

BRIEF COMMUNICATIONS

Monozygotic twins discordant for intermittent allergic rhinitis differ in mRNA and protein levels

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Abstract

Monozygotic (MZ) twins discordant for complex diseases may help to find disease mechanisms that are not due to genetic variants. Intermittent allergic rhinitis (IAR) is an optimal disease model because it occurs at defined time points each year, owing to known external antigens. We hypothesized that MZ twins discordant for IAR could help to find gene expression differences that are not dependent on genetic variants. We collected blood outside of the season from MZ twins discordant for IAR, challenged their peripheral blood mononuclear cells (PBMC) with pollen allergen *in vitro*, collected supernatants and isolated CD4+ T cells. We identified disease-relevant mRNAs and proteins that differed between the discordant MZ twins. By contrast, no differences in microRNA expression were found. Our results indicate that MZ twins discordant for IAR is an optimal model to identify disease mechanisms that are not due to genetic variants.

The existence of monozygotic (MZ) twins who are discordant for intermittent allergic rhinitis (IAR) suggests disease mechanisms that are independent of genetic variants (1). mRNA microarray studies of discordant MZ twins have been performed in other complex diseases, such as bipolar disorder (2), SLE (3) and multiple sclerosis (MS) (4). The study of twins with MS found no differences in mRNA expression. In contrast to MS, IAR occurs at defined time points each year, owing to known external antigens. Here, we, for the first time, performed mRNA and microRNA expression microarray analyses of CD4+ T cells from MZ twins discordant for IAR. We analysed the CD4+ T cells outside the pollen season, after *in vitro* allergen challenge and during the season without *in vitro* allergen challenge.

Eleven MZ twin pairs discordant for IAR were recruited, of which six were Italian. Zygosity was tested using The AmpFISTR® IdentifierTM PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA) and likelihood ratio

analysis. IAR was defined by a positive disease history and a positive RAST test (Phadia, Uppsala, Sweden) for birch and/or grass pollen. All affected Italian siblings were sensitized to grass, and one also to birch. Three Swedish twin pairs were sensitized only to grass, one pair only to birch and one pair to both grass and birch. All healthy siblings were negative to birch and grass. We collected four materials. (i) For the study of allergen- or diluent-challenged CD4+ T cells, peripheral blood mononuclear cells (PBMC) were collected outside season from all eleven twin pairs and challenged with allergen (birch or grass; ALK Abelló, Hørsholm, Denmark; 100 µg/ml at a density of 10⁶ cells/ml) for 7 days (5). CD4+ T cells were isolated using a MACS® LS column and human CD4+ microbeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Such a challenge mimics *in vivo* conditions in that PBMC contain both antigen-presenting and CD4+ T cells. (ii) We also collected CD4+ T cells from four twin pairs during the season, when the sibling with IAR was symptomatic. To

independently validate the results, we performed gene expression microarray studies of (iii) allergen- and diluent-challenged CD4⁺ T cells from 10 unrelated patients with IAR and 10 controls, and (iv) unchallenged CD4⁺ T cells from 9 patients and 10 healthy controls, obtained during the pollen season. Preparation of cRNA and hybridization to Illumina Human-6 v2 Expression BeadChips was performed according to the manufacturer's instructions (Illumina Inc., San Diego, CA, USA). We also performed microRNA expression arrays, using the Illumina Human v2 miRNA panel. Both the mRNA and microRNA expression microarray data have been deposited at the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>). The differentially expressed genes were identified using the paired lmFit function in the Bioconductor package LIMMA and false discovery rate (FDR) to correct for multiple testing. In allergic patients, allergen induces the activation of Th2 cells, which release the Th2 cytokines IL-4, IL-5 and IL-13 (6). We measured these cytokines in the supernatants of allergen-challenged PBMC with ELISAs (Bender MedSystems GmbH, Vienna, Austria).

The allergen challenge *in vitro* resulted in significant differences in mRNA and protein levels between the allergic and healthy twins. The cytokines IL-4, IL-5 and IL-13 were increased in the supernatants from allergic twins (Fig. 1A). This supported the biological relevance of the study model. The microarray analysis of CD4⁺ T cells extracted from the allergen-challenged PBMC showed altered expression of 99 genes (Table S1). These genes were validated by the analysis of GO term similarity with an independent mRNA microarray study of allergen/diluent-challenged CD4⁺ T cells from unrelated patients and controls (see Data S1). The differentially expressed genes in the twin study were significantly enriched for pathways of known relevance for allergy, for example T-helper cell differentiation, and included several key allergy genes, such as *IL17RB*, *IL13* and *IL4R* (Data S1). Quantitative RT-PCR analysis, with TaqMan[®] gene expression assays (Applied Biosystems), confirmed increased expression of these genes in allergic twins ($P \leq 0.01$; one-sample *t* tests, $\mu = 1$) (Fig. 1B). In our study, we performed microRNA expression arrays of all the twin pairs, but found no significant differences. Next, blood samples were taken during pollen season from four MZ twin pairs discordant for IAR. Gene expression microarray analysis of CD4⁺ T cells extracted from PBMC was performed and subjected to a paired Limma test and FDR correction. In contrast to allergen challenge *in vitro*, we found no significant differences. Quantitative RT-PCR showed a nonsignificant 1.1-fold difference in *IL4R* between the siblings and that *IL17RB* and *IL13* were not detectable. We also examined unchallenged CD4⁺ T cells from unrelated patients ($n = 10$) and controls ($n = 9$). Similarly, no significant differences in gene expression were found. A recent large gene expression microarray study of unstimulated CD4⁺ T cells found no differences between allergic and nonallergic individuals (7). However, using RT-PCR, which is more sensitive than microarrays, reliable differences were observed. Thus, a likely explanation is that the frequency of circulating allergen-specific Th2 cells is too low to detect gene expression changes using microarrays. Another possibility is that sensitization to

