.22 | 0.31 | 0.31 |
| CTSC | ILMN\_2242463 | rs7930237 | rs556895 | 11:88015717 | 0.90 | 0.10 | rs56375235 | 0.23 | 0.46 | 0.09 |
| FN3KRP | ILMN\_1652333 | rs898095 | rs9892064 | 17:80678628 | 0.52 | 0.48 | NA | 0.50 | 0.42 | NA |
| GAA | ILMN\_2410783 | rs11150847 | rs12602462 | 17:78096086 | 0.20 | 0.80 | rs4889970 | 0.45 | 0.39 | 0.27 |
| HNRPH1 | ILMN\_2101920 | rs6894268 | rs4700810 | 5:178978883 | 0.65 | 0.35 | rs10078796 | 0.31 | 0.38 | 0.37 |
| LAX1 | ILMN\_1769782 | rs1891432 | rs10900520 | 1:203747772 | 0.65 | 0.35 | rs2185079 | 0.38 | 0.27 | 0.32 |
| MBLN1 | ILMN\_2313158 | rs11981513 | rs13069559 | 3:152182577 | 0.92 | 0.08 | rs67903230 | 0.50 | 0.16 | 0.11 |
| MBLN1 | ILMN\_2313158 | rs16864367 | rs13079208 | 3:152182577 | 0.92 | 0.08 | rs67903230 | 0.26 | 0.50 | 0.11 |
| MBLN1 | ILMN\_2313158 | rs2030926 | rs13069559 | 3:152182577 | 0.92 | 0.08 | rs67903230 | 0.50 | 0.16 | 0.11 |
| MBLN1 | ILMN\_2313158 | rs218671 | rs13069559 | 3:152182577 | 0.92 | 0.08 | rs67903230 | 0.43 | 0.16 | 0.11 |
| MBLN1 | ILMN\_2313158 | rs2614467 | rs13069559 | 3:152182577 | 0.92 | 0.08 | rs67903230 | 0.44 | 0.16 | 0.11 |
| MBLN1 | ILMN\_2313158 | rs7710738 | rs13069559 | 3:152182577 | 0.92 | 0.08 | rs67903230 | 0.44 | 0.16 | 0.11 |
| MBP | ILMN\_2398939 | rs8092433 | rs4890876 | 18:74723459 | 0.35 | 0.65 | rs470929 | 0.46 | 0.44 | 0.40 |
| NAPRT1 | ILMN\_1710752 | rs2123758 | rs3889129 | 8:144684215 | 0.92 | 0.08 | rs10093709 | 0.34 | 0.37 | 0.17 |
| NCL | ILMN\_2121437 | rs7563453 | rs4973397 | 2:232320581 | 0.91 | 0.09 | rs13019380 | 0.25 | 0.24 | 0.10 |
| PRMT2 | ILMN\_1675038 | rs2839372 | rs11701058 | 21:47887791 | 0.46 | 0.54 | rs4819255 | 0.24 | 0.21 | 0.48 |
| SNORD14A | ILMN\_1799381 | rs2634462 | rs6486334 | 11:17230389 | 0.93\* | 0.07\* | rs2354863 | 0.26 | 0.34 | 0.07 |
| TMEM149 | ILMN\_1786426 | rs807491 | rs7254601 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.39 | 0.23 | 0.14 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs1843357 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.17 | 0.36 | 0.14 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs2351458 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.17 | 0.41 | 0.14 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs6718480 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.17 | 0.42 | 0.14 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs6926382 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.17 | 0.49 | 0.14 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs914940 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.17 | 0.43 | 0.14 |
| TMEM149 | ILMN\_1786426 | rs8106959 | rs9509428 | 19:36234489 | 0.80 | 0.20 | rs28656784 | 0.17 | 0.50 | 0.14 |
| VASP | ILMN\_1743646 | rs1264226 | rs2276470 | 19:46033382 | 0.24 | 0.77 | rs4803827 | 0.41 | 0.46 | 0.28 |

