Title

Discovering Single Nucleotide Polymorphisms Regulating Human Gene Expression Using Allele Specific Expression from RNA-seq Data

Journal

Genetics, 204(3)

ISSN

0016-6731

Authors

Kang, Eun Yong
Martin, Lisa J
Mangul, Serghei
et al.

Publication Date

2016-11-01

DOI

10.1534/genetics.115.177246

Peer reviewed

Discovering Single Nucleotide Polymorphisms Regulating Human Gene Expression Using Allele Specific Expression from RNA-seq Data

Eun Yong Kang,*,[1] Lisa J. Martin,†,[1] Serghei Mangul,*,[1] Warin Isvilanonda,* Jennifer Zou,* Eyal Ben-David,‡ Buhm Han,§, Aldons J. Lusis,† Sagiv Shifman,‡ and Eleazar Eskin*,†,[2]**

*Department of Computer Science and †Department of Human Genetics, University of California, Los Angeles, California 90095-1596, ‡Department of Genetics, Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, 91904, Israel, §Division of Genetics, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, and **Program in Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142

ABSTRACT The study of the genetics of gene expression is of considerable importance to understanding the nature of common, complex diseases. The most widely applied approach to identifying relationships between genetic variation and gene expression is the expression quantitative trait loci (eQTL) approach. Here, we increased the computational power of eQTL with an alternative and complementary approach based on analyzing allele specific expression (ASE). We designed a novel analytical method to identify *cis*-acting regulatory variants based on genome sequencing and measurements of ASE from RNA-sequencing (RNA-seq) data. We evaluated the power and resolution of our method using simulated data. We then applied the method to map regulatory variants affecting gene expression in lymphoblastoid cell lines (LCLs) from 77 unrelated northern and western European individuals (CEU), which were part of the HapMap project. A total of 2309 SNPs were identified as being associated with ASE patterns. The SNPs associated with ASE were enriched within promoter regions and were significantly more likely to signal strong evidence for a regulatory role. Finally, among the candidate regulatory SNPs, we identified 108 SNPs that were previously associated with human immune diseases. With further improvements in quantifying ASE from RNA-seq, the application of our method to other datasets is expected to accelerate our understanding of the biological basis of common diseases.

KEYWORDS Allele specific expression; expression quantitative trait loci; causal variants

Studying the genetics of gene expression has proved useful in identifying the genes and genetic variants underlying common human diseases. The usual approach to studying the genetic factors of gene expression is to map eQTL. An eQTL study is based on treating expression as a quantitative trait and associating it with genetic variation. eQTL studies have been tremendously successful, and have identified many loci involved in gene regulation (Ghazalpour *et al.* 2008; Veyrieras *et al.* 2008; Cookson *et al.* 2009; Farber *et al.* 2009; Hayes *et al.* 2009; Jiménez-Gómez *et al.* 2010; van Nas *et al.* 2010; Steibel *et al.* 2011; Gaffney *et al.* 2012; Kabakchiev and Silverberg 2013; Francesconi and Lehner 2014). However, the traditional eQTL approach has some fundamental limitations. First, due to the linkage disequilibrium (LD) or correlation structure of the genetic variation in the genome, it is difficult to distinguish between the regulatory variant and neighboring variants in LD. Second, like other quantitative traits, the total expression of a gene is influenced by multiple genetic and environmental factors. As a result, for any given variant the effect size is small, and the study requires a large sample size to identify the effect.

An alternative approach to identify genetic variants associated with variation in gene expression is based on allele specific expression (ASE). The principle behind the ASE mapping approach is that if an individual's phenotype is heterozygous for a regulatory variant, then the two copies of the gene will show different level of expression (also known as allelic expression imbalance, AEI). It was previously shown that the pattern of

doi: 10.1534/genetics.115.177246
Manuscript received April 11, 2015; accepted for publication September 7, 2016; published Early Online October 7, 2016.
Supplemental material is available online at www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.177246/-/DC1.
[1]These authors contributed equally to this work.
[2]Corresponding author: University of California, 3532-J Boelter Hall, Los Angeles, CA 90095-1596. E-mail: eeskin@cs.ucla.edu

ASE within families may follow Mendelian inheritance (Yan *et al.* 2002). Analysis of ASE is advantageous over analyzing total expression levels because the two alleles express in the same cellular environment, thus providing an internal control for each other. Consequently, *trans*-acting environmental and genetic factors that increase variation between samples are minimized to similar eQTL studies, since the analysis of ASE is influenced by the local LD structure and by the amount of allelic heterogeneity. However, the relationship between LD and variant identification has a different flavor when utilizing ASE compared with eQTL studies. Thus, ASE provides a complementary approach to identifying variants affecting expression compared with traditional eQTL studies.

ASE measured using microarrays and RNA-seq has been used for mapping variants associated with gene expression (Tao *et al.* 2006; Bjornsson *et al.* 2008; Serre *et al.* 2008; Bell and Beck 2009; Degner *et al.* 2009; Ge *et al.* 2009; Palacios *et al.* 2009; Daelemans *et al.* 2010; Gregg *et al.* 2010; Heap *et al.* 2010; Pastinen 2010; Ritchie *et al.* 2010; Sun *et al.* 2010; Wagner *et al.* 2010; Hill *et al.* 2011; Sun 2011; Wolff *et al.* 2011; Castel *et al.* 2015; van de Geijn *et al.* 2015).

In this study, we developed and used a novel analytical approach for identifying *cis*-acting regulatory variants based on ASE. Our method is based on a nonparametric approach that is robust and computationally very efficient. We demonstrated the utility of our method by analyzing RNA-seq data from 77 unrelated northern and western European individuals (CEU). For the mapping of each gene, we employed ASE measurements across a set of sequenced individuals simultaneously. We then identified genetic variants in proximity to those genes that can explain the observed patterns of ASE.