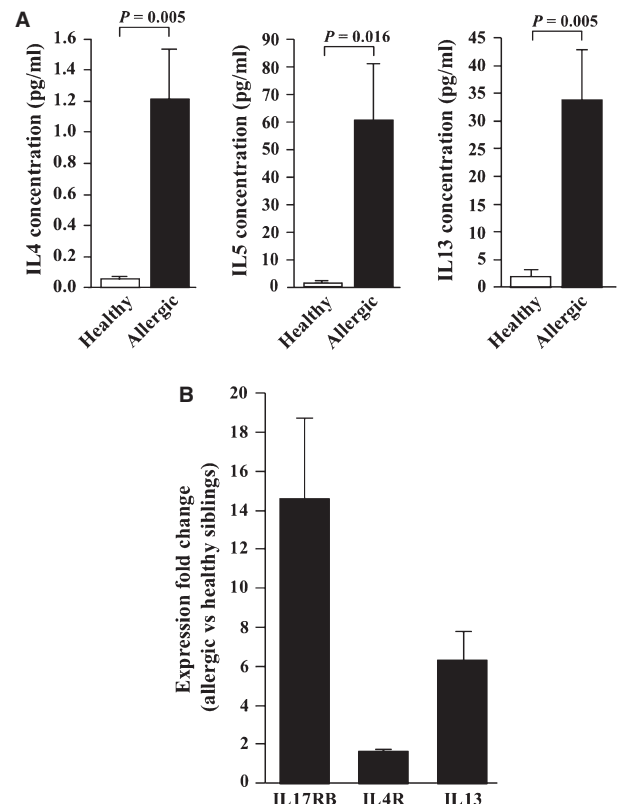


Figure 1 Monozygotic twins discordant for intermittent allergic rhinitis differ in mRNA and protein expression. (A) Cytokine concentrations in the supernatant of allergen-challenged peripheral blood mononuclear cells (PBMC) from allergic and healthy twins ($n = 11$ of each). Data are presented as mean and standard error of mean (SEM). *P* values were determined using paired two-sample *t* tests. (B) Quantitative RT-PCR analysis of key allergy genes in CD4⁺ T cells from allergen-challenged PBMC. Values are normalized against RPL13A and EF1A2 and correspond to the average fold change in expression between 9 and 10 allergic twins and their corresponding healthy siblings (1–2 pairs had to be excluded because of low mRNA quantity). Error bars represent SEM.

aeroallergens other than those tested could confound the results. On the other hand, using RT-PCR, we found no detectable levels of two of three tested genes in unchallenged cells, even though microarrays showed significant changes in gene expression following *in vitro* allergen challenge. This supports that *in vitro* challenge studies with the cognate allergen may be needed for gene expression studies of CD4⁺ T cells in IAR.

The relevance of our findings to IAR lies in that they indicate that because the MZ twins are almost genetically identical, the observed altered expression of important disease-associated genes and proteins may have epigenetic causes. Although we could not link the differences to microRNAs, future studies of other possible causes such as altered DNA methylation are warranted. In summary, our results indicate that studies of allergen-challenged CD4⁺ T cells from MZ twins discordant for IAR may help to identify genome-wide expression changes, as well as to identify epigenetic disease mechanisms.

Authors' contributions

A.K.S. designed the research, acquired and analysed the data and wrote the paper; F.B. designed the research, analysed the data and wrote the paper; M.G. performed bioinformatic array analyses; A.M. acquired the data; P.S. designed the research and analysed the data; H.W. designed the research and acquired data. M.B. designed the research and wrote the paper.

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Conflict of interest

The authors have declared that they have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supporting analyses of gene expression microarray data.

Table S1. Differentially expressed genes in CD4+ T cells extracted from allergen-challenged PBMC from MZ twins discordant for intermittent allergic rhinitis.

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¹Online supplement

²**Monozygotic twins discordant for intermittent allergic rhinitis** ³**differ in mRNA and protein levels**

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7Materials

8Four materials were included in the study:

91) The *in vitro* study of monozygotic twins. Peripheral blood mononuclear cells (PBMC)
10were collected outside season from all eleven twin pairs and challenged with allergen, and
11CD4 + T cells isolated for microarray analysis. All subjects were asymptomatic when the
12samples were obtained. The median age of the Italian twins was 26 (18-50), of which one pair
13was female. The Swedish twins were 37 (35-40) years and three were female.

142) The *in vivo* study of monozygotic twins. CD4 + T cells from four twin pairs were obtained
15during the season, when the sibling with IAR was symptomatic. These and the remaining
16subjects were Swedish.

17

18To independently validate the results we performed additional gene expression microarray
19studies:

20 3) The *in vitro* study of unrelated subjects. Allergen challenged CD4 + T cells were obtained
21from 10 unrelated patients with IAR, of which 5 were female, and from 10 healthy controls,
22of which 7 were female. These samples were obtained outside of the pollen season, when all
23subjects were asymptomatic.

244) The *in vivo* study of unrelated subjects. Unchallenged CD4 + T cells were obtained from 9
25patients, of which 4 were female, and 10 healthy controls, of which 5 were female. The
26median age of the patients was 28 (22 - 35) and of the controls 19 (16 - 37) years. These cells
27were obtained during the pollen season, when the patients with IAR were symptomatic. All
28patients and controls were examined for sensitization against birch and pollen using RAST
29(Phadia, Uppsala, Sweden). All affected Italian siblings were sensitised to grass, and one also
30to birch. All control siblings had negative tests to birch and grass. Three of the affected