\* Missing the AA homozygous

Original analysis from InCHIANTI group

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| cis/trans | GENE | Probe | INCSEQ SNP R2 | 8DF model R2 for 2 Hemani SNPs | Interaction P | Interaction P adjusted |
| cis | ADK | ILMN\_2358626 | 0.31 | 0.20 | 9.1E-04 | 8.6E-01 |
| cis | ATP13A1 | ILMN\_2134224 | 0.23 | 0.06 | 7.9E-03 | 6.4E-01 |
| cis | C21ORF57 | ILMN\_1795836 | 0.22 | 0.06 | 7.2E-03 | 4.3E-01 |
| cis | CSTB | ILMN\_1761797 | 0.18 | 0.08 | 8.3E-07 | 9.9E-01 |
| cis | CTSC | ILMN\_2242463 | 0.12 | 0.16 | 5.0E-06 | 4.2E-02 |
| cis | FN3KRP | ILMN\_1652333 | 0.33 | 0.12 | 2.9E-12 | 4.3E-01 |
| cis | GAA | ILMN\_2410783 | 0.07 | 0.01 | 1.5E-01 | 3.4E-01 |
| cis | HNRPH1 | ILMN\_2101920 | 0.03 | 0.01 | 5.3E-01 | 4.5E-01 |
| cis | LAX1 | ILMN\_1769782 | 0.14 | 0.09 | 1.6E-04 | 5.2E-01 |
| trans | MBLN1 | ILMN\_2313158 | 0.15 | 0.06 | 1.6E-02 | 1.0E-01 |
| cis | MBLN1 | ILMN\_2313158 | 0.15 | 0.09 | 2.7E-06 | 1.6E-01 |
| trans | MBLN1 | ILMN\_2313158 | 0.15 | 0.06 | 3.2E-02 | 2.1E-01 |
| trans | MBLN1 | ILMN\_2313158 | 0.15 | 0.06 | 9.0E-01 | 7.9E-01 |
| trans | MBLN1 | ILMN\_2313158 | 0.15 | 0.05 | 2.4E-01 | 5.5E-01 |
| trans | MBLN1 | ILMN\_2313158 | 0.15 | 0.04 | 2.3E-02 | 2.4E-02 |
| cis | MBP | ILMN\_2398939 | 0.09 | 0.03 | 5.5E-02 | 2.8E-01 |
| cis | NAPRT1 | ILMN\_1710752 | 0.39 | 0.32 | 6.2E-06 | 8.4E-01 |
| cis | NCL | ILMN\_2121437 | 0.03 | 0.01 | 9.7E-02 | 7.1E-01 |
| cis | PRMT2 | ILMN\_1675038 | 0.22 | 0.16 | 2.6E-04 | 3.0E-01 |
| cis | SNORD14A | ILMN\_1799381 | 0.39 | 0.06 | 3.7E-01 | 1.7E-01 |
| cis | TMEM149 | ILMN\_1786426 | 0.44 | 0.30 | 2.9E-06 | 4.1E-01 |
| trans | TMEM149 | ILMN\_1786426 | 0.44 | 0.37 | 4.4E-01 | 7.3E-01 |
| trans | TMEM149 | ILMN\_1786426 | 0.44 | 0.37 | 3.0E-01 | 4.6E-01 |
| trans | TMEM149 | ILMN\_1786426 | 0.44 | 0.37 | 4.4E-01 | 6.9E-01 |
| trans | TMEM149 | ILMN\_1786426 | 0.44 | 0.38 | 2.3E-01 | 5.3E-01 |
| trans | TMEM149 | ILMN\_1786426 | 0.44 | 0.37 | 6.2E-01 | 7.1E-01 |
| trans | TMEM149 | ILMN\_1786426 | 0.44 | 0.37 | 8.8E-02 | 3.9E-01 |
| cis | VASP | ILMN\_1743646 | 0.16 | 0.01 | 8.1E-01 | 5.6E-01 |

**Table 3 |** One important point to note is the absence of any real evidence for the removal of cis-trans effects. I don’t think this needs to be the primary focus of our response, but is very important none the less. As it currently stands Wood et al are implying they can remove all epistasis signals in their data. In their data they only show this for cis-cis effects. Given these are only 19/501 in the discovery data they represent a small proportion of our reported results.