Materials and Methods

Reads alignment and quality control

For measuring ASE, we used the RNA-seq data of 77 unrelated northern and western European individuals (CEU) whose phased SNP information is available through the 1000 Genomes Project (phase 1). For accurate ASE measurements, individual RNA-seq data were prepared, and only reads that mapped uniquely to the genome were used to calculate the allele counts. In order to be consistent with current analyses of these datasets, we used the alignments from a previous study (Lappalainen *et al.* 2013). In this study, individual human transcriptomes were sequenced using a 75-bp paired-end protocol, and the mRNA reads were mapped to the human genome with the GEM mapper. Reads mapped to multiple locations in the human genome were filtered out, together with reads having a low mapping quality score. (We included reads having a mapping quality score >10) To ensure high accuracy of the counts at each SNP position, mRNA reads were further filtered based on individual nucleotide quality scores ("phred quality scores"). (We included positions with phred quality scores >10).

Correcting for allelic bias in RNA-seq

Biases in reading and mapping of different alleles may result in inaccurate ASE measurements. To exclude SNPs with biased allele signals, we applied the method proposed in Degner *et al.* (2009). We created a simulated dataset consisting of all possible 75-bp reads (369 million reads in total) that overlap the 1000 Genomes Project exonic SNPs. For each SNP, 150 reference allele reads (75 matching each strand) and 150 nonreference allele reads were generated for the 75-bp reads. We mapped the simulated reads back to the genome using the MAQ software. Any SNP successfully mapped to the genome, yet having an unequal number of reads from the two alleles, was removed in order to filter out the SNPs showing a mapping bias in favor of a specific allele. To determine whether or not a read mapped successfully, we applied the same read mapping quality threshold that we used for RNA-seq mapping [mapping quality score (MAPQ) = 10].

Filtering out low-quality SNPs

To minimize the effect of noise in the RNA-seq data, we used a rigorous SNP filtering scheme, which helped us to accurately measure the amount of allelic expression by removing low-quality SNPs. We identified low-quality SNPs by looking at SNP quality information at the individual level and at the population level. After removing SNPs showing inherent allelic bias using the approach explained in the previous section, we had a set of SNPs for each individual and the reads mapped to them. This set and the corresponding reads represented the amount of allelic expression for the transcripts containing SNPs. In the first filtering process, we used the individual-level SNP quality information. We removed all SNPs for each individual whose coverage was <10. We chose a threshold of 10 because the transcript harboring these SNPs was not expressed or the SNPs did not accurately measure allelic expression for various reasons, including read alignment errors. Second, for each SNP we compared allele information from the 1000 Genomes Project data with the actual alleles collected from the reads mapped to the SNP position. Due to noise and inaccuracy in the RNA-seq data, we observed many reads with alleles other than those reported in the 1000 Genomes Project SNP information. When we observed many reads containing other alleles, we excluded the SNP from our allelic expression measurement process according to the rules described below. If the SNP of an individual from the 1000 Genomes Project data was heterozygous, we filtered out the SNP when (1) the frequency of the alternative allele was higher than two alleles recorded in the 1000 Genome Project data or (2) the total frequency of the third and fourth alleles combined was greater than 5%, where the third and fourth alleles are not recorded in the 1000 Genomes Project data. If the SNP of the individual from the 1000 Genomes Project data was homozygous, we filtered out the SNP when (1) the frequency of the alternative allele was higher than an allele recorded in the 1000 Genomes Project data, (2) the total frequency of the combined third and

fourth alleles was >5%, or (3) the total frequency of the combined second, third, and fourth alleles was >5%. After filtering SNPs using individual-level SNP quality information, we categorized the remaining SNPs as "good" SNPs. Next, we used the SNP quality information from the population level to exclude SNPs that did not exhibit high quality across all individuals. To achieve this, we kept SNPs only if 80% of the individuals had at least a 10-read coverage for the SNP and 80% of individuals had a "good" SNP at the same time. By filtering SNPs with individual- and population-level SNP quality information, we produced a set of high-quality "informative SNPs" allowing us to measure the amount of allelic expression accurately.

ASE calling

After removing low-quality SNPs using the filtering scheme described above, we applied a simple ASE calling for each heterozygous site. We developed the ASE calling based on the three options: (1) higher expression of the paternal chromosome, (2) balanced expression, or (3) higher expression of the maternal chromosome. For each individual and transcribed SNP, we made ASE calls by computing the ratio between allelic counts from maternal and paternal chromosomes using the following equation:

$$\frac{C_p}{C_m + C_p} \tag{1}$$

where C_m is the read count from the maternal chromosome and C_p is the read count from the paternal chromosome. The ASE calls are computed based on the allelic ratio between the allelic counts from the maternal and paternal chromosomes. If the ratio is greater than 0.65, then the paternal chromosome is more expressed. If the ratio is less than 0:35, then the maternal chromosome is more expressed. Otherwise, the expression is balanced between the two chromosomes.

Mapping regulatory variants using ASE

Our ASE mapping method fundamentally assumes that the ASE is a discrete event. As explained above, we discretize the ASE status into three groups, which can be simply expressed mathematically as -1, 0, and 1. Since we are only looking at SNPs in the proximal region of the transcript, SNPs located within $\pm$250-kb flanking sequences of the start and end of the transcript region were considered in our ASE mapping. Analyzing a large window around the gene helps to identify *cis*-acting variants located far from the gene, such as enhancers. This approach also helps to test the distribution of ASE-associated SNPs relative to the transcription start site (TSS). In addition, a larger window size was used by others for ASE mapping (Ge *et al.* 2009).

Data availability

The genomic sequencing data discussed in this paper are available as part of the 1000 Genomes Project. The ASE mapping software we developed is available for download at http://genetics.cs.ucla.edu/ase.

Results

The principle of ASE mapping

We have proposed a method that uses ASE measurements to identify the most likely regulatory variants in a genome. We measure ASE for each gene in a set of individual samples for which we know the entire sequence and have identified the genetic variations on each chromosome. (Data with a high resolution of detail are becoming available more often as full genome sequencing becomes increasingly feasible and cost-effective.) Taking one gene at a time, we attempt to infer which variants in the gene region may be responsible for the observed pattern of ASE. For example, assume we have three individuals with SNPs in the proximal region for a particular gene of interest and relative expression of the two copies of the gene (Figure 1). In our framework, ASE is a *discrete* phenomenon: individuals 1 and 3 have AEI for the gene while individual 2 does not. In this example, there is one regulatory SNP (the third SNP from the left), which is the variant that is responsible for the observed ASE. The "A" allele at the regulatory SNP site causes higher expression of the gene relative to the "T" allele.