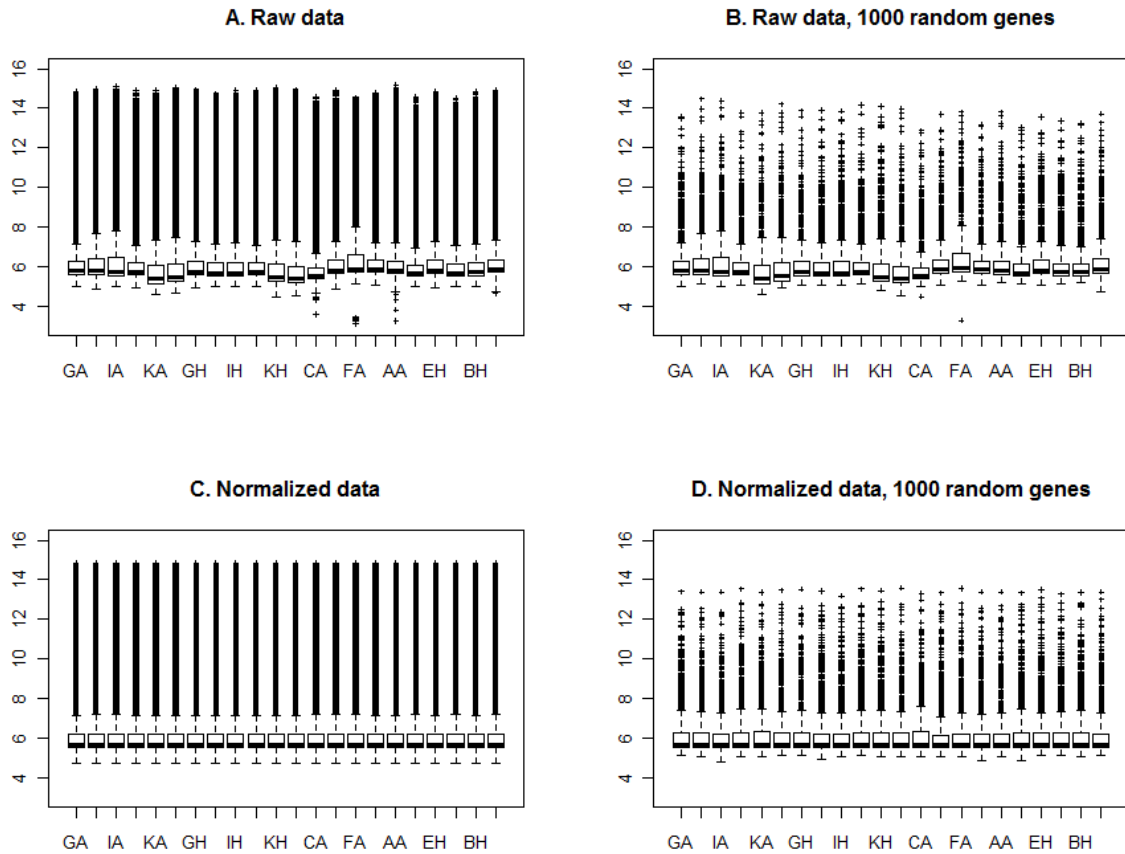
31 Swedish twin pairs were sensitised only to grass, one pair only to birch and one pair to both
32 grass and birch. All controls were negative to grass and birch. In the *in vivo* study of unrelated
33 subjects, six patients were only allergic to grass while three were allergic to birch. The
34 controls were all negative for birch and grass.

35 Data pre-processing

36 1.1 *In vitro* twin study

37 In what follows, analysis of allergen/diluent challenged CD4 + T cells from the monozygotic
38 twins will be referred to as the *in vitro* twin study, while the study of CD4 + T cells obtained
39 during the pollen season (without *in vitro* challenge) will be referred to as the *in vivo* twin
40 study. Raw microarray data was normalized using quantile normalization. This is one of the
41 most widespread normalization methods. It makes the basic assumption that most genes do
42 not differ between the two experimental groups (in this case the allergic and healthy sibling).
43 Thus, it shifts the median, upper and lower quantiles of all samples so that they are identical.

44 Before normalization the median expression values (after $\log_2$ transformation) ranged between
45 5.35 and 5.88 and the 75% quantile ranged between 5.91 and 6.62. The outcome was similar
46 in the other two datasets (not shown).



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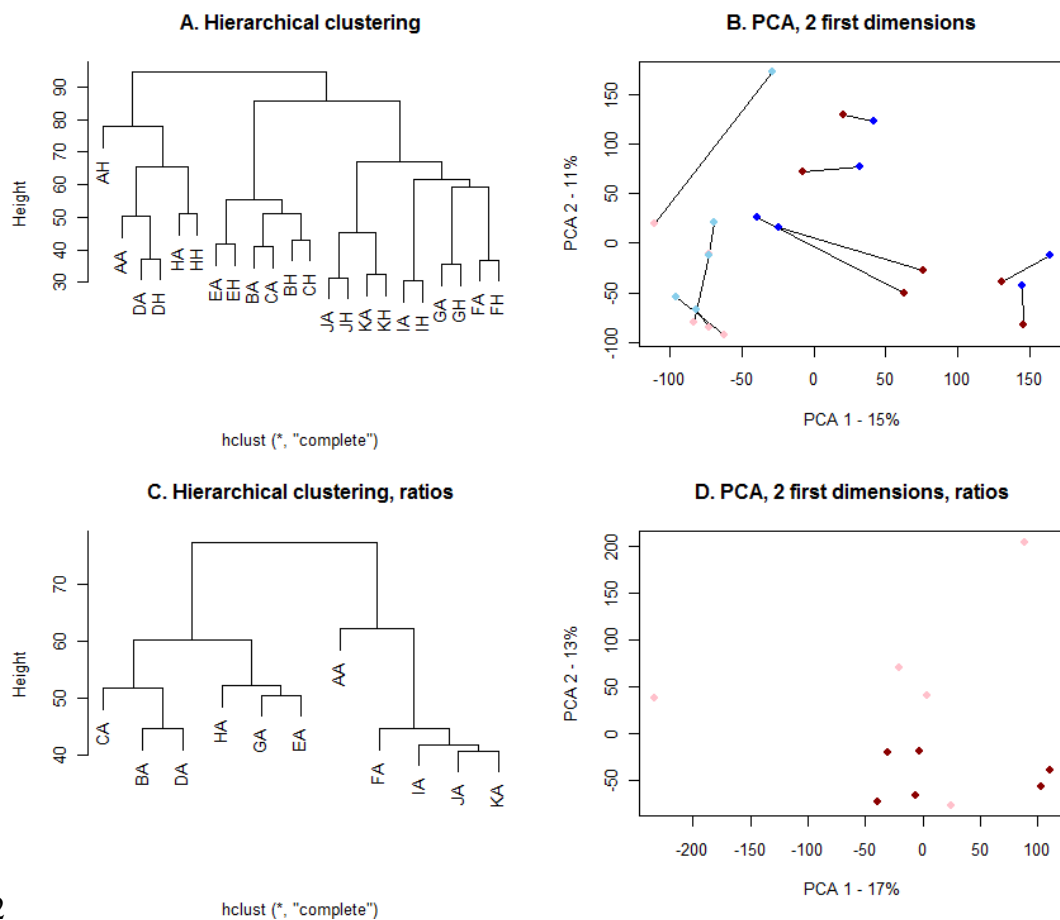
48**Supplemental figure 1.** Sample expression distribution before normalization (panel A and B)
 49and after normalization (panel C and D). Since each sample has almost 50 000 probes, the
 50panel A and C are saturated with data points. For clarity, panel B and D only shows 1000
 51random probes.