**Estimates of phenotype correlations by relationship pairs in BSGS data and additive and non-additive variance components (powell et al 2013 PG)**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ILMN\_GENE** | **PROBE\_ID** | **PP** | **PO** | **DZ** | **SIB** | **MZ** | **h2** | **d2** | **IncSNP R2** |
| ADK | ILMN\_2358626 | 0.095 | 0.143 | 0.123 | 0.093 | 0.376 | 0.41 | 0.12 | 0.31 |
| ATP13A1 | ILMN\_2134224 | -0.02 | 0.162 | 0.138 | 0.202 | 0.606 | 0.67 | 0.16 | 0.23 |
| C21ORF57 | ILMN\_1795836 | -0.023 | 0.153 | 0.169 | 0.231 | 0.47 | 0.51 | 0.08 | 0.22 |
| CSTB | ILMN\_1761797 | -0.064 | 0.156 | 0.15 | 0.171 | 0.299 | 0.25 | 0.04 | 0.18 |
| CTSC | ILMN\_2242463 | 0.122 | 0.142 | 0.202 | 0.161 | 0.374 | 0.27 | 0.08 | 0.12 |
| FN3KRP | ILMN\_1652333 | -0.071 | 0.166 | 0.138 | 0.212 | 0.434 | 0.31 | 0.11 | 0.33 |
| GAA | ILMN\_2410783 | -0.047 | 0.163 | 0.143 | 0.13 | 0.387 | 0.39 | 0.06 | 0.07 |
| HNRPH1 | ILMN\_2101920 | 0.015 | 0.152 | 0.122 | 0.129 | 0.237 | 0.17 | 0.05 | 0.03 |
| LAX1 | ILMN\_1769782 | -0.065 | 0.142 | 0.175 | 0.19 | 0.356 | 0.27 | 0.04 | 0.14 |
| MBNL1 | ILMN\_2313158 | 0.022 | 0.179 | 0.158 | 0.184 | 0.239 | 0.18 | 0.11 | 0.15 |
| NAPRT1 | ILMN\_1710752 | -0.062 | 0.186 | 0.208 | 0.283 | 0.506 | 0.37 | 0.14 | 0.39 |
| NCL | ILMN\_2121437 | -0.019 | 0.141 | 0.178 | 0.145 | 0.399 | 0.31 | 0.08 | 0.03 |
| PRMT2 | ILMN\_1675038 | -0.045 | 0.199 | 0.193 | 0.183 | 0.402 | 0.34 | 0.06 | 0.22 |
| SNORD14A | ILMN\_1799381 | 0.028 | 0.17 | 0.146 | 0.13 | 0.525 | 0.43 | 0.14 | 0.39 |
| TMEM149 | ILMN\_1786426 | 0.065 | 0.267 | 0.233 | 0.173 | 0.489 | 0.41 | 0.09 | 0.44 |
| VASP | ILMN\_1743646 | -0.003 | 0.144 | 0.271 | 0.179 | 0.52 | 0.38 | 0.13 | 0.16 |

**Table 4 |** correlation coefficients between relative pairs in BSGS and estimates of additive (h2) and non-additive (d2) variance components. All probes are within the top 10% of h2 estimates and the top 5% of d2 (from 17994 probes).

