eLife

Reviewed Preprint
v1 • October 21, 2025
Not revised

Reviewed Preprint
v2 • July 1, 2026
Revised by authors

For correspondence:
ytguan@bimsa.cn

Competing interests: No competing interests declared

Funding: See page 25

Reviewing editor: Siming Zhao, Dartmouth College, United States

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Abundant Parent-of-origin Effect eQTL: The Framingham Heart Study

Yongtao Guan, Tianxiao Huan, Daniel Levy

National Heart, Lung, and Blood Institute, Bethesda, United States

eLife Assessment

This **important** study systematically investigates parent-of-origin (POE) effects on gene expression using large trio-based data from the Framingham Heart Study, identifying thousands of potentially novel associations. However, the statistical support for classifying POE eQTLs is **incomplete**, and as a result, downstream analyses of the identified POE eQTLs are not fully supported.

https://doi.org/10.7554/eLife.107920.2.sa2

Abstract

Parent-of-origin effect (POE) is a phenomenon whereby an allele's effect on a phenotype depends both on its allelic identity and parent's gender from whom the allele is inherited, as exemplified by the polar overdominance in the ovine callypyge locus and the human obesity *DLK1* locus. Systematic studies of POE of expression quantitative trait loci (eQTL) are lacking. In this study we use trios among participants in the Framingham Heart Study to examine to what extend POE exists for gene expression of whole blood using whole genome sequencing and RNA sequencing. For each gene and the SNPs in cis, we performed eQTL analysis using genotype, paternal, maternal, and joint models, where the genotype model enforces the identical effect sizes on paternal and maternal alleles, and the joint model allows them to have different effect sizes. We compared models using Bayes factors to identify paternal, maternal, and opposing eQTL, where paternal and maternal effects have opposite directions. The resultant variants are collectively called POE eQTL. The highlights of our study include: 1) There are more than 2, 000 genes harbor POE eQTL and majority POE eQTL are not in the vicinity of known imprinted genes; 2) Among 180 genes harboring opposing eQTL, 99 harbor exclusively opposing eQTL, and 58 of the 99 are phosphoprotein coding genes, reflecting significant enrichment; 3) Paternal eQTL are enriched with GWAS hits, and genes harboring paternal eQTL are enriched with drug targets. Our study demonstrates the abundance of POE in gene expression, illustrates the complexity of gene expression regulation, and provides a resource that is complementary to existing resources such as GTEx. We revisited two previous POE findings in light of our POE results. A SNP residing in *KCNQ1* that is maternally associated with diabetes is a maternal eQTL of *CDKN1C*, not *KCNQ1*. A SNP residing in *DLK1* that showed paternal polar overdominance for human obesity is a maternal eQTL of *MEG3*, offering an explanation for the baseline risk of homozygous samples through association between *MEG3* expression and obesity. Finally, we advised caution on conducting Mendelian randomization using gene expression as the exposure.

1 Introduction

Parent-of-origin effect (POE) is a phenomenon whereby an allele's effect on a phenotype not only depends on its allelic identity, but also on the parent from whom the allele is inherited (DeChiara et al., 1991; Cockett et al., 1996). POE can be driven by factors such as sex bias in transmission of genetic variants (Tomé et al., 2011) and maternal genetic effects that influences the environment in which the offspring develops (Hager et al., 2008). The main driver, however, is genomic imprinting, which is a phenomenon where one of the two alleles at a locus is functionally silenced by methylation. Importantly, which parental copy of the allele being silenced is highly

consistent for that gene across samples. Imprinting appears to play an important role in embryonic and placental development and social behavior (Reik and Walter, 2001; Garfield et al., 2011). Genes underpinning syndromic disorders such as Prader-Willi, Beckwtih-Weidemann, and Angelman show POE (Lawson et al., 2013). The same is also true for common disease phenotypes, such as breast cancer, diabetes, and cardiovascular disease (Kong et al., 2009; Hanson et al., 2013; Mozaffari et al., 2019).

