A genome-wide association study of global gene expression

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We have created a global map of the effects of polymorphism on gene expression in 400 children from families recruited through a proband with asthma. We genotyped 408,273 SNPs and identified expression quantitative trait loci from measurements of 54,675 transcripts representing 20,599 genes in Epstein-Barr virus–transformed lymphoblastoid cell lines. We found that 15,084 transcripts (28%) representing 6,660 genes had narrow-sense heritabilities (H^2) > 0.3. We executed genome-wide association scans for these traits and found peak lod scores between 3.68 and 59.1. The most highly heritable traits were markedly enriched in Gene Ontology descriptors for response to unfolded protein (chaperonins and heat shock proteins), regulation of progression through the cell cycle, RNA processing, DNA repair, immune responses and apoptosis. SNPs that regulate expression of these genes are candidates in the study of degenerative diseases, malignancy, infection and inflammation. We have created a downloadable database to facilitate use of our findings in the mapping of complex disease loci.

Variation in gene transcription is important in mediating disease susceptibility, and global identification of genetic variants that regulate gene transcription will be helpful in mapping human disease genes. The many genome-wide association (GWA) studies currently under-way are likely to identify multiple genetic variants that are associated with multifactorial traits. We anticipate that these variants will often reside outside of coding regions and will have no known or evident functional effects. Gene transcript abundance is directly modified by polymorphism in regulatory elements and consequently may be mapped with considerable power[1,2]. As a consequence, our objective was to build a database that would allow researchers to systematically examine potential effects of disease-associated variants on transcript expression. By studying global gene expression in a comprehensive GWA study, we also hoped to add global understanding to issues of heritability, marker coverage, interactions and dominance that are of relevance to all GWA studies of complex traits.

We recruited a panel of 206 families of British descent (MRC-A) through a proband with childhood asthma, as described previously[3,4]. We included siblings regardless of asthma status[5]. The panel contained 297 sib pairs and 11 half-sib pairs. Lymphoblastoid cell lines (LCLs) were derived from peripheral blood lymphocytes on probands and siblings. Cells were harvested at log phase from roller cell cultures in the first growth after transformation. Global gene expression was measured with the Affymetrix HG-U133 Plus 2.0 chip. All analyses of transcript abundance used quantile normalization, after performing

robust multi-array averaging (RMA)[6,7], to enforce normality and deal with outliers.

RESULTS

Global gene expression

We initially considered all transcripts detected by probes on the array as independent phenotypes. As many genes are represented by more than one transcript probe, we ran parallel analyses on the mean transcript level for all probes in each gene. In general, the results were similar whether we considered transcripts or genes. We report the results for transcripts, except when biological interpretation is helped by considering genes.

The H^2 for all the expression levels after RMA and quantile normalization ranged between 0.0 and 1.0, with a mean of 0.203 and a third quartile (Q3) of 0.317 (**Fig. 1a**). We considered highly heritable expression levels to be the best evidence that a trait was genetically regulated, and we applied an arbitrary H^2 threshold of 0.3 to filter transcripts for downstream analyses. We did not apply a threshold filter for transcript abundance because we felt that genetic regulation of transcripts with low abundance might still occur and could be biologically relevant. Nevertheless, we note that the correlation between mean expression and heritability was substantial ($r = 0.45$).

Human LCLs provide general information about gene expression, even for genes whose primary function is not in these cells[1,8–10]. Although the LCLs used in our analyses were derived from children

a

b

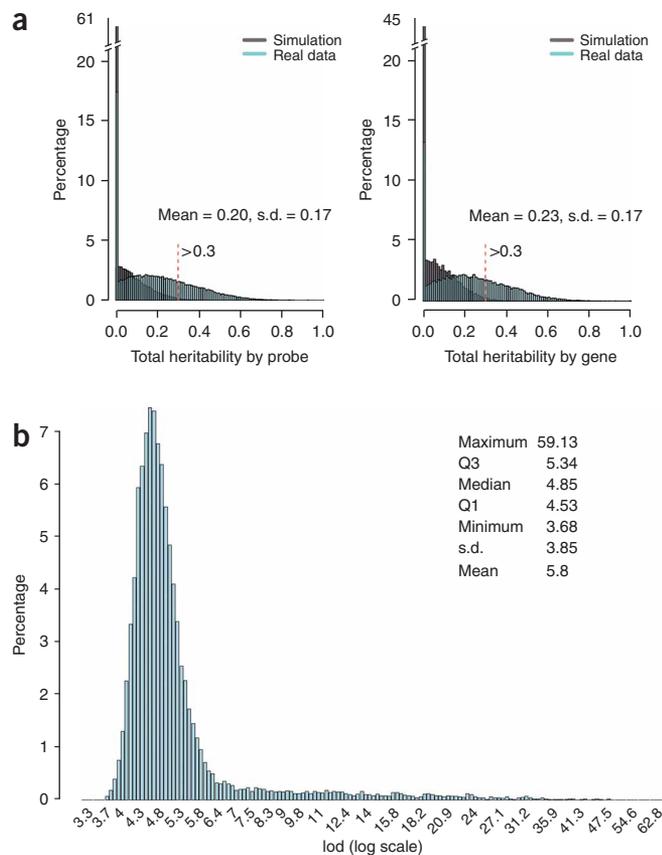|	
Maximum	59.13
Q3	5.34
Median	4.85
Q1	4.53
Minimum	3.68
s.d.	3.85
Mean	5.8