52Preliminary data analysis

53This study was based on microarray data from twin pairs in which one sibling was allergic. To
 54compare the variation between healthy and allergic sibling to the variation between unrelated
 55individuals we performed hierarchical clustering analysis and principle component analysis
 56(PCA) on the normalized microarray data.

571.2 *In vitro* twin study

58The *in vitro* microarray data was obtained from Swedish and Italian twins. The preliminary
59data analysis gave an overview of sources of variation in the data. This enabled the
60comparison of variations between (a) allergic twins and their healthy siblings, (b) individuals
61from unrelated families and (c) Swedish and Italian twin pairs.



62

63**Supplemental figure 2.** Hierarchical clustering and PCA of *in vitro* twin data. (A)
64Hierarchical clustering. The first letter denotes the twin pair, the second letter denotes allergic
65or healthy status, A and H, respectively. Swedish twin pairs: A-E, Italian twin pairs: F-K. (B)
66PCA of *in vitro* twin data. Healthy subjects are blue, allergic subjects are red. Italian subjects
67are shown in darker colours and Swedish subjects are shown in brighter colours. (C)
68Hierarchical clustering of ratios between allergic and healthy siblings. (D) PCA of ratios

69between allergic and healthy siblings. Italian subjects are shown in darker colours and
70Swedish subjects are shown in brighter colours.

71The hierarchical clustering (Fig 2A) showed that each each subject clustered closely with his
72or her sibling, meaning that the allergic phenotype caused less variation than the variation
73between different twin pairs. Most Italian subjects clustered together closely, but this was not
74the primary source of variation (the first branch in the hierarchical clustering produced one
75branch containing two Swedish and one Italian subject).

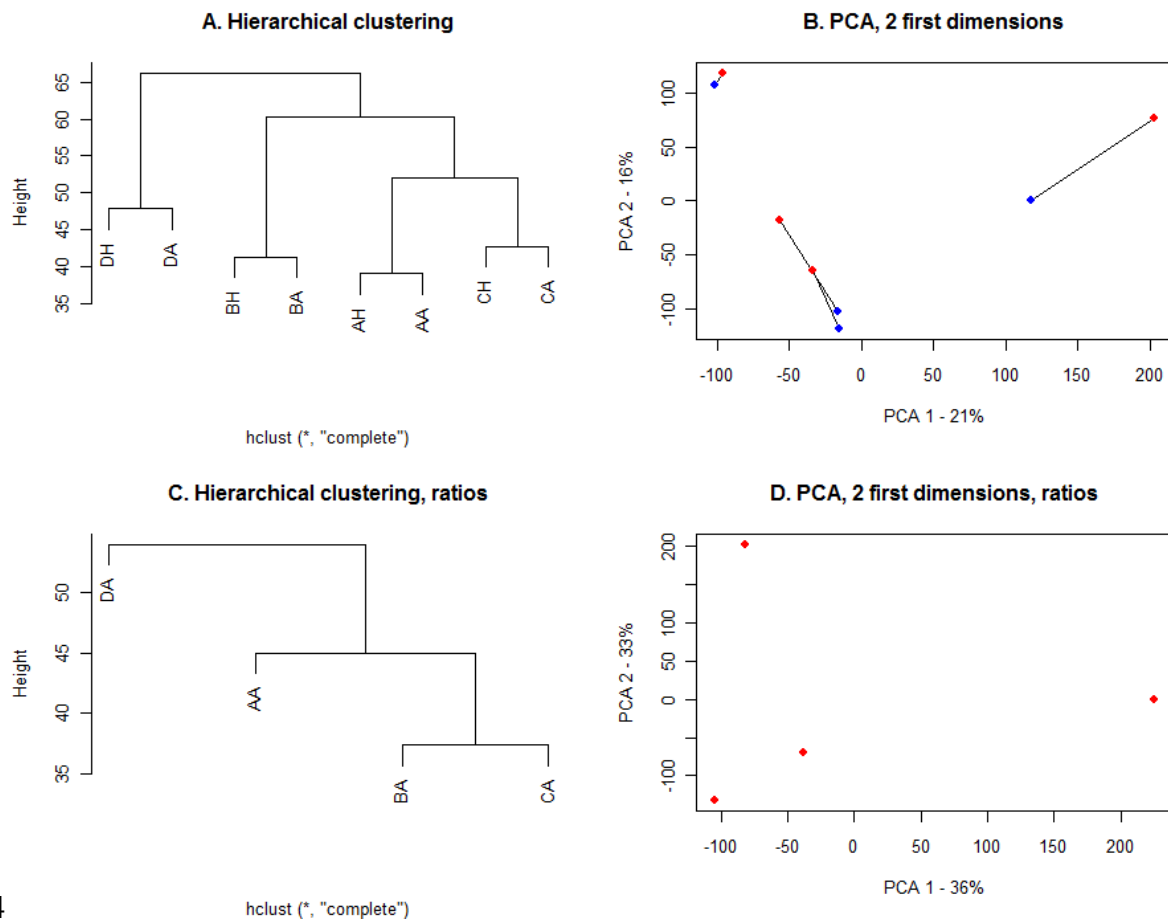
76The PCA (Fig 2B) confirmed that most siblings were quite similar in the microarray data. It
77also supported that the variation between unrelated twin pairs was greater than the variation
78between allergic and healthy siblings, and that there was some separation between the
79Swedish and Italian subjects. The second principal component (y-axis) suggested that the
80variation between the allergic and healthy siblings was consistent (in 10 of the 11 twin pairs
81the allergic twin had a lower value than its healthy sibling). However, these two components
82only captured about 25% of the total variation between individuals, so the conclusions that
83can be drawn from this plot were limited.

84One advantage of twin studies is that the family association between siblings enables the use
85of paired statistical methods, which can add considerable statistical power. To evaluate how
86the paired analysis between siblings eliminated other sources of variation (such as the
87difference between Italian and Swedish subjects) we repeated the hierarchical clustering
88analysis and PCA analysis using the $\log_2$ ratio between the allergic and healthy siblings
89instead of using each subject individually (in other words, each twin pair was treated as a
90single experimental sample, fig 2C and 2D).