Our goal is to identify the regulatory variant based on both the ASE measurements and the genotypes of all SNPs in the region. We assume that the regulatory variant is within this set of genotyped SNPs. However, only variants that are heterozygous with AEI are possible candidates. For example, even though the first SNP has AEI, it could not be the regulatory variant since the first individual is homozygous. If we apply this principle to evaluate every SNP in the region, we identify the third and seventh SNPs as the only possible variants that may be responsible for the observed pattern of ASE.

This example assumes that our calls of ASE are error free and that there is only one regulatory variant. However, we can incorporate the possibility of errors or multiple regulatory variants into our approach by allowing for a fixed number of errors when matching genotypes with the ASE pattern. If we allow for one error, assuming that one of the three individuals is called incorrectly, the first, second, and ninth SNPs can now explain the observed ASE. A mismatch between the observed ASE pattern and the genotypes for the regulatory variant can be explained by mechanisms other than measurement errors. For example, rare variants could generate or eliminate AEI (Montgomery *et al.* 2010, 2011). In our approach, analytically incorporating errors allows us to take into account biases produced by both measurement errors and other mismatches in the model, assuming that they affect only a small number of individuals.

We characterize the efficacy of this method as the "reduction rate." The reduction rate is defined as the ratio between the number of candidate regulatory SNPs to the total number of SNPs in the proximal region of the gene. This ratio indicates exactly how effective the measurements of ASE are in identifying the regulatory variant. In the example above (Figure 1), we reduced the set of candidate SNPs from ten to two (a reduction rate of 80%). Allowing for one error increases

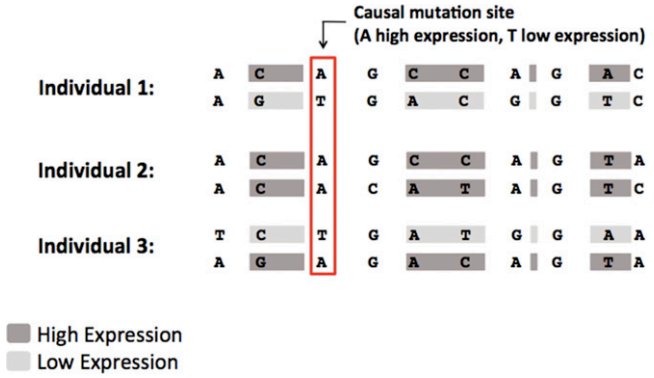


Figure 1 ASE example and the corresponding mathematical representation of three individuals (1, 2, and 3). We assume that the third SNP is the causal SNP site affecting the differential gene expression level (allele A/allele T).

the number of candidate SNPs to five, decreasing the reduction rate to 50%. We also observe that the relationship between LD and variant identification has a different flavor in ASE mapping when compared with eQTL studies. In our approach, the genotypes of each single individual with ASE provide information useful in determining which variants are potentially responsible for the observed ASE. On the other hand, in eQTL studies, each individual contributes only a limited amount to the association signal since the effect of a variant is small compared with the variance of the total expression.

Power and resolution of ASE mapping

We evaluated the analytical power and resolution of our method using data generated from the SNPs of 54 unrelated Nigerian HapMap individuals. In comparison with other modern populations, African populations have small regions of LD and higher genetic diversity. These factors allow the evaluation of our method to produce potentially higher-resolution results. Phased genotypes were obtained for the 54 unrelated Nigerian individuals from HapMap (HapMap phase 2). From this dataset, we evaluated 10.2 million polymorphic SNPs located in proximity to 18,849 human Ensembl genes (see *Materials and Methods* for details). We found 540 average SNPs per gene (Supplemental Material, Figure S1).

The efficacy of ASE mapping or the reduction rate depends on the number of individuals, the minor allele frequency (MAF) of the SNPs, and the number of SNPs in a specific gene region. We used simulations to measure the expected reduction rate as a function of the MAF. For each gene, we randomly selected an SNP to be the regulatory variant, and then generated ASE calls for each individual. We used our methodology to identify the set of possible candidate SNPs, and computed the reduction rate. Figure 2A shows the average reduction rate as a function of the MAF for the 54 unrelated Nigerian individuals. Figure 2B shows the results for a varying number of ASE measurement errors when matching the SNP genotypes to a pattern of observed ASE.

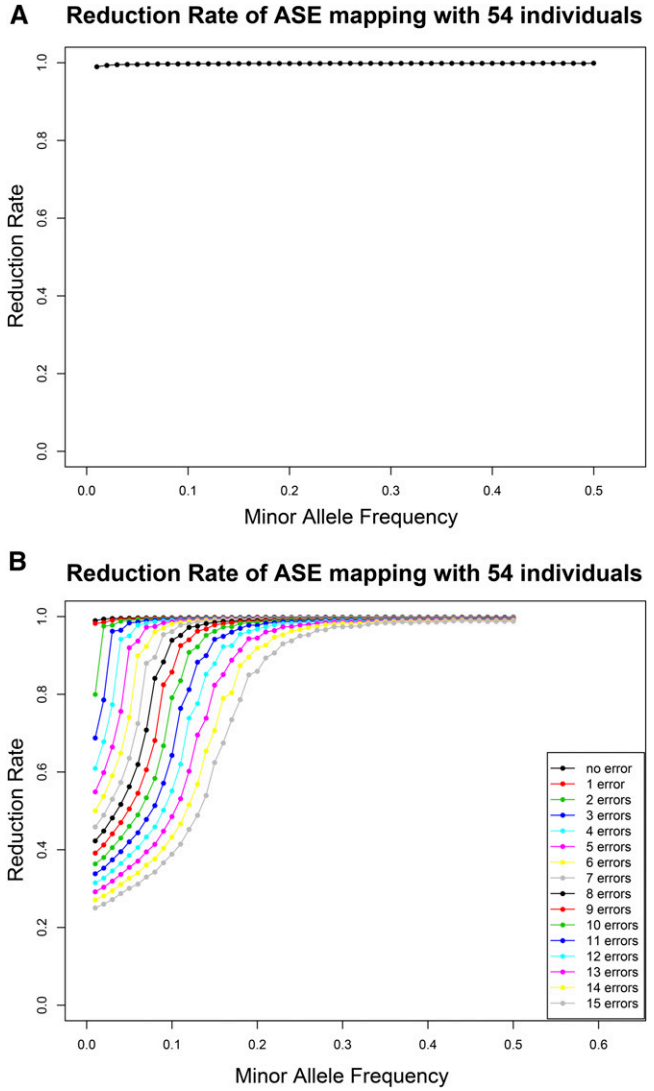


Figure 2 The reduction rate of ASE mapping was measured as a function of the MAF in the simulation experiments using 54 unrelated Nigerian individuals (HapMap phase 2). (A) Reduction rate without ASE measurement errors. (B) Reduction rate with a varying number of ASE measurement errors.

