

Allele-specific binding and expression: a uniform survey over many individuals and assays

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Abstract

Allele-specific behavior provides insight into the functional effect of the variants in personal genomes. However, since many of these allelic variants are rare, aggregation across multiple individuals is necessary to identify broadly applicable “allelic regions” and their association with a particular personal genome. Thus, we comprehensively annotate allele-specific binding and expression in 382 individuals by uniformly processing 1,263 ChIP-seq and RNA-seq datasets, developing approaches to reduce the heterogeneity between datasets due to over-dispersion. We then identify genomic regions enriched in allelic activity. We distribute our results via an online resource (alleledb.gersteinlab.org).

Deleted: Genomic variant databases are central to the large-scale annotation of personal genomes. An important class of genomic variants is associated with allele-specific behavior, which occurs when there is a differential effect between the two alleles in the human diploid genome. Many separate studies have conducted RNA sequencing (RNA-seq) and chromatin immunoprecipitation sequencing (ChIP-seq) experiments to detect variants associated with allele-specific expression (ASE) and binding (ASB). However, the heterogeneity makes it difficult to perform a naive aggregation of results to build a database. To meaningfully detect ASE and ASB variants from different studies, we reprocessed 1,141 RNA-seq and ChIP-seq experiments over 382 individuals in a uniform fashion. By taking into account varying degrees of overdispersion in the various experiments, we were able to harmonize the datasets and detect 7,462 and 85,742 allelic SNVs for ASB and ASE respectively; these represents 6% and 16% of accessible SNVs. In addition to variants, we also quantify 20,000 genes and 708 categories of coding and non-coding genomic annotations in terms of their allele-specific behavior. These results are housed in a searchable database, AlleleDB (www.alleledb.gersteinlab.org), which conveniently allows for viewing of these SNVs across multiple individuals. ¶

Introduction

In recent years, the number of personal genomes has increased dramatically, from single individuals¹⁻³ to large sequencing projects such as the 1000 Genomes Project⁴, UK10K⁵ and the Personal Genome Project⁶. These efforts have provided the scientific community with a massive catalog of human genetic variants, most of which are rare.⁴ Subsequently, a major challenge is to functionally annotate these variants.

Much of the characterization of variants so far has been focused on those found in the protein-coding regions, but the advent of large-scale functional genomic assays, such as chromatin immunoprecipitation sequencing (ChIP-seq) and RNA sequencing (RNA-seq), has facilitated the annotation of genome-wide variation. This can be accomplished by correlating functional readouts from the assays to genomic variants, particularly in identifying regulatory variants, such as mapping of expression quantitative trait loci (eQTLs)⁷⁻⁹ and allele-specific^{10,11} variants. eQTL mapping assesses the effects of variants on expression profiles across a large population of individuals and is usually used for detection of common regulatory variants. On the other hand, allele-specific approaches assess phenotypic differences directly at heterozygous loci within a single genome. Using each allele in a diploid genome as a perfectly matched control for the other allele, allele-specific variants can be detected even at low population allele frequencies. Therefore, allele-specific approaches are very **powerful**, in terms of functionally annotating personal genomes, **especially** for identifying **rare** cis-regulatory variants on a large scale.

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Early high throughput implementations of allele-specific approaches employed microarray technologies, and thus are restricted to a small subset of loci.¹²⁻¹⁴ Later studies have used ChIP-seq and RNA-seq experiments for genome-wide measurements of allele-specific variants but have been mostly limited to a single assay with a variety of individuals,¹⁵ or a few individuals with deeply-sequenced and well-annotated genomes.^{11,16} For instance, GM12878, a very well-characterized lymphoblastoid cell-line from a Caucasian female, has several RNA-seq datasets and a huge trove of ChIP-seq data for more than 50 transcription factors (TFs) distributed across multiple studies.¹⁷⁻¹⁹ Merging these datasets to create a database is advantageous. The database consolidates a catalog of annotated allele-specific variants in a central repository. Datasets belonging to the same individuals are also combined to increase statistical power in detection and simply having more features facilitates intra- and inter-individual comparisons (such as across more TFs and populations or investigating ASB-ASE coordination).

However, it is not optimal to simply aggregate results from multiple studies, even for the same biological sample. This is because disparate studies might design RNA-seq and ChIP-seq experiments with various goals in mind. Even if allele-specific analyses are conducted, they are often performed with different sets of tools, parameters and variations of the same test (**Supp Table 1**). In addition, each allele-specific analysis is also sensitive to the technical issues associated with variant calling and processing, RNA-seq and ChIP-seq experiments, such as thresholding and read mapping.²⁰⁻²³ For example, homozygous SNVs incorrectly called as heterozygous will result in reads mapping to one allele (over the other), giving rise to false signals of allelic imbalance. Variants called using shorter reads such as those in RNA-seq datasets can also contain many artefacts. Thus, it is important to have a call set, particularly obtained from whole genome DNA sequencing, such as **those from** the 1000 Genomes Project.

Also, allele-specific SNVs detected in copy number variants have a higher rate of false positives, since copy number changes can easily masquerade as allelic imbalance.

Therefore, the task of merging has to be carried out in a uniform, standardized fashion to yield interpretable results. To this end, we organize and unify datasets from eight different studies into a comprehensive data corpus and repurpose it especially for allele-specific analyses. We first take into account the overdispersion of each dataset when we harmonize them and then a second time, during the detection of allele-specific variants. Overall, we detect more than 7K and 85K single nucleotide variants (SNVs) associated with allele-specific binding (ASB) and expression (ASE) events respectively, over 382 individuals. We are able to present a survey for these allele-specific variants in various general and specific categories of coding and non-coding genomic elements and annotations (e.g. CDS regions, enhancers) in a population-aware manner. We identified genomic regions that are enriched or depleted in allelic activity. Finally, using our consolidated data, we investigate the extent of purifying selection in allele-specific SNVs and the inheritance of allele-specific expression and allele-specific binding in two different transcription factors. The variants and annotations are available as an online resource, AlleleDB (<http://alleledb.gersteinlab.org/>).

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Results

AlleleDB Workflow

In general, the AlleleDB workflow uniformly processes two pieces of information from each individual: the DNA sequence, and reads from either the ChIP-seq or RNA-seq experiment to assess SNVs associated with ASB or ASE respectively (Figure 1). Briefly, it starts by (1) constructing a diploid personal genome for each of the 382 individuals, using DNA variants from the 1000 Genomes Project. (2) It then aligns the ChIP-seq or RNA-seq dataset to each of the haploid genomes instead of the human reference genome, and chooses the better uniquely mapped alignment. This reduces reference bias that can potentially result in erroneous read mapping.¹⁶ Because each individual can have multiple ChIP-seq or RNA-seq datasets, the alignment is performed to each personal genome twice. (2a) In the first round, the alignment is performed for each of 276 ChIP-seq and 987 RNA-seq datasets to calculate a measure of overdispersion (with respect to an expected binomial distribution), ρ (see Discussion and Methods). We observe that if there is a greater overdispersion in the allelic ratio (defined as the proportion of reads that map to the reference allele) distribution of a dataset, the binomial test tends to overestimate the number of allele-specific events (Figure 2). There are varying degrees of overdispersion in our datasets, even between biological replicates; in general, RNA-seq datasets are generally more consistent in overdispersion than ChIP-seq datasets. Differing overdispersion in individual datasets poses a challenge later in step 2b when we merge, or pool, multiple datasets. In order to harmonize the datasets, we flag and filter datasets that are deemed to be more overdispersed in allelic ratio distributions, leaving us with 186 ChIP-seq and 955 RNA-seq datasets for allele-specific detection (Supp Table 2). (2b) The second alignment is performed by pooling the 186 ChIP-seq and 955 RNA-seq datasets that has not been filtered in Step 2a. The pooling is performed for each individual and each transcription factor (for ChIP-seq); e.g. CTCF ChIP-seq datasets for NA12878 that were not filtered were pooled together. An overdispersion parameter is re-calculated for each pooled set. (3) Finally, a beta-binomial test is performed using the 'pooled' overdispersion parameter calculated in Step 2b to detect allele-

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specific SNVs. For ChIP-seq data, the SNVs are further pared down to those within peak regions. We also remove SNVs if they lie in regions predicted to be copy number variants (see [Methods](#)).