Figure 1 Summaries of heritability and association analysis for all genes. (**a**) Total heritability of expression quantitative traits. The distribution of measured heritability estimates (light blue bars) may be compared with those from simulation (gray bars). Results are shown for individual transcripts, and for mean transcript abundances for individual genes. (**b**) Distribution of lod scores for association between 14,819 traits with annotation entries in the UCSC browser with $H^2 > 0.3$ and 408,273 SNP markers.

SNPs was 80.3%. We tested for association between our genotyped SNPs and expression (**Supplementary Table 1** online) using the FASTASSOC component of MERLIN[11] and including sex in the model. We applied the method of genomic control[12] to results of the association analyses and derived a coefficient of 1.0099, indicating negligible population stratification. We found that the 14,819 traits with annotation entries in the University of California Santa Cruz (UCSC) browser and $H^2 > 0.3$ had a minimum peak lod score for association of 3.683, and a maximum of 59.128 (median 4.853, Q3 5.339) (**Fig. 1b**). We estimated the threshold for genome-wide significance to be a lod score >6.076 (equivalent to $P = 0.05$). We found that the false discovery rate (FDR) was 0.152 for a lod score of 5.5, 0.056 for a lod of 6, 0.0067 for a lod of 7.0 and 0.0008 for a lod of 8.

The mean H^2 explicable by association with the SNP showing the strongest association to each trait was 0.077 (s.d. 0.049, maximum 0.707) compared with 0.429 for the overall H^2 (s.d. 0.103, maximum 1.0), indicating that, on average, the peak SNP can explain 18.2% of the H^2 in these traits. For the 1,989 transcripts where the peak lod was >6, the mean H^2 explicable by association with the SNP showing the strongest association was 0.157, and the average overall H^2 was 0.479, indicating that 32.9% of the H^2 in these traits can be explained by the peak SNP. The proportion of peak SNPs exceeding the lod significance threshold of 6 rose with the H^2 of the underlying trait, so that 81% of traits with $H^2 > 0.8$ were associated with at least one SNPs with lod > 6 (**Fig. 2** and **Supplementary Fig. 3** online). This may suggest that increases in sample size or selection for trait distribution may have a greater impact on GWA studies compared with the effects of an increase in marker density.

We carried out a sequential search to test for transcripts independently associated with multiple SNPs. This identified 88 genes that were separately associated with three or more SNPs, which typically mapped to a combination of *cis* and *trans* locations (**Supplementary Table 2** online).

both with and without asthma, only 10 of 54,675 transcripts ($\sim 0.018\%$) differed significantly ($P < 0.0001$) between asthmatics and non-asthmatics, and we did not find any significant differences after adjusting for the number of comparisons (**Supplementary Fig. 1** online). This result is not unexpected, as we measured expression in cultured, unchallenged cell lines; many of the changes in transcript abundances previously observed in asthma cells and tissues are the result of challenge with environmental and pro-inflammatory stimuli. Consequently, we expect our experiment to inform the genetics of gene expression not only for studies of asthma but also more generally.

Genome-wide association

We genotyped 830 offspring and parents successfully with the Illumina Sentrix Human-1 Genotyping BeadChip (concentrated on genes and surrounding sequences, producing 89,653,540 genotypes for 109,157 SNPs with a 36.0% average heterozygosity and a 99.0% average call rate). We genotyped 378 offspring for 317,149 SNPs using the Illumina Sentrix HumanHap300 BeadChip (derived from HapMap phase I). Of these, 18,033 overlap with Illumina Human-1 BeadChip, so that overall the Sentrix chip resulted in an additional 112,464,123 genotypes for 299,116 SNPs with 34.5% average heterozygosity (99.5% call rate). We found only 0.321 mendelian errors per SNP among the 109,157 markers typed on all family members using the Sentrix chip. Genotypes that were not consistent with mendelian inheritance were excluded from subsequent analyses.