**Prediction of the InCHIANTI seq SNP (inc\_snp) genotypes from the epistasis pair of SNPs**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| cis/trans | GENE | Probe | SNP1 | SNP2 | rs\_id\_inc\_in\_bsgs | r2\_full | r2\_snp1 | r2\_snp2 |
| cis | ADK | ILMN\_2358626 | rs2395095 | rs10824092 | rs67594352 | 0.23 | 0.21 | 0.12 |
| cis | ATP13A1 | ILMN\_2134224 | rs4284750 | rs873870 | chr19:19756073:D | 0.34\* | 0.2 | 0.08 |
| cis | C21ORF57 | ILMN\_1795836 | rs9978658 | rs11701361 | rs11702450 | 0.06 | 0.02 | 0.05 |
| cis | CSTB | ILMN\_1761797 | rs9979356 | rs3761385 | rs35285321 | 0.62\* | 0.06 | 0.23 |
| cis | CTSC | ILMN\_2242463 | rs7930237 | rs556895 | rs56375235 | 0.18 | 0.15 | 0.08 |
| cis | FN3KRP | ILMN\_1652333 | rs898095 | rs9892064 | NA | NA | NA | NA |
| cis | GAA | ILMN\_2410783 | rs11150847 | rs12602462 | rs4889970 | 0.29 | 0.28 | 0.21 |
| cis | HNRPH1 | ILMN\_2101920 | rs6894268 | rs4700810 | rs10078796 | 0.25 | 0.02 | 0.24 |
| cis | LAX1 | ILMN\_1769782 | rs1891432 | rs10900520 | rs2185079 | 0.47\* | 0.16 | 0.04 |
| trans | MBLN1 | ILMN\_2313158 | rs11981513 | rs13069559 | rs67903230 | 0.64 | 0 | 0.63 |
| cis | MBLN1 | ILMN\_2313158 | rs16864367 | rs13079208 | rs67903230 | 0.25 | 0.21 | 0.08 |
| trans | MBLN1 | ILMN\_2313158 | rs2030926 | rs13069559 | rs67903230 | 0.63 | 0 | 0.63 |
| trans | MBLN1 | ILMN\_2313158 | rs218671 | rs13069559 | rs67903230 | 0.63 | 0 | 0.63 |
| trans | MBLN1 | ILMN\_2313158 | rs2614467 | rs13069559 | rs67903230 | 0.65 | 0 | 0.63 |
| trans | MBLN1 | ILMN\_2313158 | rs7710738 | rs13069559 | rs67903230 | 0.64 | 0.01 | 0.63 |
| cis | MBP | ILMN\_2398939 | rs8092433 | rs4890876 | rs470929 | 0.3 | 0.16 | 0.26 |
| cis | NAPRT1 | ILMN\_1710752 | rs2123758 | rs3889129 | rs10093709 | 0.36 | 0.29 | 0.03 |
| cis | NCL | ILMN\_2121437 | rs7563453 | rs4973397 | rs13019380 | 0.22 | 0.17 | 0.16 |
| cis | PRMT2 | ILMN\_1675038 | rs2839372 | rs11701058 | rs4819255 | 0.27 | 0.04 | 0.27 |
| cis | SNORD14A | ILMN\_1799381 | rs2634462 | rs6486334 | rs2354863 | 0.15 | 0.14 | 0.13 |
| cis | TMEM149 | ILMN\_1786426 | rs807491 | rs7254601 | rs28656784 | 0.47 | 0.1 | 0.46 |
| trans | TMEM149 | ILMN\_1786426 | rs8106959 | rs1843357 | rs28656784 | 0.69 | 0.68 | 0 |
| trans | TMEM149 | ILMN\_1786426 | rs8106959 | rs2351458 | rs28656784 | 0.69 | 0.68 | 0 |
| trans | TMEM149 | ILMN\_1786426 | rs8106959 | rs6718480 | rs28656784 | 0.69 | 0.68 | 0 |
| trans | TMEM149 | ILMN\_1786426 | rs8106959 | rs6926382 | rs28656784 | 0.69 | 0.68 | 0 |
| trans | TMEM149 | ILMN\_1786426 | rs8106959 | rs914940 | rs28656784 | 0.68 | 0.68 | 0 |
| trans | TMEM149 | ILMN\_1786426 | rs8106959 | rs9509428 | rs28656784 | 0.68 | 0.68 | 0 |
| cis | VASP | ILMN\_1743646 | rs1264226 | rs2276470 | rs4803827 | 0.16 | 0.07 | 0.15 |

**Table 5 |** prediction of the inc\_snp genotypes by the two epistasis SNPs. The last three columns show the r^2 from the;

Full model = inc\_snp = snp1 + snp2 + snp1:snp2. The snp1 and snp2 models are inc\_snp = snp1 and inc\_snp = snp2 (i.e the r2 LD). The results show no increase in prediction of the inc\_snp genotypes when the trans snp and interactions are fitted. The prediction r2 is entirely driven by the cis snp in LD. The cis pairs show the same pattern (i.e. no increase beyond ld with one snp) apart the inc snp for; ATP13A1, CSTB, LAX (\*)

**Interactions between InCHIANTI seq SNP and the epistasis pair (InCHIANTI seq SNP replaced the original SNP in the highest LD)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| GENE | Probe | SNP1 | SNP2 | INCSEQ\_VAR | rs\_id\_inc\_in\_bsgs | Original int Pval | InCHIANTI  IntPval |
| CSTB | ILMN\_1761797 | rs9979356 | rs3761385 | 21:45201832 | rs35285321 | 1.02e-12 | 0.77 |
| HNRPH1 | ILMN\_2101920 | rs6894268 | rs4700810 | 5:178978883 | rs10078796 | 4.17e-16 | 2.51e-10 |
| MBP | ILMN\_2398939 | rs8092433 | rs4890876 | 18:74723459 | rs470929 | 3.98e-06 | 3.01e-07 |
| VASP | ILMN\_1743646 | rs1264226 | rs2276470 | 19:46033382 | rs4803827 | 8.1e-06 | 1.32e-08 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Original4dfPval | Original8dfPval | New4dfPval2 | New8dflPval | Original8dfR2 | Original4dfR2 | New8dfR2 | New4dfR2 |
| 11.99 | 17.25 | 0.77 | 25.45 | 0.1 | 0.07 | 0.14 | 0.007 |
| 15.37 | 17.09 | 9.6 | 30.76 | 0.1 | 0.08 | 0.16 | 0.06 |
| 5.4 | 16.92 | 6.52 | 37.13 | 0.1 | 0.03 | 0.19 | 0.04 |
| 5.09 | 15.58 | 7.88 | 81.86 | 0.1 | 0.03 | 0.32 | 0.05 |

**Table 6 |** 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 InCHIANTI sequence SNP and corresponding epistasis pair. Of these one is CSTB that shows no interaction effect. The remaining three have strongly significant effects.