We build a database, AlleleDB (<http://alleledb.gersteinlab.org/>), to house the annotations, the allele-specific and accessible SNVs. AlleleDB can be downloaded as flat files or queried and visualized directly as a UCSC track in the UCSC Genome browser²⁴ as specific genes or genomic locations. This enables cross-referencing of allele-specific variants with other track-based datasets and analyses, and makes it amenable to all functionalities of the UCSC Genome browser. Heterozygous SNVs found in the stipulated query genomic region are color-coded in the displayed track; [Figure 4a shows a schematic that illustrates an example of a visualization.](#)

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ASB and ASE Inheritance analyses using CEU trio

The CEU trio is a well-studied family and with multiple ChIP-seq studies performed on different TFs. Previous studies have also presented allele-specific inheritance.^{11,18} Here, after uniformly processing datasets from multiple studies, we are able to analyze and compare the heritability of ASE and ASB across two DNA-binding proteins in a consistent manner ([Figure 3](#); see [Methods](#)). For the DNA-binding protein CTCF and PU.1, we observe a high parent-child correlation ([Figure 3](#), [Supp Table 3](#)), denoting great similarity in allelic directionality (Pearson's correlation, $r \geq 0.77$ in both parent-child plots). We also observe considerable heritability in ASE, but to a lesser degree. In general, the high inheritance of allele-specific SNVs observed in the same allelic direction from parent to child also implies a sequence dependency in allele-specific behavior.

Allele-specific variants and enrichment analyses

Using the AlleleDB variants found in the personal genomes of the 2 parents of the trio and 379 unrelated individuals from Phase 1 of the 1000 Genomes Project, we focus on autosomal SNVs and detected 85,742 [unique](#) ASE and 7,462 ASB SNVs, representing [16%](#) and [6%](#) of the accessible SNVs respectively ([Table 1](#)). [15%](#) of our candidate ASE SNVs and [3%](#) of ASB SNVs are in the coding DNA sequences (CDS); [these correspond to log odds ratios of 0.3 \(enrichment\) and -0.2 \(depletion\) respectively, when compared to the non-coding regions \(Supp Figure 1\).](#)

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Of great interest, is the annotation of these allele-specific SNVs with respect to known genomic elements, both coding and non-coding. We calculate the enrichment of ASB and ASE SNVs in various genomic categories. To do so, we further define sets of 'control' SNVs. This is especially pertinent to our enrichment analyses, since the Fisher's exact test is dependent on the choice of the null expectation (i.e. controls). The control SNVs are not allele-specific and are derived from a set of 'accessible' SNVs, which are heterozygous SNVs and possess at least the minimum number of reads needed to be statistically detectable for allelic imbalance; in other words, the control SNVs are well-matched in power to the detected allele-specific SNVs (see [Methods](#)). The accessible SNVs are determined for each pooled ChIP-seq (grouped by individual and TF, not by study) or RNA-seq dataset (grouped by individual).

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To estimate the degree of allele-specificity in both coding and non-coding genomic elements, we calculate the enrichment of allele-specific SNVs by comparing allele-specific SNVs relative to the control SNVs using Fisher's exact tests. [The enrichment analyses are performed in two ways:](#)

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'expanded' and 'collapsed'. The former counts each occurrence of SNV in a population-aware manner, where each accessible or allele-specific SNV is counted for each individual at each locus. The latter collapses and counts an accessible or allele-specific SNV location as a unique SNV as long as it occurs in at least one individual (Figure 4b). Both enrichment analyses are performed in genomic annotations (or categories) with differing granularities, from broad genomic categories to individual binding motifs and genes. Broad genomic categories are grouped based on similar functional context. These include 708 non-coding genomic categories from the ENCODE project²⁵ (e.g. DNaseI hypersensitivity sites and transcription factor binding motifs) and six gene sets known to be involved in monoallelic expression (MAE)^{26,27} (e.g. imprinted genes,²⁸ olfactory receptor genes²⁹). Between the two enrichment analyses, we observe consistent trends in the odds ratios of ASB SNVs and ASE SNVs across the MAE gene sets, except for the T cell receptors. The category is only enriched in ASE SNVs when we collapsed the SNV count (Supp Fig 1) but depleted when we expand the enrichment analysis in a population-aware fashion. This suggests that the allele-specific expression in T cell receptors is not consistently observed in all individuals. Interestingly, there is a consistent depletion in ASE SNVs for the constitutively expressed housekeeping genes, implying that most housekeeping genes give a more balanced (biallelic) expression (Figure 5).

We further calculate the enrichment of allele-specific SNVs in 19,257 autosomal protein-coding genes from GENCODE³¹ in both collapsed and population-aware expanded fashion. The database allows us to visualize allele-specific SNVs across the gene region and over multiple individuals. For example, SNRPN and SNURF are maternally-imprinted genes, shown to be highly implicated in the Prader-Willi Syndrome, an imprinting disorder.³² Indeed, they are two of our most highly-ranked allele-specific genes by overall odds ratio (column 'AS.OR' in Supp file 3). When SNURF is queried in our database, we can see clearly that the allele-specificity is supported not only by evidence from 61 ASE loci across the gene but a number of variants are shown to be also allele-specific over multiple individuals, one variant even up to 169 individuals. The concurrent visualization of ASB and ASE SNVs with respect to genomic elements using the UCSC genome browser is also another advantage of AlleleDB. For example, ZNF331 gene contains a good number of both ASE and ASB loci. It has previously been shown experimentally to be consistently expressed from the paternal allele.³³ Our visualization shows ASB loci from POL2, RPB2 and MYC of several individuals coinciding near ZNF331 exons; the former two DNA-binding proteins are components of RNA polymerase II (Figure 4a).

Additionally, we extend the enrichment analyses to gene elements, such as introns and promoter regions. Figure 5 (and Supp Fig 1) shows the enrichment of allele-specific SNVs in elements closely related to a gene model, namely enhancers, promoters, CDS, introns and untranslated regions (UTR). For SNVs associated with allele-specific binding (ASB), we observe an enrichment in the 5' UTRs. This is in line with an enrichment of ASB SNVs in promoters. Though not significant, this suggests functional roles for these variants found in TF binding motifs or peaks found near transcription start sites to regulate gene expression. We see variable enrichments of ASB SNVs in the peaks of particular TFs such as POL2, SA1 and CTCF in promoter regions, while depletion in others, such as PU.1 (Figure 5, Supp file 3). These differences might imply that some TFs are more likely to participate in allele-specific regulation than others. Between the two enrichment analyses, we observe more consistent trends in the odds ratios of ASB SNVs than ASE SNVs. The differences are most likely contributed by the

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Deleted: seven other gene categories, including housekeeping and imprinted genes. Figure 5

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presence of common SNVs that are also behaving consistently (either being allele-specific or non-allele-specific) over multiple individuals.

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The population-aware analysis gives additional power to calculate enrichment for very specific genomic annotations, namely protein-coding genes, enhancers and transcription factor binding motifs; this is unlike broad genomic categories that span over multiple regions in broad genomic categories. Based on the expanded enrichment analysis, we find xxx protein-coding genes (Supp File 2), yyy enhancers and zzz motifs that are significantly enriched and aaa genes, bbb enhancer and ccc motifs significantly depleted in allele-specific SNVs; we define the enriched elements as 'allele-specific' and the depleted elements as 'balanced', the rest are considered 'indeterminate'. We also provide these lists on the AlleleDB resource.

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Rare variants and purifying selection in allele-specific SNVs

To assess the occurrence of ASB and ASB SNVs in the human population, we consider the population minor allele frequencies (MAF). Table 1 shows the breakdown of the accessible and allele-specific SNVs in six ethnic populations (we combined the results for CHB and JPT) and allele frequencies. Yoruba from Ibadan, Nigeria (YRI) contribute the most to both ASE and ASB variants at each allele frequency category. The number of rare allele-specific SNVs (MAF $\leq 5\%$) is about two folds higher in the YRI than the other European sub-populations of comparable (CEU, FIN) or larger (TSI) population sizes (see Methods for full explanation of population abbreviations). However, the percentage of allele-specific SNVs (in accessible SNVs) remain fairly consistent. In general, rare variants do not form the majority of all the allele-specific variants. For each category of allele frequency, the proportion of allele-specific SNVs detected (with respect to accessible SNVs) is fairly comparable across populations (CEU, FIN, GBR, TSI and YRI), with a slight enrichment of ASB SNVs and slight depletion of ASE SNVs as we go towards lower frequencies.