The minimum allele frequency (MAF) was predominantly > 0.1 (**Supplementary Fig. 2** online). Our 408,273 genotyped SNPs included 372,821 common SNPs (MAF > 0.05) from the HapMap database. These covered 1,794,828 HapMap SNPs (including the 372,821) at $R^2 > 0.8$, so that the total coverage of the 2,236,212 HapMap common
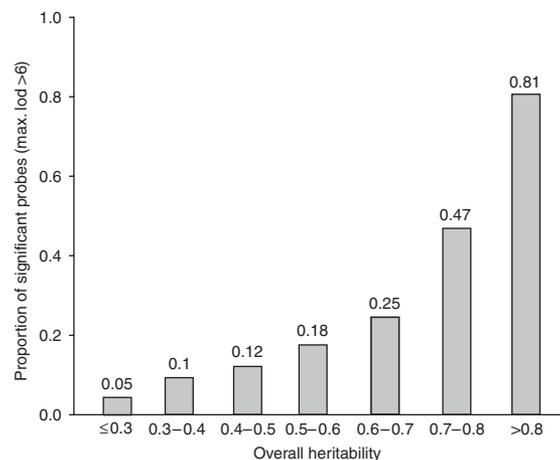

Figure 2 Proportion of significantly associated SNPs and expression trait heritability. All analyses were adjusted for sex. The proportion of significantly associated SNPs rises with the measured heritability of individual traits.
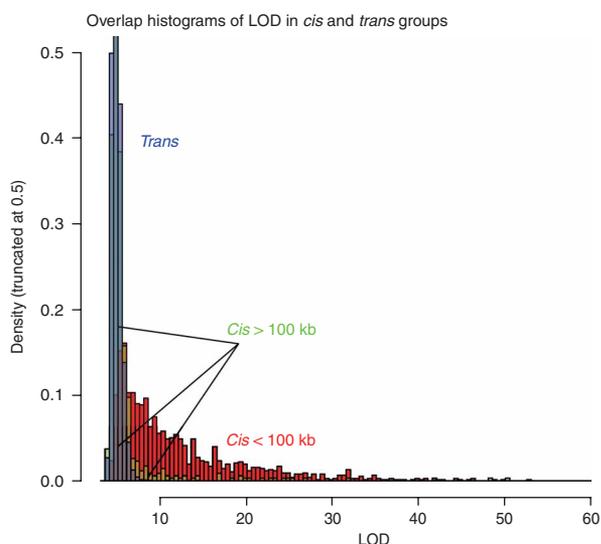
Overlap histograms of LOD in *cis* and *trans* groups

Figure 3 Associations in *cis* and *trans*. The density scale on the *y* axis is truncated at 0.5. Signals in *cis* within 100 kb of the transcription unit are shown in red; signals in *cis* distant (>100 kb) from the transcription unit are shown in green; signals in *trans* are shown in blue. The overlap of the *cis* loci >100 kb from the start with the other distributions is shown orange and gray.

Table 1 Gene Ontology of exceptionally heritable (positive *Z* score) and non-heritable (negative *Z* score) traits

Gene Ontology (GO) biological process	GO ID	H^2	Z for H^2
Response to unfolded protein	6,986	0.38	9.03
Regulation of progression through cell cycle	74	0.26	8.2
RNA processing	6,396	0.3	7.85
DNA repair	6,281	0.29	7.81
Protein folding	6,457	0.3	7.8
Immune response	6,955	0.26	7.62
Regulation of I-kappaB kinase/NF-kappaB cascade	43,123	0.28	6.84
Mitosis	7,067	0.3	5.82
Intracellular signaling cascade	7,242	0.26	5.72
Regulation of transcription	45,449	0.29	5.7
Regulation of viral genome replication	45,069	0.39	5.47
Protein biosynthesis	6,412	0.26	5.45
Vesicle-mediated transport	16,192	0.27	5.02
Cytokinesis	910	0.32	4.94
Protein complex assembly	6,461	0.25	4.67
DNA replication	6,260	0.27	4.61
Phosphoinositide-mediated signaling	48,015	0.32	4.59
Humoral immune response	6,959	0.3	4.47
Apoptosis	6,915	0.24	4.42
Phagocytosis; engulfment	6,911	0.1	–3.06
Cell adhesion	7,155	0.18	–3.12
G protein signaling	7,187	0.14	–3.14
Calcium-dependent cell-cell adhesion	16,339	0.12	–3.14
Cell-cell signaling	7,267	0.18	–3.25
Cell-cell adhesion	16,337	0.15	–3.47
Adenylate cyclase activation	7,190	0.11	–3.55
Sodium ion transport	6,814	0.13	–3.63
Phospholipase C activation	7,202	0.12	–3.74
Potassium ion transport	6,813	0.14	–4.43
Glutamate signaling pathway	7,215	0.08	–4.52
Synaptic transmission	7,268	0.16	–5.64

The analysis compared the mean total H^2 of transcripts in an individual GO category with the mean total H^2 of all 54,675 transcripts.