In genetic association studies, particularly genome-wide association studies (GWAS), the absence of POE is usually assumed implicitly. This assumption is largely out of convenience, as in most GWAS, the parent-of-origin of an allele is not directly observable, and not inferable due to lack of first and second degree relatives in the sample. Exceptions include an Icelandic cohort, with their extensively documented pedigrees and the abundance of closely related samples (Kong et al., 2009), a Hutterite pedigree (Mozaffari et al., 2019), the Framingham Heart Study with three-generations of participants (Kannel et al., 1979), and the biobank datasets, for which parent-of-origin can be inferred using distant relatives (Hofmeister et al., 2022, 2025). Another contributing factor to the implicit assumption may be the presumed scarcity of documented POE in humans. Although POE is believed to be associated with a wide range of complex traits and diseases (Lawson et al., 2013), significant associations reported in GWAS setting are of limited quantity. For example, in a Hutterite study, Mozaffari et al. (2019) examined 21 phenotypes and produced 18 significant associations with POE, fewer than one association per phenotype.

Mouse studies demonstrated that, despite a limited number of imprinted genes, most phenotypes display POE, and loci that show POE were not enriched for known imprinted genes. Using reciprocal F1 crosses, Mott et al. (2014) showed that non-imprinted genes can generate POE by interactions with imprinted loci. Focusing on metabolic traits and using a mouse population at different levels of intercrossing, Macias-Velasco et al. (2022) identified a network comprised of three imprinted and six non-imprinted genes that show POE. A recent stduy on pigs demonstrated that a large number genes show POE effect in association with back fat and longissimus dorsi (Li et al., 2025). In humans, however, loci with POE in relation to phenotypes are either near imprinted regions by design (Kong et al., 2009; Hanson et al., 2013), or near regions with characteristic of imprinting from a genome-wide scan (Mozaffari et al., 2019). To what extend POE contributes to phenotypic variation in humans and how widespread the loci with POE in the human genome remains elusive.

Using whole genome sequencing and RNA sequencing data from the Framingham Heart Study, and taking advantage of its relative abundance of trios, we set to investigate to what extent and degree gene expressions is affected by parent-of-origin. There exist multiple studies on POE on gene expression (Zhabotynsky et al., 2019; Jadhav et al., 2019; Deng et al., 2020) or POE of methylation and gene expression jointly (Zink et al., 2018), but these studies are either underpowered due to small sizes or focus on analysis of allele specific gene expression instead of eQTL. A systematic study of POE eQTL is lacking and our study aims to fill this void.

2 Results

2.1 Overview of data processing

We identified 1477 trios with whole genome sequencing (WGS) data and children within the trios having RNAseq data. The trios were selected from participants in the Framing-ham Heart Study (FHS) who are predominantly of European descent. After routine QC of genotype data, for each trio, we first phased the child based on rules of Mendelian inheritance, which resolved all non triple heterozygous genotypes. (See Methods for details on Mendelian errors rate and how to handle them.) We then marked the remaining triple heterozygous genotypes as missing, which produced phased paternal and maternal haplotypes for each child in the trio. Next we imputed missing alleles into their paternal and maternal haplotype background, and the parental haplotype with a higher imputed allele dosage was assigned the reference allele, the other

haplotype was assigned the alternative allele. Simulation studies showed this mask and impute phasing to be highly accurate (Supplementary Table S1). Thus at each SNP, we have a genotype vector, a paternal vector, and a maternal vector.

To process RNAseq data, we started from Transcript Per Million (TPM) values, and selected 16, 824 genes that have < 5% missing values (i.e., 0 TMP). We then corrected for the GC content bias using an approach that is similar to the one used by EDAseq (Risso et al., 2011). The corrected gene expression values were then quantile normalized.

2.2 Bayes factors as evidence for association

For genetic association we used a linear mixed model, fitted by a novel and efficient method that is designed for analyzing multiomics datasets (Guan and Levy, 2024a). The genetic relatedness matrix (twice of kinship matrix) used for the linear mixed model was estimated by Kindred (Guan and Levy, 2024c). The covariates included age, sex, body mass index, and white blood cell composition. This study focuses on genetic association between gene expression and genetic variants in cis, defined as within 1Mb from transcription start site.

We elected to use Bayes factors (Stephens and Balding, 2009) instead of p-values as the evidence of association for the following considerations. First, at many SNPs, paternal and maternal alleles have different allele frequencies, presumably due to sampling variation. P-values are not comparable unless they have the same power, while the same power requires equal allele frequencies. On the other hand Bayes factors are comparable because they take into account power through prior specifications. Second, Bayes factors panelize genetic variants with small allele frequencies to reduce false positives (Guan and Stephens, 2011; Zhou and Guan, 2018). This is useful as we observed extremely low paternal or maternal allele frequencies. Third, Bayes factors are also convenient in comparing different alternative models, such as paternal effects versus genotype effect, a setting that is difficult for p-values.