The reduction in the number of candidate SNPs does not necessarily mean that the association is "significant." It is possible that an SNP pattern in the gene randomly fits the observed ASE pattern. To compute the significance levels of SNP patterns in relation to ASE patterns, we permuted the ASE observations and applied our mapping method to the permuted data. We kept track of how many times an SNP in the gene fitted the permuted ASE measurements. Since our methodology was used for each gene, we applied a genome-wide threshold of 2.5×10^{-6}, which is the Bonferroni correction for an overall significance level of 0.05, assuming 20,000 genes.

We can measure the power of our ASE mapping strategy by randomly selecting a variant to be responsible for the observed ASE and then setting the ASE status accordingly and randomly

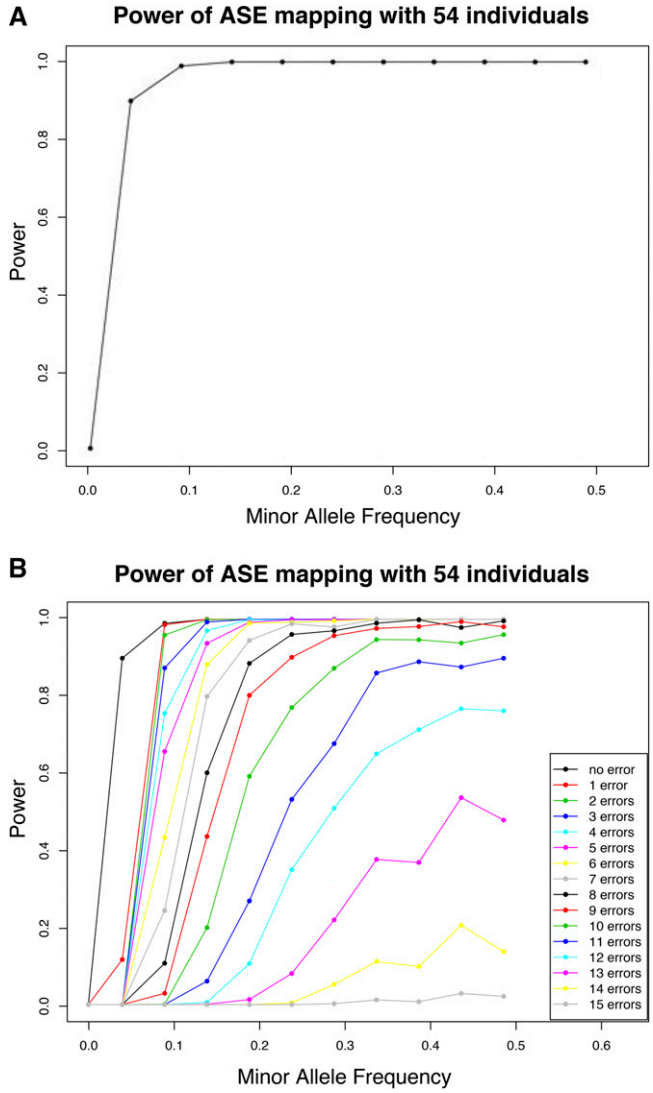


Figure 3 The power of ASE mapping is measured as a function of the MAF in the simulation experiments using 54 unrelated Nigerian individuals (HapMap phase 2). (A) Power of ASE mapping without ASE measurement errors. (B) Power of ASE mapping with a varying number of ASE measurement errors.

changing some of these statuses to simulate a specific number of errors. We can then use our ASE mapping approach to measure power. Figure 3A shows the power of our approach when applied to the 54 Nigerian individuals using the genome-wide thresholds. The power computes the percentage of simulated instances, which are more significant than the genome-wide threshold. The power is calculated as a function of different MAFs. Figure 3B shows how including ASE measurement errors affects the power. The results presented in Figure 2B and Figure 3A show that, with this sample size and 10% errors, there is still sufficient power and reduction rate to identify the potential regulatory variants affecting gene expression. As long as the sum of the error rate of the ASE measurements or the frequencies of the additional regulatory variants in the gene are $<10\%$, the method still has significant statistical power and reduction rate. Clearly, the same error rate can be achieved with even larger ASE measurement error rates given larger sample sizes.

Table 1 The number of informative SNPs per transcript

No. of distinct informative SNPs	1	2	3	4	5	>6
No. of transcripts	527	163	69	27	21	41

Of these transcripts, 62% had only one informative SNP for measurement of ASE.

Our simulation also implies that, given a sample size of 54 individuals, the observed pattern of ASE is unlikely to randomly match the genotype distribution of a random variant. Thus, other deviations from the model, such as the presence of multiple variants affecting expression, will likely avoid producing either false positives or an observed ASE pattern matching any single SNP genotype pattern.

ASE mapping in 77 European samples

We applied our methodology to the RNA sequencing data of 77 lymphoblastoid cell lines (LCLs) derived from unrelated European individuals (CEU). Phased haplotypes were available from the 1000 Genomes Project. The allelic count for each exonic SNP was calculated from a previously mapped RNA sequencing of the LCLs (Lappalainen *et al.* 2013). To maintain the integrity of the ASE calls, we applied rigorous SNP filtering schemes using individual- and population-level SNP quality information. Further, we only considered "informative SNPs," which are SNPs with at least 10 reads from each allele in an individual (see *Materials and Methods* for more details). After conducting all quality control processes, we identified 281,653 informative SNPs (counting the same SNP found in different individuals multiple times) to accurately measure the allelic expression abundance. For each transcribed SNP, the ASE calling assigned each individual into one of three different categories: (1) higher expression of the maternal allele, (2) balanced expression, and (3) higher expression of the paternal allele. In our combinatorial model, each category is denoted by -1, 0, and 1, respectively. We used the ASE calls in the mapping algorithm to identify candidate regulatory SNPs, and we tested the significance of these candidates using a permutation test (explained in *Materials and Methods*).