**Conditional analysis**

Analysis of conditional additive genetic variation at the cis locus. More analyses using the conditional model functions in GCTA and the list of cis eQTLs from Westra et al 2013 to follow.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| cis/trans | GENE | Probe | snp1\_R2 | snp1\_pval | snp2\_R2 | snp2\_pval | Incseq\_R2 | incseq\_pval | Sum R2 (snp1, snp2, incseq) |
| cis | ADK | ILMN\_2358626 | 0.04 | 7.84 | 0.00 | 0.00 | 0.11 | 20.72 | 0.15 |
| cis | ATP13A1 | ILMN\_2134224 | 0.00 | 0.34 | 0.00 | 0.35 | 0.24 | 60.04 | 0.24 |
| cis | C21ORF57 | ILMN\_1795836 | 0.01 | 1.73 | 0.00 | 0.06 | 0.25 | 65.19 | 0.26 |
| cis | CSTB | ILMN\_1761797 | 0.00 | 0.90 | 0.00 | 0.27 | 0.17 | 30.07 | 0.17 |
| cis | CTSC | ILMN\_2242463 | 0.02 | 4.64 | 0.07 | 14.13 | 0.05 | 9.66 | 0.14 |
| cis | FN3KRP | ILMN\_1652333 | NA | NA | NA | NA | NA | NA | NA |
| cis | LAX1 | ILMN\_1769782 | 0.00 | 0.26 | 0.00 | 0.71 | 0.28 | 71.59 | 0.29 |
| trans | MBLN1 | ILMN\_2313158 | 0.00 | 0.23 | 0.05 | 9.54 | 0.15 | 36.71 | 0.20 |
| cis | MBLN1 | ILMN\_2313158 | 0.01 | 2.38 | 0.00 | 0.11 | 0.15 | 36.71 | 0.16 |
| trans | MBLN1 | ILMN\_2313158 | 0.00 | 0.18 | 0.05 | 9.97 | 0.15 | 36.71 | 0.20 |
| trans | MBLN1 | ILMN\_2313158 | 0.00 | 0.73 | 0.05 | 9.57 | 0.15 | 36.71 | 0.20 |
| cis | NAPRT1 | ILMN\_1710752 | 0.02 | 3.55 | 0.00 | 0.79 | 0.25 | 58.60 | 0.26 |
| cis | PRMT2 | ILMN\_1675038 | 0.00 | 0.94 | 0.01 | 2.94 | 0.05 | 8.50 | 0.06 |
| cis | TMEM149 | ILMN\_1786426 | 0.00 | 1.14 | 0.07 | 14.66 | 0.30 | 88.19 | 0.37 |

**Table 7 |** The effect of the inc\_snp was removed from the probe expression levels by; y = inc\_snp + e = y\*. y\* was used as the new phenotype and the additive effects of the two original epistasis SNPs were tested. Secondary additive effects (p <0.01) were found for all probes with the exception of those on genes ATP13A1, CSTB and LAX.