Previous studies have shown the power of expression quantitative trait locus (eQTL) mapping, but they have examined limited numbers of transcripts or markers in a small number of CEPH pedigrees[1,2,9]. In order to investigate the impact of sample size, we repeated our analyses using only the first 50 sibships in our sample. We identified only 503 associations (for 106 transcripts) in this subset that exceed our lod threshold of 6 for genome-wide significance. Using 100 sibships, we found 4,923 such associations (for 736 transcripts), and in our full data set of 206 sibships, we found a total of 16,098 such associations (for 1,989 transcripts). These results clearly suggest that further increases in sample size will enable even more regulators of gene expression to be mapped with statistical confidence.

Dominance and interaction

We explored the heritability that was not explained by our initial association analyses by testing for dominance and interaction effects on association among the 13,095 transcripts with $H^2 > 0.3$ that could not be mapped (maximum lod < 6) under the additive model. We identified 699 transcripts under a dominant model with $P < 6.12 \times 10^{-8}$ (Bonferroni correction for $2 \times 408,273$ tests). However, this was fewer than the 1,097 transcripts that we observed in simulated null genotype data, suggesting that in these subjects, dominance had a minimal effect on gene transcription.

We conducted further tests for interactions among the top 100 SNPs for each of the 13,095 transcripts with high heritability but no significant genome-wide SNP associations. We found that 600 had a $P < 6 \times 10^{-8}$ for the interaction term (Bonferroni correction for $2 \times 408,273 + 10,000$ tests), compared to 219 in a permuted genome-wide association scan data set. Although many of the interactions were between SNPs in the same chromosome (and could simply point to a haplotype effect), we observed an excess of interacting SNPs even after removing these. Thus, our data suggest that genetic interactions may have an important influence on regulation of expression for individual genes.

Cis and trans and master regulators

Trans effects were weaker than those in *cis* (defined as a SNP within 100 kb upstream and downstream of a gene), and most lod scores >9 were in *cis*. (**Fig. 3**) This is consistent with previous studies in humans[1,2] and mice[13]. Despite the relative weakness of *trans* effects, we observed numerous distant associations (for example, the peak of association for 698 transcripts was on the same chromosome but >100 kb from the nearest transcribed gene, and for 10,382 transcripts, the peak of association was on a different chromosome), and it may be anticipated that larger samples will define more precisely the extent of *trans* regulation of human transcripts.

It has been proposed, on the basis of genetic linkage, that the human genome contains hotspots of transcriptional regulation with effects on multiple expression phenotypes[2]. We found 13 SNPs that showed association with ten or more heritable expression traits with lod scores >6 (**Supplementary Table 3a** online). These represent potential master regulators. However, when we confined the analysis to traits with $H^2 > 0.3$ and excluded markers and transcripts within the extended linkage disequilibrium of the major histocompatibility complex, only three SNPs (rs1035665, rs10509971 and rs1031808) were associated with more than five transcripts (**Supplementary Table 3b**). These markers were not in known genes, and the mean

Figure 4 Regulators of genes involved in the cell cycle or immune response. (**a**) Mapping of genes with Gene Ontology biological process descriptors for cell cycle, DNA repair and RNA processing. Genome position is shown along the *x* axis divided by chromosome, and the lod score for association is shown on the *y* axis. Genes showing significant associations (lod > 6) are labeled in red for *cis* effects (±100 kb of the gene), in green if on the same chromosome but > 100 kb away and in blue if in *trans* on a different chromosome. (**b**) Mapping of genes with Gene Ontology biological process descriptors for immune responses.


lod scores for association were only 6.7, 6.6 and 6.5. Thus, from our data, it seems that master regulators with strong effects on many genes are not widely prevalent in the human genome.

Gene Ontology

We used Gene Ontology (GO) analyses to identify genes that were significantly enriched among highly heritable traits (**Table 1** and **Supplementary Table 4** online). The most highly heritable GO biological process was "response to unfolded proteins". This group contained numerous chaperonins and heat shock proteins (*CRNN*; seven *DNAJ* family members; *HERPUD1*; 16 *HSPA*, *HSPB*, *HSPC* or *HSPD* family members; *SERPINH1*; *TOR1A* and *TOR1B*; *HSP90B1* and *TXNDC4*) (**Supplementary Table 5a** online). The individual variation in response to unfolded proteins may represent an evolutionary response to cellular stress, and these genes could be candidates in the study of neurodegenerative diseases and aging processes.