To examine selective constraints in allele-specific SNVs, we then consider the enrichment of rare variants with MAF $\leq 0.5\%$.^{4,36} Figure 6 shows a shift of the allele frequency spectrum towards very low allele frequencies in all allele-specific and non-allele-specific SNVs, peaking at MAF $\leq 0.5\%$ (Figure 6). We limit our analyses for ASE SNVs to only those found in CDS regions and ASB SNVs to only those found within known TF motifs (among the 708 non-coding categories in Supp File 1). Our results in Figure 6 show a statistically significant lower enrichment of rare variants in ASE SNVs as compared to non-ASE SNVs (Fisher's exact test odds ratio=0.2, $p < 2.2e-16$) but statistically insignificant higher enrichment of rare variants in non-ASB SNVs than ASB SNVs (Fisher's exact test odds ratio=1.4, $p=0.08$). This posits that ASE SNVs are under lesser selective constraints than non-ASE SNVs. Such weaker selection may be a result of accommodating varying levels of gene expression across individuals. In addition, ASB SNVs seem to be under less selective constraints than ASE SNVs, which agrees well with the results in a previous study where more variability is being observed in binding than expression¹⁹.

Allele-specific variants in TF binding motifs affecting TF occupancy

A pertinent ASB analysis is to identify ASB SNVs that might cause a TF binding difference. To perform this analysis, we focus on the 328 ASB SNVs found across multiple individuals that reside in the binding motifs of 16 TFs. We consider an allele to be disruptive when it occurs less frequently at the position in the motif. Thus, we compare the difference in occurrence between

the reference and the alternate allele of the ASB SNV in the position weight matrix (PWM) of a TF binding motif. For instance, if the alternate allele is disruptive, the reference allele is favored, and the difference in occurrence > 0 (see Methods). We then correlate this with the allelic ratio at the ASB SNV. We expect a TF binding motif that favors the reference allele of an ASB SNV (difference in occurrence > 0) to be associated with more binding to the reference allele (i.e. allelic ratio > 0.5). We find a statistically significant correlation between the difference in occurrence and the allelic ratio for the 328 ASB SNVs (Pearson's correlation = 0.70, $p < 2.2e-16$), showing that there is indeed an overall trend for the favored allele to correspond to increased TF binding. In general, the effects of the SNVs are consistent across individuals in the context of the same motifs. As a resource, we provide the list of ASB SNVs with the frequencies of the occurrence of their reference and alternate alleles found in the various TF motifs and their corresponding allelic ratios (Supp File 4).

Discussion

The binomial test is typically used to provide statistical significance for the identification of allele-specific SNVs. However, previous studies have observed a deviation from the binomial distribution in read count distributions in CHIP-seq and RNA-seq datasets, which in turn results in broader allelic ratio distributions, i.e. overdispersed.^{7,37-39} We generally assume that most of the SNVs in autosomes would have more balanced allelic ratios. Hence, while overdispersion could be a biological consequence of allele-specific behavior, high overdispersion in ASE distributions would imply biased autosomal gene expression and might in fact indicate potential issues, e.g. sparse uneven coverage. Since there are multiple datasets for each individual and TF, it would be reasonable to homogenize the separate datasets, so that the resultant pools for each individual and TF can facilitate detection of a more conservative set of allele-specific SNVs for AlleleDB. In addition to accounting for the overdispersion in the statistical inference of allele-specific SNVs, we propose the use of the overdispersion parameter, ρ , as a means to select datasets that are more similar in the spread of the distributions. Datasets with low overdispersion give very similar results between binomial and beta-binomial tests (Figure 2A). The binomial test tends to overestimate the number of detected allele-specific SNVs in datasets with higher overdispersion; it is too relaxed in these cases (Figure 2B). Consequently, we adopt a serial two-step approach of first segregating individual datasets with high overdispersion, and then pooling the datasets (by individual and TF) for allele-specific detection, using the beta-binomial test to account for the degree of overdispersion. We provide a more confident set of allele-specific SNVs, which are found to be in the same allelic direction (reference allele) in at least 2 individuals in AlleleDB (Supp File 6). The list of high-impact ASB SNVs that cause a change in transcription binding motif occupancy are also provided.

So far, allele-specific analyses have usually been more SNV- or gene-centric. However, many diseases have been found to implicate ASE in particular genomic regions.⁴⁰⁻⁴² Our downstream analyses focuses on relating allele-specific activity to known genomic elements and annotations, such as CDS and various non-coding regions. This is also useful, considering that a significant portion of allele-specific SNVs are rare (Figure 6 and Table 1), i.e. occurring in only a few individuals ($MAF \leq 0.5\%$), and they are often in close proximity to each other. Consolidating rare allele-specific SNVs to quantify allele-specificity is helpful in defining SNV sets which allows us to assign weights to regions or multiple variants based on allele-specific activity when

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So far, allele-specific analyses have usually been more SNV- or gene-centric. Our downstream analyses focuses on relating allele-specific activity to known genomic annotations, such as CDS and various non-coding regions, and many diseases have been found to implicate ASE in particular genomic regions.³⁹⁻⁴¹ This is useful, considering a significant portion of allele-specific SNVs are rare (Figure 6 and Table 1), i.e. occur in only a few individuals ($MAF \leq 0.5\%$), and they are often in close proximity to each other. Consolidating rare allele-specific SNVs as SNV sets is helpful in assigning weights to variants or regions based on allele-specific activity when incorporating into large-scale annotation pipelines.⁴⁵ Also, by comparing ASB and ASE enrichments within specific genomic regions or broad categories, we can provide some insights into the coordination of ASB and ASE within that category. For example, loci associated with monoallelic expression have shown to be associated with ASB of various transcription factors, such as imprinted^{42,43} and immunoglobulin genes⁴⁴. By associating allele-specific SNVs with a genomic annotation and assigning a proxy measure of allele-specific behavior, we are able to define categories of genomic regions with more allele-specific activity. ¶

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Our current catalog of allele-specific SNVs is detected from lymphoblastoid cell lines (LCLs), which is also the predominant cell-line type in the literature. However, it has already been known that there is considerable variability in regulation of gene expression in different tissues.⁴⁸

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incorporating into large-scale annotation pipelines⁴³; this is akin to the idea of burden tests for rare variants in association studies.^{44,45}

We have also adopted two ways to analyze enrichment: an expanded approach that capitalizes on the number of individuals and a collapsed approach that computes enrichment based on unique allele-specific SNVs occurring in at least one individual. A difference in results from both analyses can suggest an interplay between rare and common allele-specific SNVs or inconsistent allele-specificity at a given locus across multiple individuals. By computing the enrichment analysis in a population-aware fashion, we can also define elements based on evidence supported over multiple individuals. This allows us to quantify allele-specific consistency and enrichment even within smaller and very specific genomic annotations, and differentiate those that are significantly and more consistently enriched to be 'allele-specific', depleted to be 'balanced', or otherwise 'indeterminate'.

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Additionally, we can provide some insights into the coordination of ASB and ASE within that category, by comparing ASB and ASE enrichments within specific genomic regions or broad categories (Figure 5). For example, loci associated with monoallelic expression have shown to be associated with ASB of various transcription factors, such as imprinted^{46,47} and immunoglobulins genes⁴⁸. Also, in Figure 4a, we can visualize in AlleleDB specific sub-regions within ZNF331 gene that ASB and ASE coordination might occur.

Our current catalog of allele-specific SNVs is detected from lymphoblastoid cell lines (LCLs), which is also the predominant cell-line type in the literature. However, it has already been known that there is considerable variability in regulation of gene expression in different tissues.⁴⁹ Data from projects, such as GTEx⁴⁹, which has more functional assays and sequencing in other tissues and cell lines can be incorporated to provide a more complete allele-specific analysis. Furthermore, our search for datasets shows a dearth of personal genomes with corresponding ChIP-seq and RNA-seq data in non-European populations. It could be a strong reflection on the lack of large-scale functional genomics assays in specific ethnic groups – a concern echoed previously in population genetics and is recently being increasingly addressed.⁵⁰ Since many allele-specific variants have been found to be rare at both the individual and the sub-population level, it is of great interest and importance that more individuals of diverse ancestries be represented.

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In conclusion, there is great utility in integrating existing data. However, it is essential to harmonize heterogeneous datasets in a uniform fashion. As more diverse and accurate personal genomes with haplotype information⁵¹⁻⁵³ and their corresponding functional genomics data become available, an allele-specific approach to detect many allele-specific SNVs for a single personal genome will increase the number of rare allele-specific SNVs detected. AlleleDB is easily scaled to accommodate new individual genomes, tissue and cell types. Additionally, the database allows the visualization of ASB and ASE together conveniently. Such should be of value to researchers of various backgrounds.