For each gene and its cis-SNP, we computed four Bayes factors: genotype Bayes factor (BF_g), paternal Bayes factor (BF_1), maternal Bayes factor (BF_0), and joint Bayes factors (BF_j). When computing joint Bayes factor, we also computed paternal and maternal effect sizes and attached a p-value to test whether they differ significantly. Examples of test statistics are provided in Table 1. BF_g, BF_0 and BF_1 are one degree of freedom tests, but BF_j is a two degree of freedom test. Thus to achieve $BF_j > BF_g$, the paternal and maternal effect sizes have to differ significantly to compensate for the extra degree of freedom. Examples in Table 1 are chosen because their paternal and maternal effects differ significantly, and we have $BF_j \gg BF_g$. Another useful observation is that BF_1 is correlated with $|\beta_1|$, and BF_0 is correlated with $|\beta_0|$. But BF_1 and BF_0 are oblivious to the signs of β_1 and β_0, and signs are informative and interesting in our context.

We used $\log_{10} BF = 4$ as the significance threshold. If the prior odds for a cis-eQTL is 1 out of 1000, this threshold gives the posterior probability of association (PPA) of 0.91. If the prior odds for a cis-eQTL is 1 out of 100, this threshold gives the PPA of 0.99. $1 - PPA$ is the Bayesian counterpart of the local false discovery rate (c.f. Soloff et al., 2024). We included more discussions regarding p-values, Bayes factors, and FDR in Supplementary.

2.3 Overview from sentinel eQTL of joint analysis

Table 1 contains 10 genes and test statistics of their sentinel eQTL. A sentinel eQTL for a gene is defined as the eQTL with largest BF_j for that gene. A gene with significant eQTL is called eGene. These sentinel eQTL are either $BF_1 \gg BF_0 \approx 1$ such as *NDN*, or $BF_0 \gg BF_1 \approx 1$ such as *MEG3*, or $BF_j \gg BF_g \approx 1$ such as *NECAB3*. In Table 1 the sentinel eQTL were ordered according to column P, which measures the significance of differences between paternal and maternal effects. Reassuringly, six out of top ten eQTL with most significant P are from bona fide imprinted genes (colored in blue) according to *http://geneimprint.com*.

Figure 1 plots paternal effect vs maternal effect for all sentinel eQTL whose $\log_{10} BF_j > 3$. The color of dots corresponding to degree of difference between paternal and maternal effects. The marked sentinel eQTL include known imprinted genes such as maternally expressed *ZNF331* and

Gene	TSS	SNP	$lgBF_g$	$lgBF_1$	$lgBF_0$	$lgBF_j$	β_1	β_0	P
MEG3	100.78	rs12881545	35.7	-0.7	86.3	85.7	1.0	-21.2	54.0
PPIEL	39.53	rs79473113	47.6	84.6	0.5	85.8	-20.5	-2.7	38.3
NDN	23.69	rs3743340	36.5	68.5	-0.2	67.7	18.6	0.8	33.2
PEG10	94.66	rs7801134	2.6	13.3	-0.1	13.6	-8.2	2.4	12.6
ZNF331	53.52	rs1284523	6.3	-0.9	15.9	14.9	-0.5	8.8	10.3
ZNF890P	5.12	rs112844843	125.8	94.7	31.1	133.9	23.2	13.6	10.1
FAM50B	3.85	rs111515624	3.5	11.5	-0.5	10.9	-7.4	0.6	8.6
NECAB3	33.66	rs2626556	-0.7	1.2	4.6	5.5	-2.9	4.9	7.8
SNURF	24.95	rs4906936	5.1	12.1	-0.9	11.2	7.7	-0.1	7.6
LSM7	2.32	rs393651	-1.0	3.5	1.6	4.9	4.4	-3.3	7.5

Table 1. Example of eQTL statistics.

$lgBF_x$ columns are $\log_{10} BF_x$. Column β_j's are effect estimates in joint analysis used to compute BF_j. P column contains $-\log_{10}$ p-value with the null hypothesis $\beta_0 = \beta_1$ and alternative hypothesis $\beta_0 \neq \beta_1$. Transcript start site (TSS) is in Mb, and the coordinate is from HG38. Bona fide imprinted genes are highlighted.