Among 18,849 Ensembl transcripts, we found 850 transcripts with at least one informative SNP that allowed us to measure ASE. Table 1 shows the number of informative SNPs observed per transcript. There were 1706 informative SNPs in at least one individual. Across the 1706 SNPs, the average number of individuals with AEI and balanced expression was 12 and 10, respectively (Figure 4).

We used our ASE mapping method for each transcript. We considered only SNPs that were proximal to or within the transcript (250-kb flanking sequences of the start and end of the transcript). To identify candidate regulatory variants, we examined in total 6,445,845 SNPs in the proximal region of the 850 gene. We found at least one significant SNP ($P < 2.5 \times 10^{-6}$) for 104 transcripts (the total number of

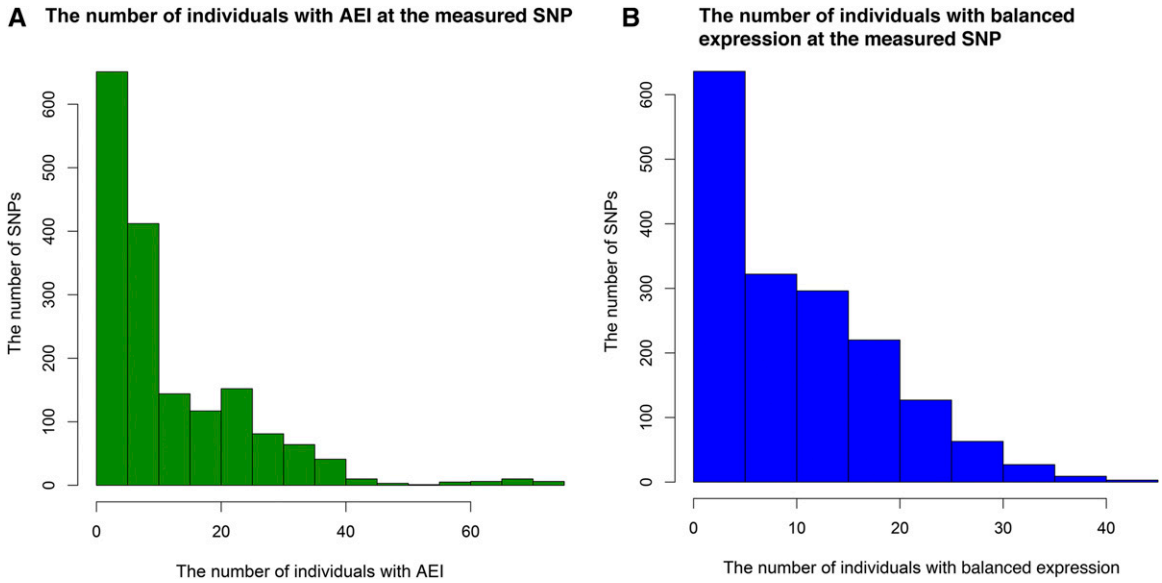


Figure 4 The number of individuals with AEI or balanced expression.

SNPs associated with ASE was 2309). The average number of candidate SNPs after the ASE mapping was 22.2. For 85 transcripts (81.7%), we found that either one variant or multiple variants in perfect LD could explain the ASE patterns. Of these transcripts, 53.8% had <10 candidate SNPs, and 21.1% had one or two candidate SNPs. The average reduction rate was 98.7%. File S1 reports all the transcripts and their significantly associated SNPs.

The mapped SNPs are enriched in promoter regions and known functional regulatory variants and are associated with immune-related diseases

The SNPs mapped to the ASE are expected to be highly enriched for regulatory SNPs (some may be in LD with such variants). Therefore, we expect those candidate SNPs to be distributed nonrandomly relative to the distribution of known regulatory sequences. The major regulatory element of transcription is the region upstream of the transcription start site (TSS), known to contain a core promoter sequence.

To see if the 2309 significantly associated SNPs identified by the ASE mapping are within possible promoter regions, we computed the distance between the associated SNPs and TSSs. As shown in Figure 5, the mapped SNPs (denoted by a red line) were enriched near the TSS. Furthermore, the SNPs were more abundant downstream of the TSS in the gene bodies and less abundant in the region upstream of the TSS ($P < 6.5 \times 10^{-85}$). To further test the significance of these observations, we compared the density of SNPs associated with ASE to the density of SNPs not associated with ASE as a function of distance from the TSS (Figure 5). These comparisons showed a striking difference in the distance from the TSS between the two types of SNPs ($P < 5.8 \times 10^{-195}$). This difference indicates that the enrichment around the TSS cannot be attributed to the initial distribution of distances between the candidate SNPs and the TSS.

To further test the functional evidence for the variants associated with ASE, we used the list of functionally annotated SNPs in the RegulomeDB database. RegulomeDB integrates a large collection of regulatory information collected from high-throughput experimental datasets from ENCODE and other sources. In addition, RegulomeDB uses computational predictions and manual annotations to identify putative regulatory potential and functional variants (Boyle *et al.* 2012). The annotation information contained in RegulomeDB is particularly useful for genome-wide association studies (GWASs), because the database includes functional annotations of genomic regions outside of genes as well as those in regions showing direct changes in protein-coding genes. To evaluate the functional evidence, we tested the enrichment of ASE-mapped SNPs among the annotated SNPs in RegulomeDB. We used a Fisher's exact test to assess the relationship between the ASE-mapped SNPs and the category 1 annotated SNPs in RegulomeDB. The RegulomeDB category 1 SNPs are most likely to be functional, as they are predicted to alter transcription factor binding and were previously found to be linked to gene expression. We found that the ASE-mapped SNPs were significantly enriched for the functional annotations category 1 ($P = 4.6 \times 10^{-17}$, odds ratio = 4.0). Out of the 1809 uniquely ASE-mapped SNPs, 55 were in category 1 (File S2), although only 13 would be expected by chance.