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Materials and Methods

Construction of diploid personal genomes

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There is a total of 382 genomes used in this study: 379 unrelated genomes, of low-coverage (average depth of 2.2 to 24.8) from Utah residents in the United States with Northern and Western European ancestry (CEU), Han Chinese from Beijing, China (CHB), Finnish from Finland (FIN), British in England and Scotland (GBR), Japanese from Tokyo, Japan (JPT), Toscani from Italy (TSI), and Yorubans from Ibadan, Nigeria (YRI) and 3 high-coverage genomes from the CEU trio family (average read depth of 30x from Broad Institute's, GATK Best Practices v3; variants are called by UnifiedGenotyper). Each diploid personal genome is constructed from the SNVs and short indels (both autosomal and sex chromosomes) of the corresponding individual found in the 1000 Genomes Project. This is constructed using the tool, *vcf2diploid*.¹⁶ Essentially, each variant (SNV or indel) found in the individual's genome is incorporated into the human reference genome, hg19. Most of the heterozygous variants are phased in the 1000 Genomes Project; those that are not, are randomly phased. As a result, two haploid genomes for each individual are constructed. When this is applied to the family of CEU trio, for each child's genome, these haploid genomes become the maternal and paternal genomes, since the parental genotypes are known. Subsequently, at a heterozygous locus in the child's genome, if at least one of the parents has a homozygous genotype, the parental allele can be known. However, for each of the genomes of the 379 unrelated individuals and the 2 parents from the CEU trio, the alleles, though phased, are of unknown parental origin.

CNV genotyping is also performed for each genome by CNVnator,⁵⁴ which calculates the average read depth within a defined window size, normalized to the genomic average for the region of the same length. For each low coverage genome, a window size of 1000 bp is used, while for the high coverage genomes, a window size of 100 bp is used. SNVs found within genomic regions with a normalized abnormal read depth <0.5 or >1.5 are filtered out, since these would mostly likely give rise to spurious allele-specific detection.

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RNA-seq and ChIP-seq datasets

In total, we reprocessed 287 ChIP-seq and 993 RNA-seq datasets for 382 individuals from eight different studies (Supp Table 2).

RNA-seq datasets are obtained from the following: gEUVADIS¹⁵, ENCODE²⁵, Lalonde *et al.* (2011)⁵⁵, Montgomery *et al.* (2010)⁵⁶, Pickrell *et al.* (2010)⁷, Kilpinen *et al.* (2013)¹⁸ and Kasowski *et al.* (2013)¹⁹.

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ChIP-seq datasets are obtained from the following: ENCODE²⁵, Kilpinen *et al.* (2013)¹⁸, Kasowski *et al.* (2013)¹⁹ and McVicker *et al.* (2013)⁵⁷.

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Read alignment and estimation of ρ in individual and pooled datasets

Reads are aligned against each of the derived haploid genome (maternal/paternal genome for trio) using Bowtie 1.⁵⁸ No multi-mapping is allowed and only a maximum of 2 mismatches per alignment is permitted. This enables the calculation of the proportion of reads that align to the reference allele, or the allelic ratio, at each heterozygous SNV.

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To estimate ρ , we adopt a three-step approach. We first obtain the empirical histogram for the allelic ratios of all heterozygous SNVs with read counts ≥ 6 . Next, we calculate the expected null

distribution (where there is no allelic imbalance) using the probability density function (pdf) of the beta-binomial distribution using the R package, VGAM⁵⁹.

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$$P_{betabin}(X = k|n, a, b) = \binom{n}{k} \frac{B(k + a, n - k + b)}{B(a, b)}$$

where n represents the total number of reads at a particular locus, $B(x,y)$ represents the beta function with variables x and y , a and b represent the shape parameters of the beta distribution. For computational efficiency, if $n \geq 1000$, we set it to a maximum of 1000, but retain the allelic ratio at the SNV. The VGAM beta-binomial routines require the input of the overdispersion parameter, ρ , and probability of success (also the mean of the beta distribution), which we fix at $p=0.5$ since the null hypothesis assumes no allelic imbalance. We then obtain the expected beta-binomial distributions for $\rho=0$ to $\rho=1$ with increment of 0.1, and choose ρ that minimizes the least sum of squared errors (LSSE) between the empirical and the expected distributions. Lastly, to further refine our estimate, we iterate a bisection method to arrive at a LSSE (R pseudo-code available in Supp file [6](#)).

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After removing 11 ChIP-seq and 6 RNA-seq datasets that have insufficient read alignments, we calculate ρ for each 276 ChIP-seq and 987 RNA-seq individual datasets. For RNA-seq datasets, we removed 32 datasets with $\rho \geq 0.125$, which is one standard deviation higher than the mean ρ amongst the RNA-seq datasets. For ChIP-seq datasets, because many of the datasets have considerable ρ , we use a less stringent arbitrary threshold of $\rho \geq 0.3$ to remove 90 ChIP-seq datasets. Using the resultant 186 ChIP-seq and 955 RNA-seq datasets, we pool datasets by TF and individual for ChIP-seq and by individual for RNA-seq and re-calculate ρ for each pooled dataset. This final ρ is used in the beta-binomial test for allele-specific SNV detection.

Allele-specific SNV detection

Allele-specific SNV detection is performed on the pooled datasets, as mentioned above. Here, a beta-binomial p-value is derived based on the VGAM R package as described in the previous section. Similarly for computational efficiency, if $n \geq 1000$, we set it to a maximum of 1000, but retain the allelic ratio at the SNV. To correct for multiple hypothesis testing, FDR is calculated. Since statistical inference of allele-specificity of a locus is dependent on the number of reads of the ChIP-seq or RNA-seq dataset, this is performed using an explicit computational simulation.¹⁶ Briefly, for each iteration of the simulation, a mapped read is randomly assigned to either allele at each heterozygous SNV and performs a beta-binomial test using the estimated ρ . At a given p-value threshold, the FDR can be computed as the ratio of the number of false positives (from the simulation) and the number of observed empirical positives. An FDR cutoff of 10% is used for ChIP-seq data and 5% for RNA-seq data, since the latter is typically of deeper coverage. Furthermore, we allow only significant allele-specific SNVs to have a minimum of 6 reads.

For ChIP-seq data, allele-specific SNVs have to be also within peaks. Peak regions are determined by first performing PeakSeq⁶⁰ for each of the personal haploid genome. Only a single read per strand per position is kept and duplicates removed. The fragment length is set to 200 bps. Peak calling is performed with default parameters and the final peak set for each transcription factor is identified at a false discovery rate of 5%. Finally, the coordinates of the

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peaks (based on the respective personal haploid genomes) are mapped to the reference genome and then finally being merged between the haploid genomes.

Allele-specific detection for all TFs and gene expression of 382 individuals took about 600 days in CPU time (1.6 years), but the pipeline is highly parallelizable, thereby streamlining the process.

AlleleDB

The final data and results are organized into a resource, AlleleDB (<http://alleledb.gersteinlab.org/>), which conveniently interfaces with the UCSC genome browser for query and visualization. Since many in the scientific community are familiar with the genome browser, we hope that this would increase the accessibility and usability of AlleleDB. The query results are also available for download in BED format, which is compatible with other tools, such as the Integrated Genome Viewer⁶¹. More in-depth analyses can be performed by downloading the full set of allele-specific results. For ASB, the output will be delineated by the sample ID and the associated TFs; for ASE, the output will be categorized by individual and the associated gene. We also provide the raw counts for each accessible SNV and indicate if it is identified as an allele-specific SNV. AlleleDB also serves as an annotation of allele-specific regulation of the 1000 Genomes Project SNV catalog.

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Allele-specific inheritance analyses

The conventional measure of ‘heritability’ allows the estimation of (additive) genetic contribution to a certain trait. The population genetics definition of ‘heritability’ in a parent-offspring setting is described by the slope, β , of a regression ($Y = \beta X + \alpha$), with the dependent variable being the child’s trait value (Y) and the independent variable (X) being the average trait values of the father and the mother (‘midparent’).⁶² This is a population-based measure typically performed on a large set of trios for a particular trait (e.g. height) and β is not necessarily bound between 0 and 1.