Genes regulating progression through the cell cycle, RNA processing and DNA repair were also exceptionally heritable (**Supplementary Table 5b** online and **Fig. 4a**). We speculate that expression of these genes is under very tight genetic control, with little stochastic noise, so that nearby polymorphisms can more easily influence expression in a detectable manner. The evolutionary advantage of individual variation in these genes is unclear. These genes may be relevant candidates for the investigation of inherited susceptibility to cancer.

Genes expressed in LCLs have been shown to be enriched in GO categories of immune response[14], and the significant heritability that we observed for these traits emphasizes the value of our data for the study of infectious and inflammatory diseases (**Supplementary Table 5c** and **Fig. 4b**). Genetic variation of *HLA-DQ* expression has been observed[15], but effects that we found on *HLA-DR* and *HLA-DP*, as well as the smaller effects on *HLA-A* and *HLA-C*, have not been reported previously (**Fig. 4b**). The strength of these effects suggests that associations of major histocompatibility complex class I and class II polymorphism with diseases may depend on the amount of gene transcription as much as the restriction of response to antigen.
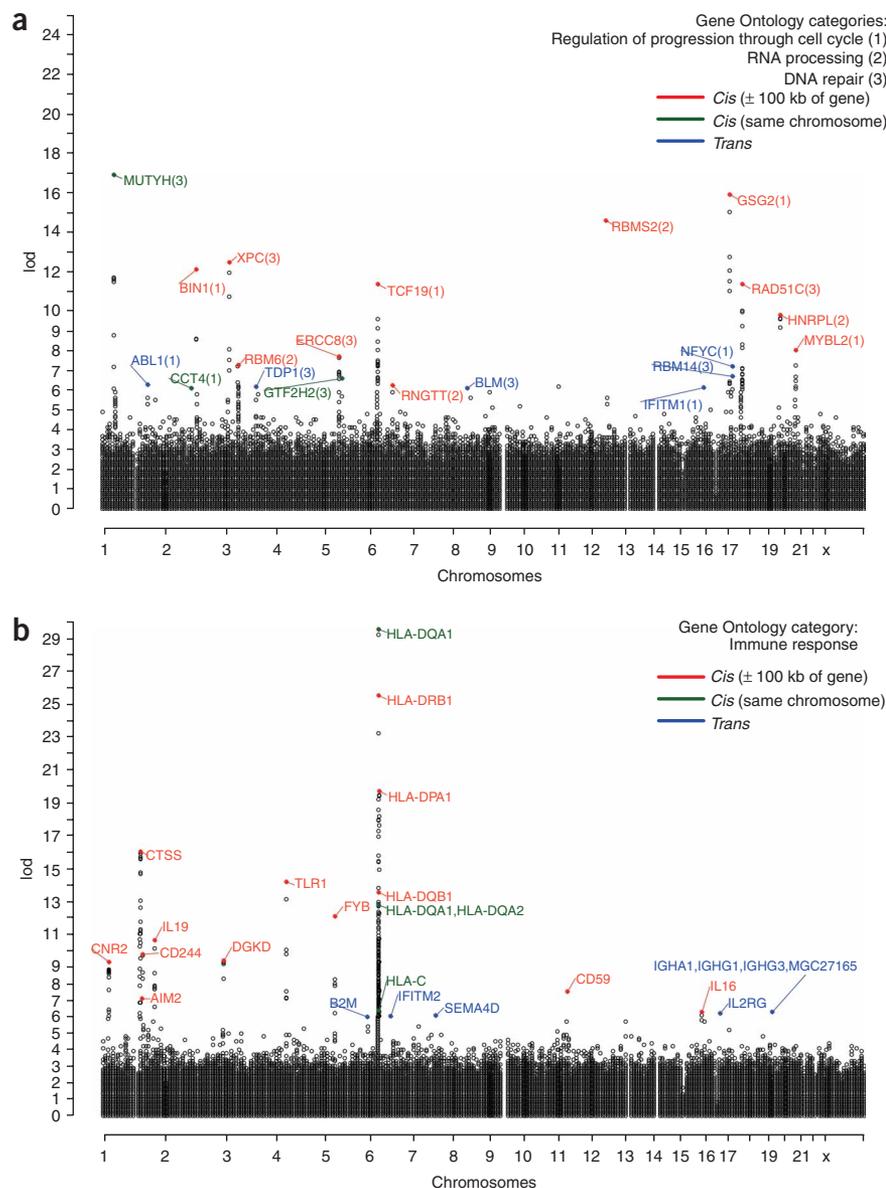
Applications