We next investigated whether the SNPs identified in our study were reported in GWASs. A large number of SNPs have been identified as associated with different human traits and diseases in GWASs; however, in most cases the molecular mechanism is unknown. To study the connection between GWASs and the ASE mapping results, we matched the list of candidate regulatory SNPs to the list of significant SNPs reported by GWASs (based on GWAS Central). We included high-LD SNPs ($r^2 > 0.8$) with the GWAS-reported SNPs, since GWASs use SNP arrays that do not cover all SNPs in the

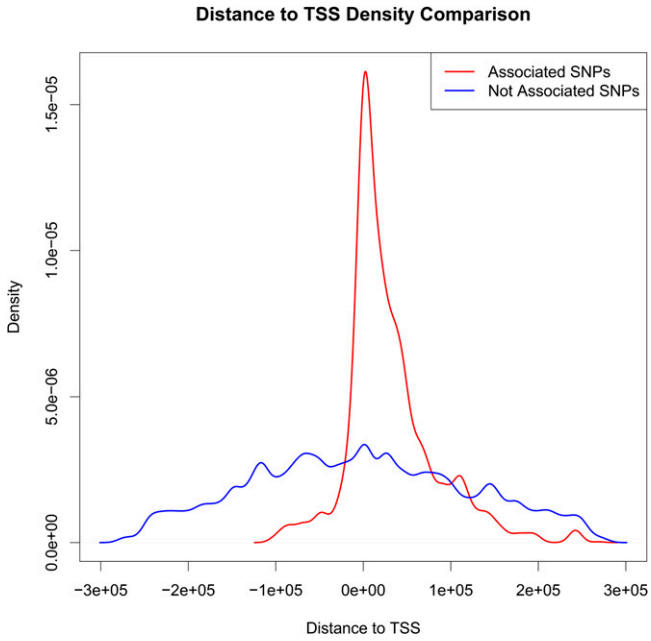


Figure 5 Distance to TSS comparison between associated SNPs and not associated SNPs. As can be seen, there is significant enrichment around the TSS site for the ASE-mapped SNPs.

genome. We found six clusters of SNPs with high LD ($r^2 > 0.7$) that were reported in multiple GWASs to be associated with ASE (File S1). The diseases were all immune related, and included inflammatory bowel disease, Crohn's disease, type 1 diabetes, and asthma.

Discussion

We have developed a novel method based on ASE for identifying *cis*-regulatory variants. The fundamental assumption of the proposed approach is that the AEI is a discrete event [*i.e.*, balanced (0), or imbalanced in two different directions (-1 or 1)]. The discretization may cause errors in the ASE calling. However, as we show with our simulation experiments, a strong correlation between observed ASE calls and a random set of genotypes is uncommon, even for samples with only 54 individuals. Further, the proposed mapping approach is resistant to ASE calling errors with increased sample size. Our proposed approach is simple, powerful, and capable of flexibly handling a wide range of sample sizes.

Our approach has several limitations. First, our method depends on accurate measurement of ASE from RNA-seq data. Obtaining accurate measurement of ASE has been shown to be challenging, as the reads from each allele are subject to various biases including the alignment procedure (Degner *et al.* 2009; Stevenson *et al.* 2013). In addition, a single clone can take over in cell lines grown for many generations, increasing the chance of random monoallelic expression (Eckersley-Maslin and Spector 2014).

Second, like any other method based on ASE, we could identify regulatory variants acting in *cis*, but we could not identify regulatory variants acting in *trans*. Third, with the current coverage of RNA-seq, we mainly rely on transcribed SNPs. Thus, not all genes have informative SNPs to measure ASE in each individual. Instead, to expand the number of genes having sufficient informative SNPs to measure ASE, we rely on both rare and common variants, and use intronic SNPs, larger sample sizes, and increased sequencing depths.

Third, our approach assumes that one causal variant drives the observed ASE. However, it is likely that in each region there is more than one variant affecting expression. Thus, more than one variant can be responsible for the observed ASE. In this scenario, each secondary variant that affects ASE will appear as an error in our framework. If the number of individuals affected by these secondary variants is smaller than the error threshold, our framework for mapping will still correctly identify the variant primarily responsible for the observed ASE. In the future, we plan to extend our framework to incorporate the possibility of multiple variants affecting ASE.

Finally, our approach assumes that the haplotype phase is both known and accurately inferred. In practice, errors in haplotype inference may manifest as errors in our ASE mapping approach. In the event that the haplotype phase is unavailable, we can use a modified version of our approach where we consider variants as being either homozygous or heterozygous, and consider a match between an ASE observation and a variant if the variant is heterozygous. In this scenario, the lack of phase will lead to a loss of power, but our framework will still control false positives.

Our method inherently assumes that the ASE calls are discrete. This has several advantages. First of all, in principle, it should be easier to make accurate discrete ASE calls when we are allowed to declare a call as ambiguous, rather than estimating the quantity of ASE. In addition, the discrete nature of our calls naturally predisposes the nonparametric mapping methodology we have presented. However, this approach has several disadvantages, including loss of information regarding the effect size of the variants that are causing ASE and loss of information about the confidence in the ASE calls themselves.

Despite the above limitations, ASE studies are a powerful approach to identifying associations between genetic variation and gene expression. When ASE is accurately measured, it has a high power in identifying *cis*-acting regulatory variants associated with common diseases. Our developed method for ASE mapping is a step forward in establishing the functional risk alleles for these diseases and using this information to develop new hypotheses about their causes and treatment.

Acknowledgments

E.Y.K., S.M., and E.E. are supported by National Science Foundation grants 0513612, 0731455, 0729049, 0916676, 1065276, 1302448, 1320589, and 1331176 and National Institutes of Health (NIH) grants K25-HL080079, U01-DA024417, P01-HL30568, P01-HL28481, R01-GM083198,

R01-ES021801, R01-MH101782, and R01-ES022282. E.E. is supported in part by the NIH BD2K award U54EB020403. S.S. was supported by the Israel Science Foundation grant 940/13.

Literature Cited

Bell, C. G., and S. Beck, 2009 Advances in the identification and analysis of allele-specific expression. Genome Med. 1: 56.