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Given we have only a single trio, we adapt the definition of ‘heritability’ to quantify allele-specific inheritance for each TF. For each TF and parent-child comparison, we consider ASB SNVs from two scenarios: (1) when an allele-specific SNV is heterozygous in all three individuals but common to the two individuals being compared, and (2) when an allele-specific SNV is heterozygous in two individuals and homozygous (reference or alternate) in the third. We define the allelic ratio as the ‘trait’, which is a continuous value and computed as the proportion of reads that align to the reference allele with respect to the total number of reads mapped to either allele of a particular site. We perform the analyses separately for father-child and mother-child pair to maximize statistics, since a midparent calculation will require that a SNV is allele-specific in all three individuals (Scenario 1).

Given that Pearson’s correlation coefficient, r , always gives a value between 0 and 1, we use r instead of β , as our measure of ‘heritability’. We also compute and include β values in Supplementary Table 2. The parent-parent comparison is provided as a source of comparison for two unrelated individuals with shared ancestry. For parent-parent β , the maternal allelic ratio is chosen arbitrarily to be the independent variable.

Genomic annotations

Categories of gene elements from [Figure 5](#), such as promoters, CDS regions and UTRs, and 19,257 autosomal protein-coding gene annotations (HGNC symbols) are obtained from GENCODE version 17.³¹ Promoter regions are set as 2.5kbp upstream of all transcripts annotated by GENCODE.

Gene annotations also include 2.5kbp upstream of the start of gene. 708 categories of non-coding annotations are obtained from ENCODE Integrative release,²⁵ which includes broad categories such as TF binding sites and annotations such as distal binding sites of particular TFs, e.g. ZNF274. The details of TF family classification is first described in Vaquerizas *et al.*⁶³ and then also in Gerstein *et al.*⁶⁴ Note that these TF binding sites are separate from those sites in promoter regions in [Figure 5](#), which are based on the 44 TFs and peaks from the CHIP-seq experiments used in our pipeline.

The olfactory receptor gene list is from the HORDE database²⁹; immunoglobulin, T cell receptor and MHC gene lists are from IMGT database⁶⁵. Imprinted genes are merged from the Catalog of Parent-of-origin Effects (<http://igc.otago.ac.nz/home.html>),⁶⁶ the GeneImprint website⁶⁷ and also Lo *et al.*⁶⁸ We performed enrichment analyses on a number of enhancer lists, which are derived using the ChromHMM and Segway algorithms (Ernst and Kellis (2012)⁶⁹, Hoffman *et al.* (2013)⁷⁰, and data from distal regulatory modules from Yip *et al.* (2012)⁷¹. The result for the enhancers in [Figure 5](#) is based on the union of these lists. The lists can be found at <http://info.gersteinlab.org/Encode-enhancers>. An additional enhancer list for experimentally validated enhancers is obtained from VISTA enhancer browser database⁷² (<http://enhancer.lbl.gov/>). Housekeeping gene list is obtained from Eisenberg and Levanon (2013) (<http://www.tau.ac.il/~elieis/HKG/>)⁷³.

All enrichment analyses results with respect to these annotations are provided in the supplementary files, which are provided for download on the AlleleDB website (<http://alleledb.gersteinlab.org/download/>).

Enrichment analyses

Enrichment analyses were performed in two ways: 'collapsed' and 'expanded' (Figure 4b). In both cases we use the Fisher's exact test to calculate the odds ratio and the hypergeometric p value, to test for the enrichment of allele-specific SNVs compared to accessible non-specific SNVs. Accessible SNVs are heterozygous, and they exceed the minimum number of reads detectable statistically by the beta-binomial test, for each dataset. This is an additional criterion imposed, on top of the minimum threshold of 6 reads used in the AlleleSeq pipeline. The minimum number of reads varies with the pooled size (coverage) of the CHIP-seq or RNA-seq dataset. Given a fixed FDR cutoff, for a larger dataset, the beta-binomial p-value threshold is typically lower, making the minimum number of reads (N) that will produce the corresponding p-value, larger. This alleviates a bias in the enrichment test for including SNVs that do not have sufficient reads in the first place. Considering an extreme allelic imbalance case where all the reads are found on one allele (all successes or all failures, i.e. allelic ratio is 0 or 1), this minimum N can be obtained from a table of expected two-tailed beta-binomial probability density function, such that accessible SNVs are all SNVs with number of reads, $n = \max(6, N)$. By considering only cases with the largest effect size, we underestimate the number of control

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Deleted: Genes for random monoallelic expression are from Gimelbrant *et al.* (2007)³⁰ The olfactory receptor gene list is from the HORDE database²⁹; immunoglobulin, T cell receptor and MHC gene lists are from IMGT database⁶³. Imprinted genes are merged from the Catalog of Parent-of-origin Effects (<http://igc.otago.ac.nz/home.html>),⁶⁴ the GeneImprint website⁶⁵ and also Lo *et al.*⁶⁶ We performed enrichment analyses on a number of enhancer lists, which are derived using the ChromHMM and Segway algorithms (Ernst and Kellis (2012)⁶⁹, Hoffman *et al.* (2013)⁶⁸, and data from distal regulatory modules from Yip *et al.* (2012)⁶⁹. The result for the enhancers in [Figure 4](#) is based on the union of these lists. The lists can be found at <http://info.gersteinlab.org/Encode-enhancers>. An additional enhancer list for experimentally validated enhancers is obtained from VISTA enhancer browser database⁷⁰ (<http://enhancer.lbl.gov/>). Housekeeping gene list is obtained from Eisenberg and Levanon (2013) (<http://www.tau.ac.il/~elieis/HKG/>)⁷¹.¶

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SNVs and this provides a conservative approximation of the statistical significance of the enrichment (or depletion). In the ‘collapsed’ enrichment analysis, each accessible or allele-specific SNV is counted once uniquely, as long as it occurs in at least one individual in AlleleDB. The ‘expanded’ analysis is performed in a population-aware manner, where each accessible or allele-specific SNV is counted once for each occurrence in an individual. To use the Fisher’s exact test for enrichment analyses in each genomic annotation, we further exclude the respective ASB or ASE SNVs from the control binding or expression SNVs (accessible and non-allele-specific) in the corresponding annotations. P-values are Bonferroni-corrected and considered significant if ≤ 0.05 .

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‘Allele-specific’ and ‘balanced’ autosomal protein-coding genes, enhancers and transcription factor binding motifs are defined based on statistically significant (Bonferroni-corrected p value ≤ 0.05) enrichments (odds ratio ≥ 1.5) or depletions (odds ratio < 1.5) respectively, as obtained from the ‘expanded’ enrichment analysis; the rest of the elements with non-significant odds ratios are considered ‘indeterminate’.

Analysis of ASB SNVs found in TF motifs

We obtain a list of all TF motifs and their corresponding position weight matrices (PWMs) from Kheradpour and Kellis⁷⁴ (<http://compbio.mit.edu/encode-motifs/>), using the 2013 version. This set of motifs and PWMs is derived from the ENCODE project and include motifs from TRANSFAC and JASPAR. We then take two approaches to find the effects of ASB SNVs. (1) For all ASB SNV positions in the motifs detected by Kheradpour and Kellis, we obtain the occurrence (frequency) of their reference and alternate allele in the respective PWMs. This first approach is only able to find motif-breaking events that disrupt existing motifs in the reference genome. The PWMs of motifs are defined based on the ENCODE project. (2) Our second approach attempts to include both motif-breaking and motif-gaining events caused by ASB SNVs in AlleleDB. Based on each PWM, we further scan a 59-bp window around the ASB SNV (± 29 bp of the SNV) separately for both the reference and alternate alleles for potential motifs. For each candidate motif, we compute the sequence score using the tool TFM-Pvalue⁷⁵, where sequence score is defined by summing up the log likelihoods of each position of the PWM. A motif is identified when the P value on its sequence score $\leq 1e-6$.

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We then merge the results from both approaches. The allelic ratio is defined as before, i.e. the ratio of number of reference reads to the total number of reads, thus when the ratio > 0.5 , there are more reads that align to the reference allele, signifying more binding to the motif with the reference allele. We compute the difference in occurrence between the reference and alternate allele (occurrence of reference allele minus occurrence of alternate allele) based on the PWM of the motif, thus a positive value indicates that the reference allele is favored (i.e. less disruptive). The Pearson’s correlation is calculated between this difference and the allelic ratio.