91 **1.3 *In vivo* twin study**

92The *in vivo* data was obtained from 4 Swedish twin pairs. The hierarchical clustering and

93PCA was also performed on this material (supplemental figure 3).



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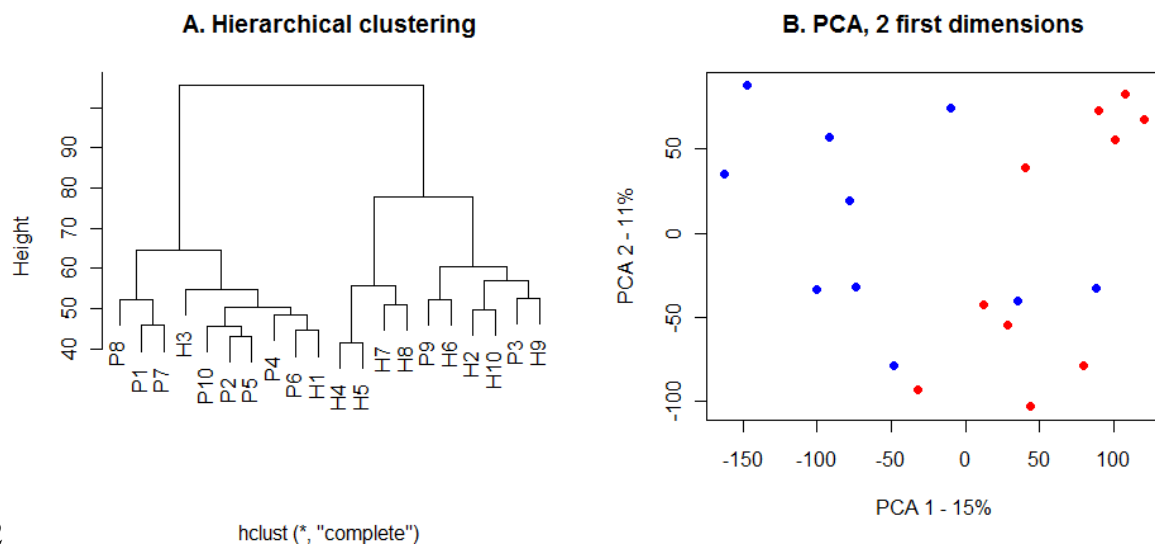
95**Supplemental figure 3.** Hierarchical clustering and PCA of *in vivo* twin data. (A)

96Hierarchical clustering. The first letter denotes the twin pair, the second letter denotes allergic
 97or healthy status. (B) PCA of *in vivo* twin data. Healthy subjects are blue, allergic subjects are
 98red. (C) Hierarchical clustering of ratios between allergic and healthy siblings. (D) PCA of
 99ratios between allergic and healthy siblings.

100The hierarchical clustering and PCA plot (panel A and B) showed that under *in vivo*
 101conditions, the variation between unrelated families was greater than the variation between
 102twin siblings. However, the relation between the healthy and allergic sibling in twin pair D
 103differed from the remaining twin pairs. A possible explanation was that both siblings in this
 104twin pairs had diabetes.

105**1.4 In vitro study of unrelated subjects with and without IAR**

106In order to examine if the differentially expressed genes from the *in vitro* twin studies also
107differed in unrelated subjects, we performed an extended analysis of allergen-challenged CD4
108+ T cells from 10 allergic patients and 10 healthy controls. This study will henceforth be
109referred to as the *in vitro* study of unrelated subjects. All the subjects were Swedish, meaning
110that the two sources of variation were (a) variance between patients and healthy controls and
111(b) the variation between individuals.



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hclust (*, "complete")

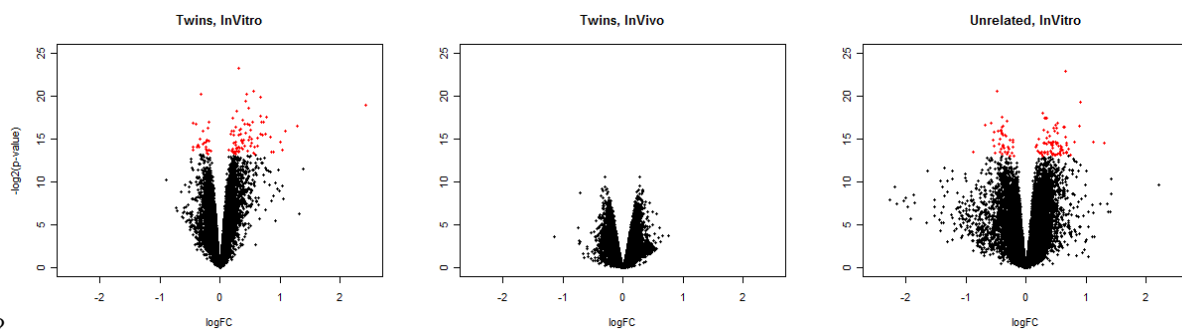
113**Supplemental figure 4.** Hierarchical clustering and PCA of *in vitro* data from unrelated
114subjects. (A) Hierarchical clustering. Allergic patients and healthy subjects are distinguished
115by the first letter, P and H, respectively. (B) PCA of *in vitro* data from unrelated subjects.
116Healthy subjects are blue, allergic subjects are red.

117The hierarchical clustering and PCA showed that when patients and healthy controls were
118not twins, allergic disease caused some separation.

119 **Analysis to define differentially expressed genes in the *in vitro* and *in vivo***

120 **materials from twins and unrelated subjects**

121 Differentially expressed genes were determined using the `lmFit` function from the
122 Bioconductor package LIMMA in the R language. The *in vitro* twin study detected 102
123 probes (supplementary table 1) and the *in vitro* study of unrelated subjects detected 1655
124 probes (the differences in the number of differentially expressed genes is discussed in section
1257). A gene was determined as differentially expressed if a corresponding probe had an
126 adjusted p-value < 0.05. The *in vivo* analysis did not show significant differential expression
127 in any probe. This was not changed by removing twin pair D.