Bjornsson, H. T., T. J. Albert, C. M. Ladd-Acosta, R. D. Green, M. A. Rongione *et al.*, 2008 Snp-specific array-based allele-specific expression analysis. Genome Res. 18: 771–779.

Boyle, A. P., E. L. Hong, M. Hariharan, Y. Cheng, M. A. Schaub *et al.*, 2012 Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 22: 1790–1797.

Castel, S. E., A. Levy-Moonshine, P. Mohammadi, E. Banks, and T. Lappalainen, 2015 Tools and best practices for data processing in allelic expression analysis. Genome Biol. 16: 1.

Cookson, W., L. Liang, G. Abecasis, M. Moffatt, and M. Lathrop, 2009 Mapping complex disease traits with global gene expression. Nat. Rev. Genet. 10: 184–194.

Daelemans, C., M. E. Ritchie, G. Smits, S. Abu-Amero, I. M. Sudbery *et al.*, 2010 High-throughput analysis of candidate imprinted genes and allele-specific gene expression in the human term placenta. BMC Genet. 11: 25.

Degner, J. F., J. C. Marioni, A. A. Pai, J. K. Pickrell, E. Nkadori *et al.*, 2009 Effect of read-mapping biases on detecting allele-specific expression from RNA-sequencing data. Bioinformatics 25: 3207–3212.

Eckersley-Maslin, M. A., and D. L. Spector, 2014 Random monoallelic expression: regulating gene expression one allele at a time. Trends Genet. 30: 237–244.

Farber, C. R., J. E. Aten, E. A. Farber, V. de Vera, R. Gularte *et al.*, 2009 Genetic dissection of a major mouse obesity QTL (Carfhg2): integration of gene expression and causality modeling. Physiol. Genomics 37: 294–302.

Francesconi, M., and B. Lehner, 2014 The effects of genetic variation on gene expression dynamics during development. Nature 505: 208–211.

Gaffney, D. J., J.-B. B. Veyrieras, J. F. Degner, R. Pique-Regi, A. A. Pai *et al.*, 2012 Dissecting the regulatory architecture of gene expression QTLs. Genome Biol. 13: R7.

Ge, B., D. K. Pokholok, T. Kwan, E. Grundberg, L. Morcos *et al.*, 2009 Global patterns of *cis* variation in human cells revealed by high-density allelic expression analysis. Nat. Genet. 41: 1216–1222.

Ghazalpour, A., S. Doss, H. Kang, C. Farber, P.-Z. Z. Wen *et al.*, 2008 High-resolution mapping of gene expression using association in an outbred mouse stock. PLoS Genet. 4: e1000149.

Gregg, C., J. Zhang, J. E. Butler, D. Haig, and C. Dulac, 2010 Sex-specific parent-of-origin allelic expression in the mouse brain. Science 329: 682–685.

Hayes, K. R., B. M. Young, and M. T. Pletcher, 2009 Expression quantitative trait loci mapping identifies new genetic models of glutathione S-transferase variation. Drug Metab. Dispos. 37: 1269–1276.

Heap, G. A., J. H. M. Yang, K. Downes, B. C. Healy, K. A. Hunt *et al.*, 2010 Genome-wide analysis of allelic expression imbalance in human primary cells by high-throughput transcriptome resequencing. Hum. Mol. Genet. 19: 122–134.

Hill, M. J., E. Kenny, S. Roche, D. W. Morris, A. Corvin *et al.*, 2011 Allelic expression imbalance of the schizophrenia susceptibility gene CHI311: evidence of *cis*-acting variation and tissue specific regulation. Psychiatr. Genet. 21: 281–286.

Jiménez-Gómez, J. M., A. D. Wallace, and J. N. Maloof, 2010 Network analysis identifies ELF3 as a QTL for the shade avoidance response in arabidopsis. PLoS Genet. 6: e1001100.

Kabakchiev, B., and M. S. Silverberg, 2013 Expression quantitative trait loci analysis identifies associations between genotype and gene expression in human intestine. Gastroenterology 144: 1488–1496, 1496.e1–3.

Lappalainen, T., M. Sammeth, M. R. Friedländer, P. A. C. 't Hoen, J. Monlong *et al.*, 2013 Transcriptome and genome sequencing uncovers functional variation in humans. Nature 501: 506–511.

Montgomery, S. B., M. Sammeth, M. Gutierrez-Arcelus, R. P. Lach, C. Ingle *et al.*, 2010 Transcriptome genetics using second generation sequencing in a Caucasian population. Nature 464: 773–777.

Montgomery, S. B., T. Lappalainen, M. Gutierrez-Arcelus, and E. T. Dermitzakis, 2011 Rare and common regulatory variation in population-scale sequenced human genomes. PLoS Genet. 7: e1002144.

Palacios, R., E. Gazave, J. Goñi, G. Piedrafita, O. Fernando *et al.*, 2009 Allele-specific gene expression is widespread across the genome and biological processes. PLoS One 4: e4150.

Pastinen, T., 2010 Genome-wide allele-specific analysis: insights into regulatory variation. Nat. Rev. Genet. 11: 533–538.

Ritchie, M. E., M. S. Forrest, A. S. Dimas, C. Daelemans, E. T. Dermitzakis *et al.*, 2010 Data analysis issues for allele-specific expression using Illumina's GoldenGate assay. BMC Bioinformatics 11: 280.

Serre, D., S. Gurd, B. Ge, R. Sladek, D. Sinnett *et al.*, 2008 Differential allelic expression in the human genome: a robust approach to identify genetic and epigenetic *cis*-acting mechanisms regulating gene expression. PLoS Genet. 4: e1000006.

Steibel, J. P., R. O. Bates, G. J. Rosa, R. J. Tempelman, V. D. Rilington *et al.*, 2011 Genome-wide linkage analysis of global gene expression in loin muscle tissue identifies candidate genes in pigs. PLoS One 6: e16766.

Stevenson, K. R., J. D. Coolon, and P. J. Wittkopp, 2013 Sources of bias in measures of allele-specific expression derived from RNA-sequence data aligned to a single reference genome. BMC Genomics 14: 536.

Sun, C., C. Southard, D. B. Witonsky, O. I. Olopade, and A. Di Rienzo, 2010 Allelic imbalance (AI) identifies novel tissue-specific *cis*-regulatory variation for human UGT2B15. Hum. Mutat. 31: 99–107.