**Fine mapping**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | Original 4df test | | No adjustment 4df test | | | After InCHIANTI SNP adjustment 4df test | | |
| probe | gene | 4df\_p\_original | R2\_original | fullP | intP | R2\_new | fullP | intP | R2\_new\_adj |
| ILMN\_2358626 | ADK | 6.69 | 4.08 | 17.54 | 7.88 | 4.83 | 4.59 | 4.41 | 2.93 |
| ILMN\_2134224 | ATP13A1 | 5.30 | 3.36 | 16.90 | 6.89 | 4.31 | 0.60 | 0.67 | 0.68 |
| ILMN\_1795836 | C21ORF57 | 9.42 | 5.46 | 16.33 | 10.25 | 6.04 | 3.85 | 3.19 | 2.25 |
| ILMN\_1761797 | CSTB | 11.99 | 6.72 | 16.96 | 12.96 | 7.39 | 3.96 | 3.78 | 2.58 |
| ILMN\_2242463 | CTSC | 7.16 | 4.33 | 18.85 | 7.97 | 4.88 | 16.54 | 6.72 | 4.18 |
| ILMN\_1652333 | FN3KRP | 16.16 | 8.66 | 20.01 | 17.91 | 9.72 | NA | NA | NA |
| ILMN\_2410783 | GAA | 13.91 | 7.62 | 19.46 | 15.98 | 8.83 | 12.15 | 8.50 | 5.10 |
| ILMN\_2101920 | HNRPH1 | 15.38 | 8.31 | 19.95 | 16.51 | 9.08 | 4.89 | 3.94 | 2.67 |
| ILMN\_1769782 | LAX1 | 19.16 | 10.00 | 9.53 | 7.59 | 4.68 | 0.02 | 0.09 | 0.18 |
| ILMN\_2313158 | MBNL1 | 7.71 | 4.61 | 27.65 | 8.94 | 5.38 | 1.86 | 2.76 | 2.00 |
| ILMN\_2313158 | MBNL1 | 13.49 | 7.43 | 27.14 | 15.58 | 8.64 | 3.99 | 3.49 | 2.42 |
| ILMN\_2313158 | MBNL1 | 7.10 | 4.30 | 23.95 | 9.45 | 5.64 | 3.34 | 3.24 | 2.28 |
| ILMN\_2313158 | MBNL1 | 7.63 | 4.57 | 24.54 | 8.51 | 5.16 | 3.04 | 2.46 | 1.82 |
| ILMN\_2313158 | MBNL1 | 5.74 | 3.59 | 26.90 | 6.25 | 3.97 | 1.95 | 3.18 | 2.24 |
| ILMN\_2313158 | MBNL1 | 7.92 | 4.71 | 28.03 | 8.75 | 5.28 | 3.40 | 3.07 | 2.18 |
| ILMN\_2398939 | MBP | 5.40 | 3.41 | 16.41 | 6.38 | 4.04 | 3.34 | 3.13 | 2.21 |
| ILMN\_1710752 | NAPRT1 | 8.45 | 4.98 | 32.08 | 9.49 | 5.66 | 3.54 | 2.53 | 1.86 |
| ILMN\_2121437 | NCL | 7.31 | 4.40 | 14.20 | 8.80 | 5.31 | 4.64 | 4.04 | 2.72 |
| ILMN\_1675038 | PRMT2 | 4.81 | 3.09 | 12.49 | 8.01 | 4.90 | 11.59 | 5.49 | 3.52 |
| ILMN\_1799381 | SNORD14A | 7.31 | 4.40 | 17.08 | 9.21 | 5.52 | 3.55 | 3.33 | 2.33 |
| ILMN\_1786426 | TMEM149 | 12.16 | 6.80 | 59.73 | 13.84 | 7.81 | 2.96 | 3.44 | 2.39 |
| ILMN\_1786426 | TMEM149 | 6.21 | 3.84 | 58.02 | 6.86 | 4.29 | 3.12 | 3.52 | 2.43 |
| ILMN\_1786426 | TMEM149 | 7.30 | 4.40 | 38.83 | 8.63 | 5.22 | 3.88 | 3.14 | 2.22 |
| ILMN\_1786426 | TMEM149 | 8.55 | 5.03 | 58.70 | 7.96 | 4.87 | 4.16 | 4.37 | 2.91 |
| ILMN\_1786426 | TMEM149 | 5.80 | 3.62 | 59.14 | 6.98 | 4.36 | 2.67 | 3.28 | 2.29 |
| ILMN\_1786426 | TMEM149 | 6.22 | 3.84 | 59.59 | 6.94 | 4.34 | 4.36 | 4.04 | 2.72 |
| ILMN\_1786426 | TMEM149 | 9.44 | 5.48 | 38.87 | 11.23 | 6.54 | 2.47 | 2.70 | 1.96 |
| ILMN\_1743646 | VASP | 5.09 | 3.24 | 32.38 | 10.79 | 6.32 | 4.57 | 3.98 | 2.70 |

**Table 8 |** -log10 pvalues and estimates of the R2 from the top interacting pair of SNPs when a 5kb region surrounding each epistasis snp. The analysis was done using the original phenotype and a phenotype adjusted for the InCHIANTI SNP. In support of the haplotype effect for the three probes, no fined mapped epistasis signals are found for LAX and ATP13A1.