Our data set has wide application to the study of genetic markers associated with disease or other biological phenotypes. We have recently used the genome-wide SNP data to map a new susceptibility locus for childhood asthma to noncoding SNPs residing within a 206-kb segment on chromosome 17q23 (ref. 3). Our expression database showed that transcripts from *ORMDL3*, one of the nineteen genes within and around this segment, were strongly ($P < 10^{-22}$) and consistently positively associated with exactly the same SNPs showing association with childhood asthma. The correlation between the *P* values from the test statistics for association with asthma and *ORMDL3* expression for markers across the 206-kb segment was 0.67 ($P = 0.004$). The disease-associated markers accounted for 29.5% of the variance of expression. After we accounted for the effects of the disease-associated markers, residual heritability of the *ORMDL3* expression was not significant ($P = 0.29$, compared with $P = 0.0009$ before adjustment).

These results focus attention on *ORMDL3* as a strong candidate gene in asthma, and they illustrate how the combination of gene

expression with genetic data can be much more powerful than differential gene expression alone in identifying candidate disease genes.

Our database has also been of use in the identification of a new susceptibility locus from Crohn's disease on chromosome 5 (ref. 16). A GWA study had identified markers with a strong disease association within a 1.25-Mb gene desert. Examination of our database showed that these markers are also associated with expression of *PTGER4*, a gene that resides on chromosome 5 outside of the 1.25-Mb segment. This led to the identification of *PTGER4* as the primary candidate gene for this disease susceptibility locus[16].

A recent GWA for type II diabetes (non–insulin-dependent diabetes mellitus (NIDDM)) identified four putative loci modulating disease risk[17]: three of these were not associated with significant transcript variation, but the fourth on chromosome 11 at 44.2 Mb showed strong association in our database with variation in *PHACS* ($P < 10^{-33}$). *PHACS* could now be considered a potential candidate for NIDDM.

Finally, we cite the example of intergenic variants between *HBS1L* and *MYB* on chromosome 6q23 that are responsible for a major QTL influencing fetal hemoglobin expression ($\alpha 2 \gamma 2$) in adults, a hereditary character that underlies the remarkable diversity in phenotypic severity of sickle cell disease and β thalassemia[18]. The trait-associated markers are located upstream of *HBS1L* and *MYB*, both of which are plausible biological candidates. Our database showed expression of *HBS1L* to be highly heritable and strongly associated with fetal hemoglobin QTL markers ($P < 10^{-16}$), whereas *MYB* expression had lower heritability and no marker association. Thus, *HBS1L* seems to be the principal candidate gene for this QTL, as also suggested by other data[18].

DISCUSSION

Our findings show excellent concordance with previous studies that have identified smaller numbers of eQTLs, despite the use of different platforms for genotyping and measuring expression. Five of our fifty most significant eQTLs (*RPS26*, *IRF5*, *LDLRAP1*, *CHI3L2* and *HSD17B12*) had been identified previously by linkage analyses in CEPH pedigrees[2]. Our study included nine mRNA traits that had shown ancestral differences in abundance in a previous study[19], and we identified significant *cis* associations for eight of these with peak lod scores within 20 kb of the previously reported association peak. Another recent study contrasted the impact of SNP variation and copy number polymorphism on gene expression[20]. In our data, we evaluated 13 of the 20 transcripts reported as showing the strongest associations with *cis* SNPs in the HapMap CEU panel. Ten of these thirteen showed similar strong *cis*-acting signals that reached genome-wide significance in our data. Two of the remaining three showed weaker evidence for *cis* association (peak lod scores within 100 kb of the transcript were 3.89 for *UBA52* and 4.513 for *USMG5*).

Our results have identified an important part of the natural variation in the human transcriptome and may be used as a general tool to investigate whether SNPs associated with any disease alter transcription of genes in *cis* or *trans*. We have created a downloadable database (the mRNA by SNP Browser) for the interrogation of our data (see Methods). The tool allows users to browse the results by transcript or by position as well as search for information on specific SNPs. Linkage disequilibrium and tag information is provided for SNPs not in our database but evaluated by the International HapMap Consortium. We are currently investigating copy number variation in our data.

Our results suggest that increases in sample size will provide greater power to detect SNP association with particular transcript abundances. In addition, it will be of interest to test global gene expression with other platforms and to investigate the LCLs with denser transcript arrays. eQTL mapping of other cell types is also desirable. Finally, exposing our LCLs directly to inflammatory and other stimuli will allow investigation of the effects of polymorphism on a wider range of transcripts outside of their basal state.

METHODS

Subjects. The recruitment of the subjects was as described previously[4]. All subjects or their parents gave written informed consent. Ethical approval was given by the Multicentre Research Ethics Committees (UK).