Sun, W., 2011 A statistical framework for eQTL mapping using RNA-seq data. Biometrics 68: 1–11.

Tao, H., D. R. Cox, and K. A. Frazer, 2006 Allele-specific KRT1 expression is a complex trait. PLoS Genet. 2(6): e93.

van de Geijn, B., G. McVicker, Y. Gilad, and J. K. Pritchard, 2015 Wasp: allele-specific software for robust molecular quantitative trait locus discovery. Nat. Methods 12: 1061–1063.

van Nas, A., L. Ingram-Drake, J. S. Sinsheimer, S. S. Wang, E. E. Schadt *et al.*, 2010 Expression quantitative trait loci: replication, tissue- and sex-specificity in mice. Genetics 185: 1059–1068.

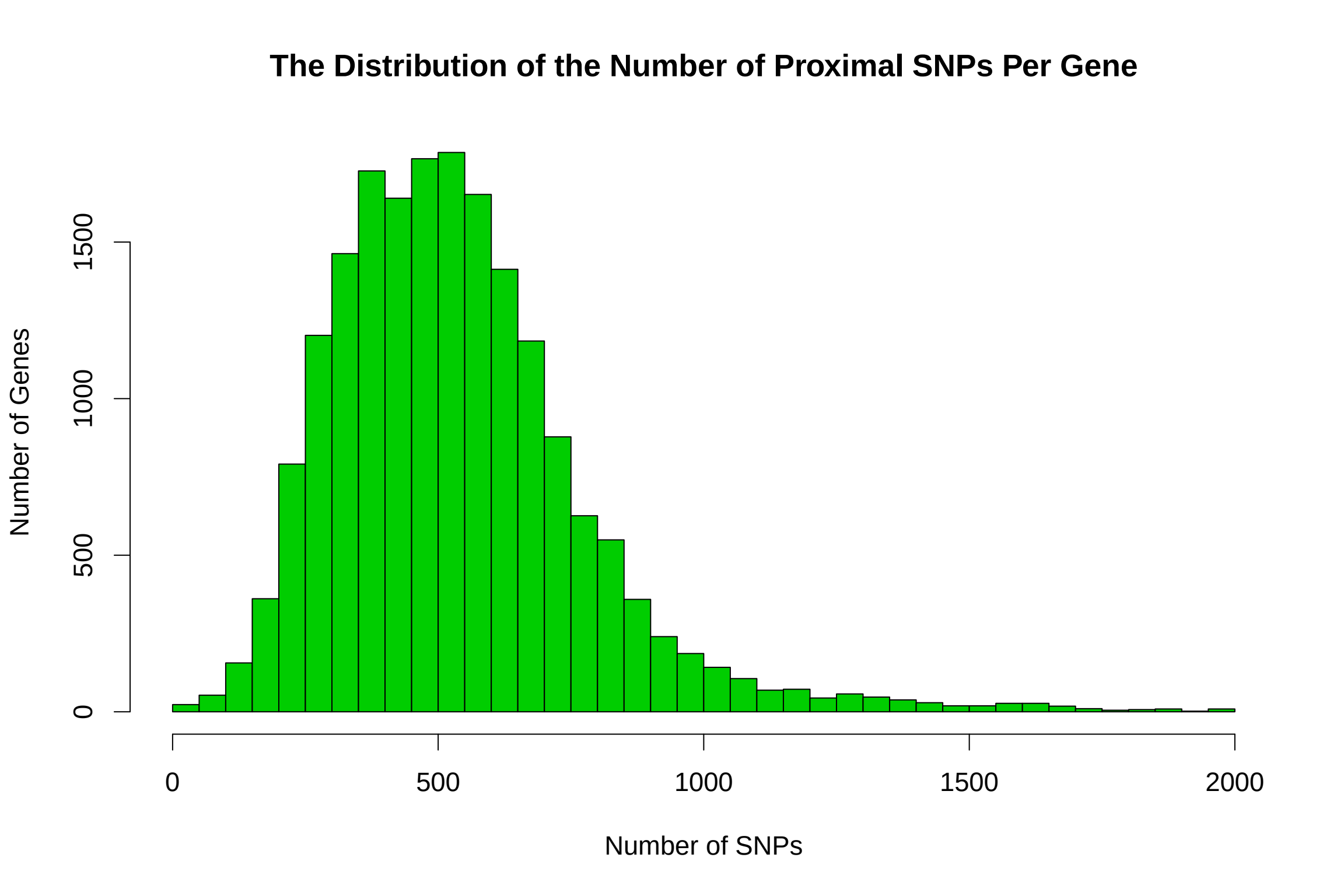
Veyrieras, J.-B., S. Kudaravalli, S. Y. Kim, E. T. Dermitzakis, Y. Gilad *et al.*, 2008 High-resolution mapping of expression-QTLs yields insight into human gene regulation. PLoS Genet. 4: e1000214.

Wagner, J. R., B. Ge, D. Pokholok, K. L. Gunderson, T. Pastinen *et al.*, 2010 Computational analysis of whole-genome differential allelic expression data in human. PLOS Comput. Biol. 6: e1000849.

Wolff, P., I. Weinhofer, J. Seguin, P. Roszak, C. Beisel *et al.*, 2011 High-resolution analysis of parent-of-origin allelic expression in the arabidopsis endosperm. PLoS Genet. 7: e1002126.

Yan, H., W. Yuan, V. E. Velculescu, B. Vogelstein, and K. W. Kinzler, 2002 Allelic variation in human gene expression. Science 297: 1143.

Communicating editor: J. M. Akey


The Distribution of the Number of Proximal SNPs Per Gene
Number of Genes
0
500
1000
1500
0
500
1000
1500
2000
Number of SNPs

File S1. A list of SNPs observed as consistent between ASE mapping and GWAS.

(.xlsx, 20 KB)

Available for download as a .xlsx file at
www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.177246/-/DC1/FileS1.xlsx

File S2. A list of ASE-mapped SNPs which have a functional annotation. (.xlsx, 50 KB)

www.genetics.org/lookup/suppl/doi:10.1534/genetics.115.177246/-/DC1/FileS2.xlsx