Acknowledgements

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References

1. Wheeler, D. A. *et al.* The complete genome of an individual by massively parallel DNA sequencing. *Nature* **452**, 872–6 (2008).
2. Lupski, J. R. *et al.* Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. *N. Engl. J. Med.* **362**, 1181–91 (2010).
3. Levy, S. *et al.* The diploid genome sequence of an individual human. *PLoS Biol.* **5**, e254 (2007).
4. Abecasis, G. R. *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012).
5. Muddyman, D., Smee, C., Griffin, H. & Kaye, J. Implementing a successful data-management framework: the UK10K managed access model. *Genome Med.* **5**, 100 (2013).
6. Church, G. M. The personal genome project. *Mol. Syst. Biol.* **1**, 2005.0030 (2005).
7. Pickrell, J. K. *et al.* Understanding mechanisms underlying human gene expression variation with RNA sequencing. *Nature* **464**, 768–72 (2010).
8. Majewski, J. & Pastinen, T. The study of eQTL variations by RNA-seq: from SNPs to phenotypes. *Trends Genet.* **27**, 72–9 (2011).
9. Montgomery, S. B., Lappalainen, T., Gutierrez-Arcelus, M. & Dermitzakis, E. T. Rare and common regulatory variation in population-scale sequenced human genomes. *PLoS Genet.* **7**, e1002144 (2011).
10. Djebali, S. *et al.* Landscape of transcription in human cells. *Nature* **489**, 101–8 (2012).
11. McDaniel, R. *et al.* Heritable individual-specific and allele-specific chromatin signatures in humans. *Science* **328**, 235–9 (2010).
12. Yan, H., Yuan, W., Velculescu, V. E., Vogelstein, B. & Kinzler, K. W. Allelic variation in human gene expression. *Science* **297**, 1143 (2002).
13. Ge, B. *et al.* Global patterns of cis variation in human cells revealed by high-density allelic expression analysis. *Nat. Genet.* **41**, 1216–22 (2009).
14. Lo, H. S. *et al.* Allelic variation in gene expression is common in the human genome. *Genome Res.* **13**, 1855–62 (2003).

15. Lappalainen, T. *et al.* Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* **501**, 506–11 (2013).
16. Rozowsky, J. *et al.* AlleleSeq: analysis of allele-specific expression and binding in a network framework. *Mol. Syst. Biol.* **7**, 522 (2011).
17. Engström, P. G. *et al.* Systematic evaluation of spliced alignment programs for RNA-seq data. *Nat. Methods* **10**, 1185–91 (2013).
18. Kilpinen, H. *et al.* Coordinated effects of sequence variation on DNA binding, chromatin structure, and transcription. *Science* **342**, 744–7 (2013).
19. Kasowski, M. *et al.* Extensive variation in chromatin states across humans. *Science* **342**, 750–2 (2013).
20. Harismendy, O. *et al.* Evaluation of next generation sequencing platforms for population targeted sequencing studies. *Genome Biol.* **10**, R32 (2009).
21. Stevenson, K. R., Coolon, J. D. & Wittkopp, P. J. Sources of bias in measures of allele-specific expression derived from RNA-sequence data aligned to a single reference genome. *BMC Genomics* **14**, 536 (2013).
22. Hansen, K. D., Brenner, S. E. & Dudoit, S. Biases in Illumina transcriptome sequencing caused by random hexamer priming. *Nucleic Acids Res.* **38**, e131 (2010).
23. Degner, J. F. *et al.* Effect of read-mapping biases on detecting allele-specific expression from RNA-sequencing data. *Bioinformatics* **25**, 3207–12 (2009).
24. Kent, W. J. *et al.* The human genome browser at UCSC. *Genome Res.* **12**, 996–1006 (2002).
25. Bernstein, B. E. *et al.* An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).
26. Goldmit, M. & Bergman, Y. Monoallelic gene expression: a repertoire of recurrent themes. *Immunol. Rev.* **200**, 197–214 (2004).
27. Zakharova, I. S., Shevchenko, A. I. & Zakian, S. M. Monoallelic gene expression in mammals. *Chromosoma* **118**, 279–90 (2009).
28. Morison, I. M., Paton, C. J. & Cleverley, S. D. The imprinted gene and parent-of-origin effect database. *Nucleic Acids Res.* **29**, 275–6 (2001).
29. Olender, T., Nativ, N. & Lancet, D. HORDE: comprehensive resource for olfactory receptor genomics. *Methods Mol. Biol.* **1003**, 23–38 (2013).

30. Gimelbrant, A., Hutchinson, J. N., Thompson, B. R. & Chess, A. Widespread monoallelic expression on human autosomes. *Science* **318**, 1136–40 (2007).
31. Harrow, J. *et al.* GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res.* **22**, 1760–74 (2012).
32. Horsthemke, B. & Buiting, K. Imprinting defects on human chromosome 15. *Cytogenet. Genome Res.* **113**, 292–9 (2006).
33. Pollard, K. S. *et al.* A genome-wide approach to identifying novel-imprinted genes. *Hum. Genet.* **122**, 625–634 (2008).
34. Wang, Z., Gerstein, M. & Snyder, M. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* **10**, 57–63 (2009).
35. Nagalakshmi, U. *et al.* The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science* **320**, 1344–9 (2008).
36. Khurana, E. *et al.* Integrative annotation of variants from 1092 humans: application to cancer genomics. *Science* **342**, 1235587 (2013).
37. Anders, S. & Huber, W. Differential expression analysis for sequence count data. *Genome Biol.* **11**, R106 (2010).
38. Skelly, D. A., Johansson, M., Madeoy, J., Wakefield, J. & Akey, J. M. A powerful and flexible statistical framework for testing hypotheses of allele-specific gene expression from RNA-seq data. *Genome Res.* **21**, 1728–1737 (2011).

39. [Zhang, S. *et al.* Genome-wide identification of allele-specific effects on gene expression for single and multiple individuals. *Gene* **533**, 366–373 \(2014\).](#)

Deleted: 39.

40. Amin, A. S. *et al.* Variants in the 3' untranslated region of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. *Eur. Heart J.* **33**, 714–23 (2012).

41. Anjos, S. M., Shao, W., Marchand, L. & Polychronakos, C. Allelic effects on gene regulation at the autoimmunity-predisposing CTLA4 locus: a re-evaluation of the 3' +6230G>A polymorphism. *Genes Immun.* **6**, 305–11 (2005).

Deleted: 40

42. Valle, L. *et al.* Germline allele-specific expression of TGFBR1 confers an increased risk of colorectal cancer. *Science* **321**, 1361–5 (2008).

Deleted: 41

43. [Fu, Y. *et al.* FunSeq2 : a framework for prioritizing noncoding regulatory variants in cancer. \(2014\). doi:10.1186/s13059-014-0480-5](#)

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44. Price, A. L. *et al.* Pooled Association Tests for Rare Variants in Exon-Resequencing Studies. *Am. J. Hum. Genet.* **86**, 832–838 (2010).
45. Han, F. & Pan, W. A data-adaptive sum test for disease association with multiple common or rare variants. *Hum. Hered.* **70**, 42–54 (2010).
46. Boodhoo, A. *et al.* A promoter polymorphism in the central MHC gene, IKBL, influences the binding of transcription factors USF1 and E47 on disease-associated haplotypes. *Gene Expr.* **12**, 1–11 (2004).
47. Kim, J. Do *et al.* Identification of clustered YY1 binding sites in imprinting control regions. *Genome Res.* **16**, 901–911 (2006).
48. Chaumeil, J. & Skok, J. A. The role of CTCF in regulating V(D)J recombination. *Current Opinion in Immunology* **24**, 153–159 (2012).
49. The Genotype-Tissue Expression (GTEx) project. *Nat. Genet.* **45**, 580–5 (2013).
50. Bustamante, C. D., Burchard, E. G. & De la Vega, F. M. Genomics for the world. *Nature* **475**, 163–5 (2011).
51. Kitzman, J. O. *et al.* Haplotype-resolved genome sequencing of a Gujarati Indian individual. *Nat. Biotechnol.* **29**, 59–63 (2011).
52. Peters, B. A. *et al.* Accurate whole-genome sequencing and haplotyping from 10 to 20 human cells. *Nature* **487**, 190–5 (2012).
53. Fan, H. C., Wang, J., Potanina, A. & Quake, S. R. Whole-genome molecular haplotyping of single cells. *Nat. Biotechnol.* **29**, 51–7 (2011).
54. Abyzov, A., Urban, A. E., Snyder, M. & Gerstein, M. CNVnator: an approach to discover, genotype, and characterize typical and atypical CNVs from family and population genome sequencing. *Genome Res.* **21**, 974–84 (2011).
55. Lalonde, E. *et al.* RNA sequencing reveals the role of splicing polymorphisms in regulating human gene expression. *Genome Res.* **21**, 545–54 (2011).
56. Montgomery, S. B. *et al.* Transcriptome genetics using second generation sequencing in a Caucasian population. *Nature* **464**, 773–7 (2010).
57. McVicker, G. *et al.* Identification of genetic variants that affect histone modifications in human cells. *Science* **342**, 747–9 (2013).
58. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* **10**, R25 (2009).