Epstein-Barr virus (EBV). The transformation of the peripheral blood lymphocytes in all children in the panel was carried out by the European Collection of Cell Cultures. Previously transformed cryopreserved EBV cell lines were grown as 500-ml roller cultures. Once the log phase was reached, cells were pelleted, medium was discarded and a mixture of RLT buffer and β-mercaptoethanol was added. Pellets were vortexed to ensure thorough resuspension, after which they were frozen at –70 °C and stored at –80 °C. RNA was extracted in batches using the RNeasy Maxi Kit, and quality and quantity of RNA were assessed.

Microarray hybridization. We used 10 μg of RNA to synthesize double-stranded cDNA using the One-Cycle cDNA Synthesis Kit (Affymetrix). Using the cDNA as a template, *in vitro* transcription (IVT) of cRNA was carried out using the IVT Labeling Kit (Affymetrix), following the manufacturer's protocol. A hybridization cocktail was made according to the Affymetrix protocol using 15 μg of labeled, fragmented cRNA and was then hybridized to U133 Plus 2.0 GeneChips (Affymetrix) for 16 h at 45 °C in a rotating oven. GeneChips were washed and stained following the protocol and were then scanned on a high-resolution scanner (Affymetrix).

Genotyping. Whole-genome genotyping was carried out according to manufacturers' instructions using the Sentrix Human-1 Genotyping BeadChip[21] and the HumanHap300 Genotyping BeadChip[22] (Illumina) in a BeadLab with full automation. All DNA samples were subjected to rigorous quality control to check for fragmentation and amplification. We used 20 μl of DNA at a concentration of 50 ng/μl for each array. DNA samples were tracked using a laboratory information management system (LIMS). The HumanHap300 Genotyping BeadChip was used with an Illumina LIMS, and the Sentrix Human-1 Genotyping BeadChip was tracked through the Illumina process by hand. Groups of 24 samples were batched. In order to determine the degree of error due to mix-ups and plate inversions, 5% of the samples were selected from the different batches for re-genotyping, and the results were compared. We did not detect any discrepancies. Raw data were analyzed using GTS Image and were extracted for statistical analysis.

Association. All data from the gene expression experiment were normalized together using the RMA (robust multi-array average) package[6,7] to remove any technical or spurious background variation. A second inverse normal transformation step was also applied to each trait to avoid any outliers. We did not test for batch effects in the data. Before performing association analyses, we used a variance components method to estimate narrow-sense heritability of each trait using MERLIN[23].

Association analysis was applied with MERLIN (with the FASTASSOC option)[11,23]. We fitted a simple regression model to each trait and used a variance component approach to account for correlation between different observed phenotypes within each family. For individuals for whom genotype data were available, we coded genotypes as 0, 1 or 2 depending on the number of copies of the allele being tested. For individuals with missing genotype data, we used the Lander-Green algorithm to estimate an expected genotype score between 0 and 2 (ref. 23). Briefly, to estimate each genotype score, we first calculated the likelihood of the observed genotype data. Then, we instantiated each missing genotype to a specific value and updated the pedigree likelihood. The ratio of the two likelihoods gives a posterior probability that the

instantiated genotype is true, conditional on all available data. As our model uses information from all available individuals and does not focus on the offspring of heterozygous parents, it is potentially sensitive to stratification. Consequently, we tested for the effects of population structure and cryptic relatedness between sampled individuals with the genomic control method[12]. The genomic control parameter was close to its expected value of 1.0, indicating no stratification.

A significance level of 0.05 and a Bonferroni correction applied to each expression trait individually led to a P value threshold of 1.2×10^{-7}, corresponding to a lod score of 6.076. This equates to a false discovery rate of 0.049 for 408,273 SNPs tested for association with 15,084 transcripts[24].

Heritable genes used in the main analysis were annotated with UCSC refGenes based on NCBI's RefSeq. Expression traits shown to be heritable were annotated using probe IDs and the programs NetAffx (see URL below) and EASE.

Gene Ontology. Transcripts and genes were matched to Gene Ontology[25] categories using the HG-U133 Plus 2.0 annotation file (accessed 19 December 2005) available from Affymetrix NetAffx (see URL below). We excluded GO annotations inferred from electronic annotation only, as these are considered less reliable[26]. We investigated the average heritability of the transcripts assigned to each GO category using the statistic

$$Z_{H_i^2} = \frac{H_i^2 - \mu}{\sigma/\sqrt{n_i}}$$

where H_i^2 is the average heritability of the transcripts assigned to the ith GO category, n_i is the number of transcripts in the ith category and μ and σ are the overall mean and s.d. of all heritability estimates, respectively. We also investigated the proportion of genes and transcripts that were associated with specific SNPs at genome-wide significance levels within each category using the statistic

$$Z_{p_i} = \frac{p_i - p}{\sqrt{p(1-p)/n_i}}$$

where p_i is the proportion of significant transcripts in the ith category, and p is the overall proportion of significant transcripts.