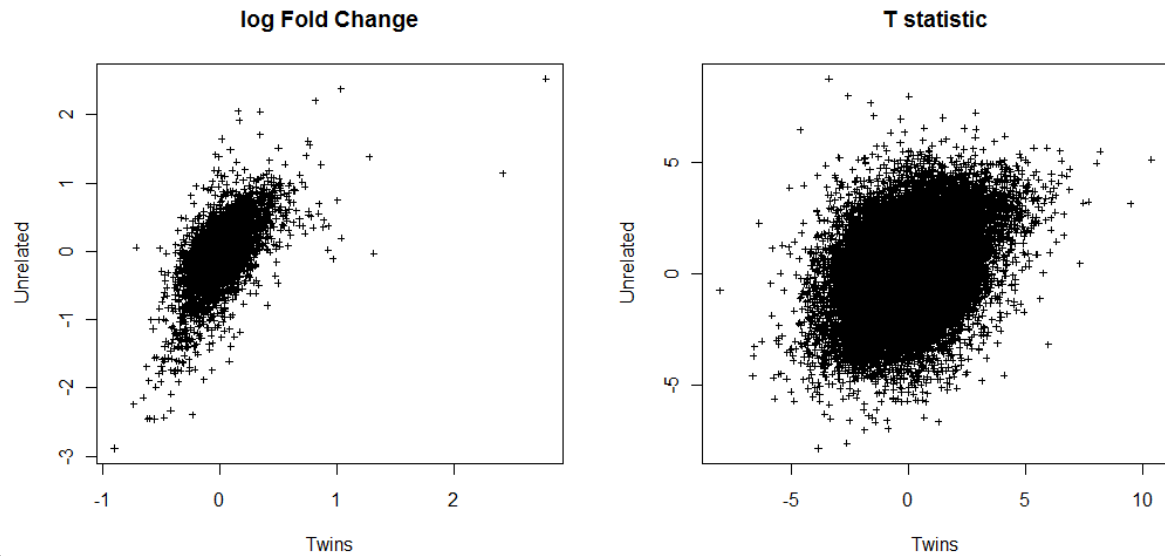


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129 **Supplemental figure 5.** Volcano plots of the differential expression analysis in the *in vitro*
130 twin study (left), *in vivo* twin study (centre) and the *in vitro* study of unrelated subjects (right).
131 Differentially expressed genes are colored red.

132 **Reproducibility analysis between twins and unrelated subjects**

133 To test the reproducibility between the results from the *in vitro* twin dataset and the dataset
134 from *in vitro*-challenged unrelated individuals, we compared log fold change and T statistic
135 for each probe in the respective datasets.

136 The Pearson correlation coefficient between the log fold changes was 0.52 ($p < 10^{-15}$). The
137 corresponding correlation coefficient for the T statistic was 0.30 ($p < 10^{-15}$).



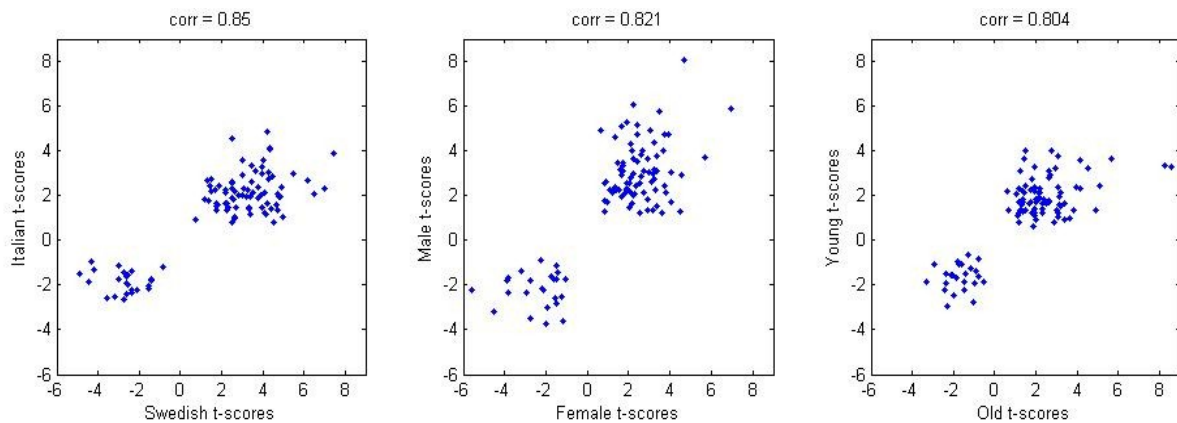
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139**Supplemental figure 6.** (Left) The log fold change in the *in vitro* twin analysis compared to
 140the *in vitro*-challenged unrelated subjects. (Right) The T statistic in the *in vitro* twin analysis
 141compared to the unrelated subjects.

142**Analysis of possible effects of nationality, gender and age in the *in vitro* twin study**

144In order to examine if the differentially expressed genes in the whole *in vitro* twin data set
 145only differed in subsets of the data (male/female, Swedish/Italian, younger/older), we
 146analyzed subsets of the data, stratified for these variables. We found that the tendencies of the
 147whole dataset remained in all the stratified subsets of the data. This was evident from
 148supplemental figure 6, where we have plotted the t-scores of the differentially expressed
 149genes from the different stratified subsets against each other. We found very good correlations
 150between the stratified subgroups and also a high correlation of all individual subsets to the full
 151data (all were above 0.88).

152



153

154**Supplemental figure 7.** Scatter plot of the t-scores of the differentially expressed genes in
 155stratified subgroups of the whole *in vitro* data. The stratification was performed based on
 156nationality, gender and age (defined as above/below median age).

157**Analysis of GO term similarity between twins and unrelated subjects**

158In order to systematically compare similarity between the differentially expressed genes in
 159the *in vitro* twin study and the *in vitro* study of unrelated individuals, we focused on the
 160biological processes that those genes were involved in. Here, we utilized GOSim (1) for
 161biological processes, as implemented by Yang and colleagues

162[<http://www.cs.rhul.ac.uk/home/haixuan/GOSIM.html>], which measures the pairwise distance

163between the genes in the GO tree to form a similarity score. We formed a similarity score

164between the gene lists from the pairwise gene similarities. This was made by normalizing

165each similarity score between gene pairs by the geometric mean of the gene self-similarities.