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Moved down [2]: - Gerstein, M. B. *et al.* Architecture of the human regulatory network derived from ENCODE data. *Nature* **489**, 91–100 (2012).¶

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Deleted: 46. - Cusanovich, D. A., Pavlovic, B., Pritchard, J. K. & Gilad, Y. The functional consequences of variation in transcription factor binding. *PLoS Genet.* **10**, e1004226 (2014).¶

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59. Yee, T. VGAM: Vector Generalized Linear and Additive Models. (2014). at <http://cran.r-project.org/package=VGAM>
60. Rozowsky, J. *et al.* PeakSeq enables systematic scoring of ChIP-seq experiments relative to controls. *Nat. Biotechnol.* **27**, 66–75 (2009).
61. Robinson, J. T. *et al.* Integrative genomics viewer. *Nat. Biotechnol.* **29**, 24–6 (2011).
62. Visscher, P. M., Hill, W. G. & Wray, N. R. Heritability in the genomics era--concepts and misconceptions. *Nat. Rev. Genet.* **9**, 255–66 (2008).
63. Vaquerizas, J. M., Kummerfeld, S. K., Teichmann, S. A. & Luscombe, N. M. A census of human transcription factors: function, expression and evolution. *Nat. Rev. Genet.* **10**, 252–263 (2009).
64. Gerstein, M. B. *et al.* Architecture of the human regulatory network derived from ENCODE data. *Nature* **489**, 91–100 (2012).
65. Lefranc, M.-P. *et al.* IMGT-Choreography for immunogenetics and immunoinformatics. *In Silico Biol.* **5**, 45–60 (2005).
66. Morison, I. M., Ramsay, J. P. & Spencer, H. G. A census of mammalian imprinting. *Trends Genet.* **21**, 457–65 (2005).
67. GeneImprint. at <http://www.geneimprint.com/>
68. Lo, H. S. *et al.* Allelic variation in gene expression is common in the human genome. *Genome Res.* **13**, 1855–1862 (2003).
69. Ernst, J. & Kellis, M. ChromHMM: automating chromatin-state discovery and characterization. *Nat. Methods* **9**, 215–6 (2012).
70. Hoffman, M. M. *et al.* Integrative annotation of chromatin elements from ENCODE data. *Nucleic Acids Res.* **41**, 827–41 (2013).
71. Yip, K. Y. *et al.* Classification of human genomic regions based on experimentally determined binding sites of more than 100 transcription-related factors. *Genome Biol.* **13**, R48 (2012).
72. Visel, A., Minovitsky, S., Dubchak, I. & Pennacchio, L. A. VISTA Enhancer Browser--a database of tissue-specific human enhancers. *Nucleic Acids Res.* **35**, D88–92 (2007).
73. Eisenberg, E. & Levanon, E. Y. Human housekeeping genes, revisited. *Trends Genet.* **29**, 569–74 (2013).

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74. Kheradpour, P. & Kellis, M. Systematic discovery and characterization of regulatory motifs in ENCODE TF binding experiments. *Nucleic Acids Res.* **42**, 2976–87 (2014).

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75. Touzet, H. & Varré, J.-S. Efficient and accurate P-value computation for Position Weight Matrices. *Algorithms Mol. Biol.* **2**, 15 (2007).

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Figure and table legend

Figure 1. Workflow for uniform processing of data from 382 individuals and construction of AlleleDB. For each of the 382 individuals, (1) a diploid personal genome is first constructed using the variants from the 1000 Genomes Project. Next, reads from individual (2a) and pooled (2b) ChIP-seq or RNA-seq datasets are mapped onto each of the haploid genome of the diploid genome. In (2a), overdispersion (OD) is measured for each dataset and used to segregate highly overdispersed datasets. (2b) The resultant datasets are pooled and the overdispersion parameter is estimated based on the pooled datasets. To determine if a heterozygous SNV is allele-specific (allele-specific), the numbers of reads that map to either allele is being compared. A statistical significance is computed (after multiple hypothesis test correction) based on the beta-binomial test using the ‘pooled’ overdispersion parameter in Step 2b to account for overdispersion. All the candidate allele-specific variants are then deposited in AlleleDB database. Additional information, such as raw read counts of both accessible non-allele-specific and allele-specific variants, can be downloaded for further analyses.

Figure 2. Comparing the effects of the binomial and beta-binomial tests in datasets with low and intermediate level of overdispersion. The grey bars represent the empirical allelic ratio distribution, while the red and blue lines represent the expected allelic ratio distribution using the binomial and beta-binomial tests respectively. Figure 2A shows the empirical and expected distributions for one of the individual RNA-seq datasets for the individual HG00096. It has a low overdispersion parameter, $\rho=0.0205$. The empirical distribution does not have heavy tails and the binomial and beta-binomial tests give very similar results. This differs from Figure 2B, which shows the empirical and expected distributions for one of the individual RNA-seq datasets for the individual NA11894. Overdispersion is higher at $\rho=0.1234$, and the beta-binomial null distribution provides a better fit to the empirical allelic ratio distribution than the binomial distribution. The empirical distribution (grey bars) also show heavier tails, signifying more SNVs with allelic imbalance.

Figure 3. Inheritance of allele-specific behavior. The left panel shows plots for the TF CTCF (top row) and ASE (bottom row) being examined for inheritance in the CEU trio (Father: NA12891, blue; Mother: NA12892, red; Child: NA12878, green). Each point on the plot represents the allelic ratio of a common ASB SNV between the parent (x-axis) and the child (y-axis), by computing the proportion of reads mapping to the reference allele at that SNV. High Pearson’s correlations, r , observed in both parent-child comparisons for CTCF ($r \geq 0.77$) signify strong heritability in allele-specific behavior. ASE also shows considerably strong evidence of heritability but has comparatively lower r values. The table at the top right panel presents the r values for ASB in two TFs and ASE in our analyses.

Figure 4. (a) ASB and ASE SNVs in allele-specific gene ZNF331 (chromosome 19, position 54,041,333-54,083,523). From AlleleDB, we can observe the ASB SNVs (filled red bars with the name of the transcription factor (TF) above the bars) and ASE SNVs (filled black bars) found in each individual (row) and genomic positions (columns) along the ZNF331 gene. We can see that many of these SNVs are sparsely distributed across a single individual. By collapsing or combining information from multiple individuals, we can identify genomic regions or elements that are enriched for allele-specific activity. Unfilled black and red bars denote accessible SNVs are heterozygous SNVs that have enough reads to be tested but are non-allele-specific. **(b) Two approaches for enrichment analyses are performed for each genomic element.** (1) The 'expanded' enrichment is performed in a population-aware fashion, in which each occurrence of allele-specific or accessible non-allele-specific SNV in each individual is counted. (2) The 'collapsed' enrichment conflates all occurrences over multiple individuals into a single unique SNV position as long as an allele-specific or accessible non-allele-specific SNV occurs in at least one individual.

Deleted: 5. Some genomic regions are more inclined to allele-specific regulation.

Figure 5. The 'expanded' enrichment analysis is population-aware and shows that some genomic regions are more inclined to allele-specific regulation. We map variants associated with allele-specific binding (ASB; green) and expression (ASE; blue) to various categories of genomic annotations, such as coding DNA sequences (CDS), untranslated regions (UTRs), enhancer and promoter regions, to survey the human genome for regions more enriched in allelic behavior. Using the accessible non-allele-specific SNVs as the expectation, we compute the log odds ratio for ASB and ASE SNVs separately, via Fisher's exact tests. The number of asterisks depicts the degree of significance (Bonferroni-corrected): *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. For each transcription factor (TF) in AlleleDB, we also calculate the log odds ratio of ASB SNVs in promoters, providing a proxy of allele-specific regulatory role for each available TF. Genes known to be mono-allelically expressed such as imprinted and MHC genes (CDS regions) are highly enriched for both ASB and ASE SNVs. The actual log odds ratio of ASB SNVs in imprinted genes, both ASB and ASE SNVs in immunoglobulin genes and ASE SNVs for 3'UTR, MHC and olfactory receptor genes are indicated on the bars.