Accession number. The microarray data have been deposited in the Gene Expression Omnibus (GEO) database under accession number GSE8052.

URLs. MRBS browser: http://www.sph.umich.edu/csg/liang/asthma/. RefSeq: http://www.ncbi.nlm.nih.gov/RefSeq/. NetAffx: http://www.affymetrix.com. Human Genome U133 Plus 2.0 Array: http://www.affymetrix.com/support/technical/byproduct.affx?product=hg-u133-plus.

Requests for materials: w.cookson@imperial.ac.uk.

Note: Supplementary information is available on the Nature Genetics website.

1. Schadt, E.E. *et al.* Genetics of gene expression surveyed in maize, mouse and man. *Nature* **422**, 297–302 (2003).
2. Morley, M. *et al.* Genetic analysis of genome-wide variation in human gene expression. *Nature* **430**, 743–747 (2004).
3. Moffatt, M.F. *et al.* Genetic variants regulating ORMDL3 expression are determinants of susceptibility to childhood asthma. *Nature* **448**, 470–473 (2007).
4. British Thoracic Society and Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. *Thorax* **58** (Suppl.), i1–i94 (2003).
5. Abecasis, G.R., Cookson, W.O. & Cardon, L.R. Selection strategies for disequilibrium mapping of quantitative traits in nuclear families. *Am. J. Hum. Genet.* **65**, A245 (1999).
6. Irizarry, R.A. *et al.* Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264 (2003).
7. Bolstad, B.M., Irizarry, R.A., Astrand, M. & Speed, T.P. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* **19**, 185–193 (2003).
8. Yan, H., Yuan, W., Velculescu, V.E., Vogelstein, B. & Kinzler, K.W. Allelic variation in human gene expression. *Science* **297**, 1143 (2002).
9. Cheung, V.G. *et al.* Natural variation in human gene expression assessed in lymphoblastoid cells. *Nat. Genet.* **33**, 422–425 (2003).
10. Gretarsdottir, S. *et al.* The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nat. Genet.* **35**, 131–138 (2003).
11. Chen, W.-M. & Abecasis, G.R. Family based association tests for genome wide association scans. *Am. J. Hum. Genet.* (in the press).
12. Devlin, B., Roeder, K. & Wasserman, L. Genomic control, a new approach to genetic-based association studies. *Theor. Popul. Biol.* **60**, 155–166 (2001).
13. Hubner, N. *et al.* Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. *Nat. Genet.* **37**, 243–253 (2005).
14. Monks, S.A. *et al.* Genetic inheritance of gene expression in human cell lines. *Am. J. Hum. Genet.* **75**, 1094–1105 (2004).
15. Beaty, J.S., West, K.A. & Nepom, G.T. Functional effects of a natural polymorphism in the transcriptional regulatory sequence of HLA-DQB1. *Mol. Cell. Biol.* **15**, 4771–4782 (1995).
16. Libioulle, C. *et al.* Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. *PLoS Genet.* **3**, e58 (2007) (doi:10.1371/journal.pgen.0030058).
17. Sladek, R. *et al.* A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* **445**, 881–885 (2007).
18. Thein, S.L. *et al.* Intergenic variants of HBS1L-MYB are responsible for a major QTL on chromosome 6q23 influencing HbF levels in adults. *Proc. Natl. Acad. Sci. USA* **104**, 11346–11351 (2007).
19. Spielman, R.S. *et al.* Common genetic variants account for differences in gene expression among ethnic groups. *Nat. Genet.* **39**, 226–231 (2007).
20. Stranger, B.E. *et al.* Relative impact of nucleotide and copy number variation on gene expression phenotypes. *Science* **315**, 848–853 (2007).
21. Gunderson, K.L., Steemers, F.J., Lee, G., Mendoza, L.G. & Chee, M.S. A genome-wide scalable SNP genotyping assay using microarray technology. *Nat. Genet.* **37**, 549–554 (2005).
22. Steemers, F.J. *et al.* Whole-genome genotyping with the single-base extension assay. *Nat. Methods* **3**, 31–33 (2006).
23. Abecasis, G.R., Cherny, S.S., Cookson, W.O. & Cardon, L.R. Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. *Nat. Genet.* **30**, 97–101 (2002).
24. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Statist. Soc. Ser. B* **57**, 289–300 (1995).
25. Ashburner, M. *et al.* Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* **25**, 25–29 (2000).
26. Harris, M.A. *et al.* The Gene Ontology (GO) database and informatics resource. *Nucleic Acids Res.* **32**, D258–D261 (2004).