166Then we performed a Kolmogorov-Smirnov test of the empirical distribution of the similarity

167scores versus similarity scores between all annotated genes. This test is distribution free, and

168generates a low p-value for the null hypothesis if an unexpectedly high proportion of the

169samples have too high values. The test utilizes the maximum distance (greater than 0)

170between the empirical cumulative distributions of all the gene GO-similarities of the lists

171versus all the gene GO-similarities of all genes (Smirnov-Kolmogorov test).

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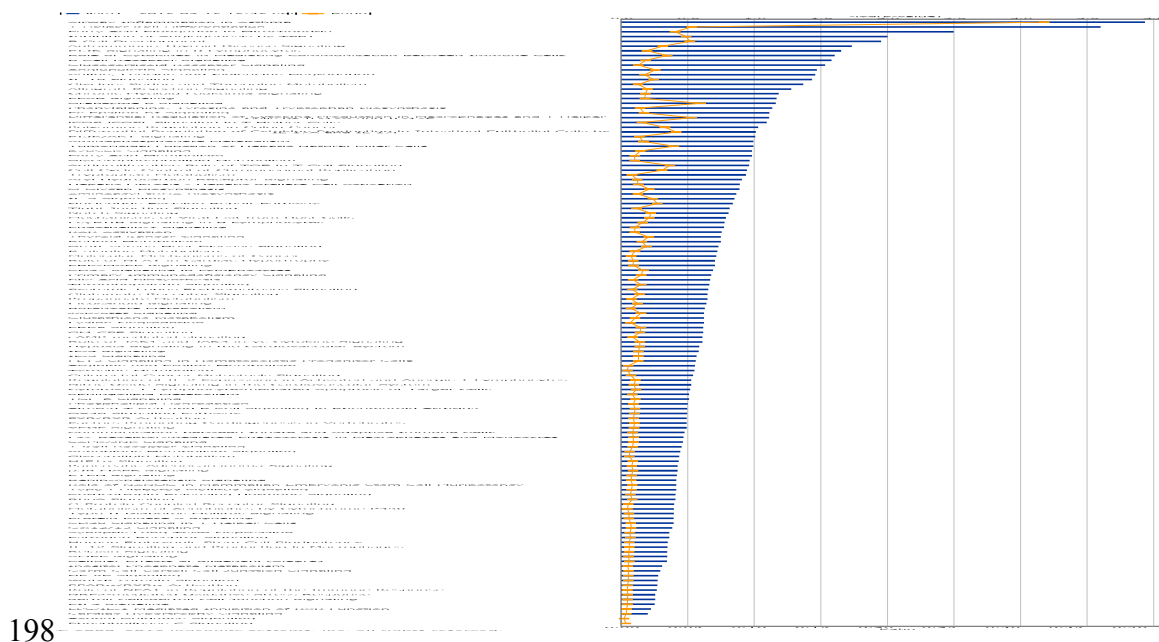
173Here, we found that that the list of differentially expressed genes from the *in vitro* twin study
174contained 99 genes (0.40%), and from the non-twin study 1655 (6.86%) of all the genes.
175Therefore, the overlapping 35 genes (0.15%) was 5.3 times higher than expected by chance
176only. Moreover, we found that the test-statistics of the similarity between the genes within
177each of the lists for the *in vitro* twin study and the *in vitro* study of unrelated subjects were
1786.2% ($p=5e-13$) and 1.2% ($p=6e-112$) respectively. For the cross-similarity (i.e. the similarity
179between the gene lists) we found a test-statistic of 2.9% ($P=9e-45$). This meant that we had
180the greatest GO-similarity in the twin list, but owing to the smaller size it had the highest P-
181value.

182The larger number of differentially expressed genes in the *in vitro* study of unrelated subjects
183compared to the *in vitro* twin study, probably depended on larger inter-individual differences,
184since the differentially expressed genes in the *in vitro* twin study were involved in more
185similar biological processes.

186Pathway analysis

187The differentially expressed genes in the *in vitro* twin study included several of known
188relevance for allergy, such as *IL5*, *IL13*, *IL17RB*, *IL4R*, *TNFRSF4* and *GZMB*. The Ingenuity
189Pathways Analysis (IPA) software (<http://www.ingenuity.com/>) was used to map the genes
190that differed in expression between the allergic and healthy monozygotic twin pairs onto
191known canonical, cellular and molecular pathways (2). We employed a Fisher's exact test to
192calculate a p value determining the probability whether the association between the proteins in
193the dataset and the canonical pathway can be explained by chance alone. Pathways with a p
194value less than 0.05 were considered to be statistically significant. The most significant
195pathways were *airway inflammation in asthma* and *T helper cell differentiation*, Other

196disease-relevant pathways were *glucocorticoid receptor signaling* and *IL10 signaling*
197(supplemental figure 8)



199**Supplemental figure 8.** Pathways that were enriched in allergen-challenged CD4 + T cells
200from allergic compared to healthy monozygotic twins. A total of 99 transcripts were identified
201by microarray analysis of the CD4 + T cells and mapped onto canonical pathways using the
202IPA software. The yellow threshold indicates a 95% confidence.

203

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Supplementary Table. Differentially expressed genes in CD4+ T cells extracted from allergen-challenged PBMC from MZ twins discordant for intermittent allergic rhinitis.

<i>Probe ID</i>	<i>Gene name</i>	<i>Fold change</i>	<i>P value</i>	<i>Adjusted P value</i>
ILMN_1767523	IL17RB	6,9	0,000000	0,001475
ILMN_1654429	C12orf48	5,3	0,000002	0,012144
ILMN_1667711	HRASLS3	2,4	0,000011	0,021559
ILMN_1798984	DACT1	2,1	0,000015	0,023893
ILMN_1725791	PTPLA	2,0	0,000038	0,034276
ILMN_1709300	IL5	1,8	0,000086	0,044999
ILMN_1800225	PPARG	1,8	0,000089	0,044999
ILMN_1676964	IL13	1,8	0,000025	0,029162
ILMN_1714335	RDH10	1,7	0,000005	0,020057
ILMN_1704369	LIMA1	1,7	0,000020	0,026471
ILMN_1691881	IL4R	1,7	0,000008	0,020057
ILMN_1760247	CD70	1,6	0,000023	0,026933
ILMN_1780599	LIF	1,6	0,000007	0,020057
ILMN_1671486	HOMER2	1,6	0,000020	0,026471
ILMN_1653280	GZMB	1,6	0,000001	0,008561
ILMN_1808391	DUSP4	1,5	0,000029	0,031947
ILMN_1790350	FAM79B	1,5	0,000055	0,040289
ILMN_1690970	LOC642233	1,5	0,000099	0,048101
ILMN_1667239	INPP1	1,5	0,000055	0,040289
ILMN_1660462	MCOLN2	1,5	0,000001	0,007860
ILMN_1782729	CLECL1	1,5	0,000095	0,046664
ILMN_1653573	LOC644846	1,5	0,000008	0,020057
ILMN_1806754	GLDC	1,4	0,000031	0,032468
ILMN_1756953	GBP6	1,4	0,000043	0,035429
ILMN_1668218	IL31	1,4	0,000014	0,023893
ILMN_1806681	CHDH	1,4	0,000053	0,040289