Moved down [3]: We map variants associated with allele-specific binding (ASB; green) and expression (ASE; blue) to various categories of genomic annotations, such as coding DNA sequences (CDS), untranslated regions (UTRs), enhancer and promoter regions, to survey the human genome for regions more enriched in allelic behavior. Using the accessible non-allele-specific SNVs as the expectation, we compute the log odds ratio for ASB and ASE SNVs separately, via Fisher's exact tests. The number of asterisks depicts the degree of significance (Bonferroni-corrected): *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. For each transcription factor (TF) in AlleleDB, we also calculate the log odds ratio of ASB SNVs in promoters, providing a proxy of allele-specific regulatory role for each available TF. Genes known to be mono-allelically expressed such as imprinted and MHC genes (CDS regions) are highly enriched for both ASB and ASE SNVs. The actual log odds ratio of ASB SNVs in imprinted genes, both ASB and ASE SNVs in immunoglobulin genes and ASE SNVs for MHC genes are indicated on the bars.

Figure 6. A considerable fraction of allele-specific variants are rare but do not form the majority. A lower proportion of allele-specific SNVs than non-allele-specific SNVs are rare, suggesting less selective constraints in allele-specific SNVs. The minor allele frequency (MAF) spectra of ASB (green filled circle), accessible non-ASB SNVs (green open circle), ASE (blue filled circle) and accessible non-ASE SNVs (blue open circle) are plotted at a bin size of 100. The peaks are in the bin for $MAF \leq 0.5\%$. The inset zooms in on the histogram at $MAF \leq 2.5\%$. The proportion of rare variants in descending order: $ASE^- > ASE^+ > ASB^+ > ASB^-$. Comparing ASE^+ to ASE^- gives an odds ratio of 0.2 (Bonferroni-corrected hypergeometric $p < 2.2e-16$), while comparing ASB^+ to ASB^- , gives an odds ratio of 1.4 ($p=0.08$), signifying statistically significant depletion of ASE SNVs but statistically insignificant enrichment of ASB SNVs relative to the respective non-allele-specific accessible SNVs. Statistically significant depletion in ASE suggests that ASE SNVs are under less purifying selection.

Table 1. Breakdown of SNVs in each ethnic population: heterozygous (HET), accessible (ACC) and ASE SNVs in Table 1A and ASB SNVs in Table 1B for 381 unrelated individuals.

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Table 1C shows the same HET, ACC and both ASE and ASB SNVs detected in a single individual, NA12878, who is also part of the trio family. For each of the last 3 columns, each category of HET, ACC and allele-specific SNVs is further stratified by the population minor allele frequencies: common (MAF > 0.05), rare (MAF ≤ 0.01) and very rare (MAF ≤ 0.005). The number of allele-specific SNVs is given as a percentage of the ACC SNVs. Table 1 also provides the number of individuals from each ethnic population with RNA-seq and ChIP-seq data available for the ASE and ASB analyses respectively.

Supplementary Figure

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Supplementary Figure 1

This figure shows the results for the ‘collapsed’ enrichment analysis. We map variants associated with allele-specific binding (ASB; green) and expression (ASE; blue) to various categories of genomic annotations, such as coding DNA sequences (CDS), untranslated regions (UTRs), enhancer and promoter regions, to survey the human genome for regions more enriched in allelic behavior. Using the accessible non-allele-specific SNVs as the expectation, we compute the log odds ratio for ASB and ASE SNVs separately, via Fisher’s exact tests. The number of asterisks depicts the degree of significance (Bonferroni-corrected): *, p<0.05; **, p<0.01; ***, p<0.001. For each transcription factor (TF) in AlleleDB, we also calculate the log odds ratio of ASB SNVs in promoters, providing a proxy of allele-specific regulatory role for each available TF. Genes known to be mono-allelically expressed such as imprinted and MHC genes (CDS regions) are highly enriched for both ASB and ASE SNVs. The actual log odds ratio of ASB SNVs in imprinted genes, both ASB and ASE SNVs in immunoglobulin genes and ASE SNVs for MHC genes are indicated on the bars.

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Supplementary Table

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Supplementary Table 1

This table shows the inconsistencies of the eight studies performing allele-specific analyses using different tools and parameters, e.g. read mapping with a range of read aligners, alignment to different reference genomes and variations of statistical tests in detecting the allele-specific variants. We uniformly processed the tools and parameters in AlleleDB.

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Supplementary Table 2

This table shows the number of individual datasets being flagged and segregated due to insufficient reads and due to having an “overdispersed” allelic ratio distribution.

*We define an “overdispersed” ChIP-seq dataset as those with $\rho \geq 0.3$, while an “overdispersed” RNA-seq dataset is defined more strictly by $\rho \geq 0.125$, which is one standard deviation more than the mean overdispersion in the RNA-seq datasets in our processing.

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Supplementary Table 3

This table shows the slope and Pearson’s correlation results for two DNA-binding proteins, PU.1 and CTCF, and ASE for parent-child and parent-parent comparisons.

Supplementary Files

Supplementary File 1

This Excel file contains results from our allele-specific analyses for 708 categories from ENCODE, including the Fisher's exact test odds ratios, p-values (original and Bonferroni-corrected), the number of allele-specific SNVs and accessible non-allele-specific SNVs found in each category. The results for five gene element categories from GENCODE and 16 enhancer categories are also included. 'NA' is marked in categories where odds ratio cannot be calculated due to insufficient numbers in non-allele-specific SNVs. These are tabulated for ASB, ASE and allele-specific SNVs; the latter is the results for the combined unique number of ASB and ASE SNVs.

Supplementary File 2

This Excel file contains results from our allele-specific analyses for the 19,257 autosomal protein-coding genes (HGNC symbols) from GENCODE, including the Fisher's exact test odds ratios, p-values (original, Bonferroni-corrected), the number of allele-specific SNVs and accessible non-allele-specific SNVs found in the gene region and the promoter region (upstream 2500bp). The results for housekeeping genes and 5 monoallelically-expressed gene categories are also included. 'NA' is marked in categories where odds ratio cannot be calculated due to insufficient numbers in non-allele-specific SNVs. These are tabulated for ASB, ASE and allele-specific SNVs; the latter is the combined unique number of ASB and ASE SNVs.

Supplementary File 3

This Excel file contains the ASB enrichment in promoter regions for 44 TFs used in our database, including the Fisher's exact test odds ratios, p-values (original, Bonferroni-corrected), the number of ASB SNVs, accessible non-allele-specific SNVs both found and not found in the gene region. ASB SNVs for each TF are contributed by different individuals. If either of the parents in the CEU trio is involved, ASB SNVs for NA12878 are not included. Those TFs with only ASB SNVs from NA12878 are annotated '1' under the column 'NA12878 only'. 'NA' is marked in categories where odds ratio cannot be calculated due to insufficient numbers in any of the last three columns.

Supplementary File 4

This Excel file contains the ASB SNVs that reside in TF motifs described in Kheradpour and Kellis⁷⁴. Under the column 'motif', the information is delimited by "#" in this order: motif identifier (as defined in Kheradpour and Kellis), start position of motif (0-based), end position of motif (1-based), strand and position of SNV in motif. Allelic ratios at each SNV position are defined above, i.e. ratio of number of reference reads to number of alternate reads.

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Supplementary File 5

This Word file contains the R pseudocode for the bisection method that is used to estimate the overdispersion parameter.

Supplementary File 6

This Excel file contains sets of more confident ASB and ASE SNVs. For the more confident 2,394 ASE SNVs, they are identified because at least 38 individuals (column 'indCount' ≥ 38) possess each of them. At the same time, for each of the SNV, the allele that has more reads for

each individual (columns 'winningAllele' and 'alleleCounts') are consistently found in 80% of the individuals (column 'freq' ≥ 0.8) that possess this ASE SNV. The more confident 183 ASB SNVs are defined by having \geq two individuals possessing that ASB SNV, regardless of the identities of TFs (columns ind_TF and indCount ≥ 3). Also, the allele that has more reads for each ind_TF (columns 'winningAllele' and 'alleleCounts') are found in 80% of ind_TF (column 'freq' ≥ 0.8).

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Supplementary File 7¶

This Excel file contains the information for the minimum of the reads to be defined an accessible SNV in each pooled RNA-seq (grouped by individual) and ChIP-seq dataset (grouped by individual and TF).