ILMN_1738207	CISH	1,4	0,000026	0,029805
ILMN_1707312	NFIL3	1,4	0,000009	0,020057
ILMN_1674046	C10orf128	1,4	0,000003	0,013680
ILMN_1771964	GSTA4	1,4	0,000032	0,032468
ILMN_1761820	EDARADD	1,4	0,000081	0,044234
ILMN_1793476	PRKCDBP	1,4	0,000009	0,020057
ILMN_1726913	RBPJ	1,4	0,000001	0,007860
ILMN_1823231		1,4	0,000019	0,026471
ILMN_1668411	FHL2	1,3	0,000001	0,009789
ILMN_1710204	TNFRSF4	1,3	0,000054	0,040289
ILMN_1737441	CD79B	1,3	0,000009	0,020057
ILMN_1666940	PTGER2	1,3	0,000034	0,032671
ILMN_1712413	RPL39L	1,3	0,000065	0,041880
ILMN_1783931	PPAP2A	1,3	0,000042	0,035429
ILMN_1763907	C6orf173	1,3	0,000069	0,041880
ILMN_1682054	SRI	1,3	0,000006	0,020057
ILMN_1657547	CCDC34	1,3	0,000067	0,041880
ILMN_1652797	LOC400451	1,3	0,000036	0,033604
ILMN_1665964	GAB2	1,3	0,000061	0,040559
ILMN_1745420	PHF19	1,3	0,000032	0,032468
ILMN_1708743	NT5DC2	1,3	0,000014	0,023893
ILMN_1785191	TMEM14A	1,3	0,000020	0,026471
ILMN_1708105	EZH2	1,3	0,000015	0,023893
ILMN_1716895	RPA3	1,2	0,000062	0,040559
ILMN_1660199	ACAA2	1,2	0,000084	0,044575
ILMN_1691436	BLVRA	1,2	0,000055	0,040289
ILMN_1791211	DOK2	1,2	0,000000	0,002375
ILMN_1693490	SEC11A	1,2	0,000079	0,044234
ILMN_1812503	PHKA1	1,2	0,000021	0,026471

ILMN_1653652	PTPRC	1,2	0,000082	0,044575
ILMN_1740349	AUH	1,2	0,000003	0,015765
ILMN_1715569	CCDC53	1,2	0,000061	0,040559
ILMN_1775224	NOS3	1,2	0,000067	0,041880
ILMN_1723962	LXN	1,2	0,000038	0,034276
ILMN_1781536	FAH	1,2	0,000012	0,022987
ILMN_1696627	LHFP	1,2	0,000100	0,048101
ILMN_1714951	NUDT6	1,2	0,000089	0,044999
ILMN_1738767	PLP2	1,2	0,000050	0,038884
ILMN_1766918	KCNK5	1,2	0,000084	0,044575
ILMN_1801313	SIAH2	1,2	0,000015	0,023893
ILMN_1793732	FARS2	1,2	0,000095	0,046664
ILMN_1747839	ANKRD20A1	1,2	0,000089	0,044999
ILMN_1799969	SNAPAP	1,2	0,000023	0,026933
ILMN_1896967		1,2	0,000069	0,041880
ILMN_1767691	VPS25	1,2	0,000005	0,020057
ILMN_1770936	COQ5	1,1	0,000016	0,024082
ILMN_1655046	NUTF2	1,1	0,000078	0,044044
ILMN_1804007	NANOS3	1,1	0,000021	0,026471
ILMN_1773247	ZNF410	1,1	0,000075	0,043790
ILMN_1785424	ABLIM1	-1,4	0,000008	0,020057
ILMN_1789793	NUAK2	-1,4	0,000059	0,040367
ILMN_1786429	P2RY5	-1,4	0,000070	0,041880
ILMN_1747305	GIMAP8	-1,3	0,000009	0,020057
ILMN_1748473	GIMAP4	-1,3	0,000056	0,040310
ILMN_1677186	MGC52498	-1,3	0,000047	0,037749
ILMN_1724666	INADL	-1,3	0,000059	0,040367
ILMN_1744862	TGFBR2	-1,3	0,000031	0,032468
ILMN_1718629	NRIP1	-1,2	0,000001	0,007860

ILMN_1800401	ZNF69	-1,2	0,000046	0,037742
ILMN_1791890	SPON1	-1,2	0,000016	0,023893
ILMN_1714700	TRIB2	-1,2	0,000041	0,035429
ILMN_1746436	HCG27	-1,2	0,000039	0,034276
ILMN_1667449	GCET2	-1,2	0,000067	0,041880
ILMN_1761101	CCDC112	-1,2	0,000058	0,040367
ILMN_1773427	ANKRD15	-1,2	0,000090	0,045386
ILMN_1693766	CEP135	-1,2	0,000033	0,032468
ILMN_1672940	ZNF562	-1,2	0,000077	0,044044
ILMN_1651819	GALNT11	-1,2	0,000012	0,023043
ILMN_1883154		-1,1	0,000101	0,048101
ILMN_1723287	SLC6A16	-1,1	0,000035	0,033010
ILMN_1741281	RNF175	-1,1	0,000076	0,044044
ILMN_1652762	HIC2	-1,1	0,000008	0,020057
ILMN_1701642	ABCA12	-1,1	0,000081	0,044234

Differentially expressed genes were determined using a paired Limma test and correcting for multiple testing using false discovery rate (adjusted P value).