MEG3, and paternally expressed *FAM50B*, *GNAS*, *SGCE*, *NDN*, *SNURF*, *SNRPN*, and *PEG10*. Imprinted genes are located along the x and y-axes. At least two genes appear to be novel imprinted genes: *PPIEL* and *CCR9*. Gene *ZNF890P* provides an example of paternal and maternal effects being in the same direction, but their sizes differ significantly. Finally, *NECAB3* and *LSM7* are two examples of paternal and maternal effects that are in opposite directions.

This approach based on the sentinel eQTL suggests that 1) the combined effect of paternal and maternal allele can be across all 360 degrees; 2) when paternal and maternal effects are in opposite directions, both effects sizes are modest compared to when they are in the same direction; and 3) there are quite a few sentinel eQTL whose paternal and maternal effects show opposite directions.

2.4 *NECAB3* : opposite paternal and maternal effects

The existence of eGenes whose eQTL show opposite paternal and maternal effects is intriguing. We focus on the example of *NECAB3* to examine further details. Figure 2 left panel shows that all eQTL of *NECAB3* have opposite paternal and maternal effects. Consequently, the joint Bayes factors BF_j's are much larger than their corresponding genotype Bayes factors BF_g's (right bottom). We select as an example SNP *rs4911348*, which is different from the sentinel eQTL shown in Figure 1, and looked into details of its association with the gene expression of *NECAB3* (phenotype). Three panels of boxplots (right top) show that genotype has no association with the phenotype, and both paternal alleles and maternal alleles are associated with the phenotype, but in opposite directions. Opposite paternal and maternal effects were also observed in Hutterite POE study, where ten associations of opposite effects across nine traits were reported (Mozaffari et al., 2019).

2.5 POE eQTL are abundant

Next we looked beyond the sentinel eQTL and examined all significant eQTL. We noted that there are 15, 893 eQTL from 14, 733 SNPs and 1, 824 eGenes that are insignificant for the SNP test, but significant for the paternal, or maternal, or joint test. That is, $\log_{10} BF_g < 4$, but either $\log_{10} BF_1 > 4$, or $\log_{10} BF_0 > 4$, or $\log_{10} BF_j > 4$. In other words, these eQTL cannot be detected without analyzing POE, which implies that POE can contribute to recover missing heritability.

The minor allele frequencies of the POE eQTL are balanced. Supplementary Figure S4 empirically demonstrates that our findings are not disproprtionately driven by low-frequency variants and provide a more complete picture of the genetic architecure underlying these POE signals.

With reference Figure 1, we proposed criteria to define subsets of eQTL. The first subset eQTL locate along the x-axis, mimicking sentinel eQTL of *NDN* and *FAM50B* ; the second subset locate along the y-axis, mimicking sentinel eQTL of *ZNF331* and *MEG3* ; the third subset locate along the secondary diagonal, mimicking sentinel eQTL of *NECAB3* and *LSM7* ; and the fourth subset follows (blues dots) along the diagonal line. Note Figure 1 only involves BF_j, our criteria to define gene sets also takes into account of BF_1, BF_0, and BF_g (details in Methods).

The first set of eQTL have significant paternal effects but insignificant maternal effects. We identified 15, 576 such *paternal eQTL* (set S_P) from 14, 372 SNPs associated with 1, 188 eGenes. The second set of eQTL have significant maternal effects but insignificant paternal effects. We identified 14, 783 such *maternal eQTL* (set S_M) from 13, 293 SNPs associated with 1, 209 eGenes. We refer to these paternal and maternal eQTL as *imprinting eQTL*. The third set of eQTL have opposite paternal and maternal effects, such that joint Bayes factors are much larger than genotype Bayes factor $BF_j \gg BF_g$. We identified 688 such *opposing eQTL* (set S_O) from 485 SNPs that are associated with 180 eGenes. Imprinting eQTL and opposing eQTL are referred to as *POE eQTL*.

The fourth set of eQTL require that both paternal and maternal alleles alone are associated with an expression phenotype, and the two effects are in the same direction. For these eQTL $BF_g > BF_j$ because similar effect sizes favor one degree of freedom test. We identified 884, 119 such *genotype*

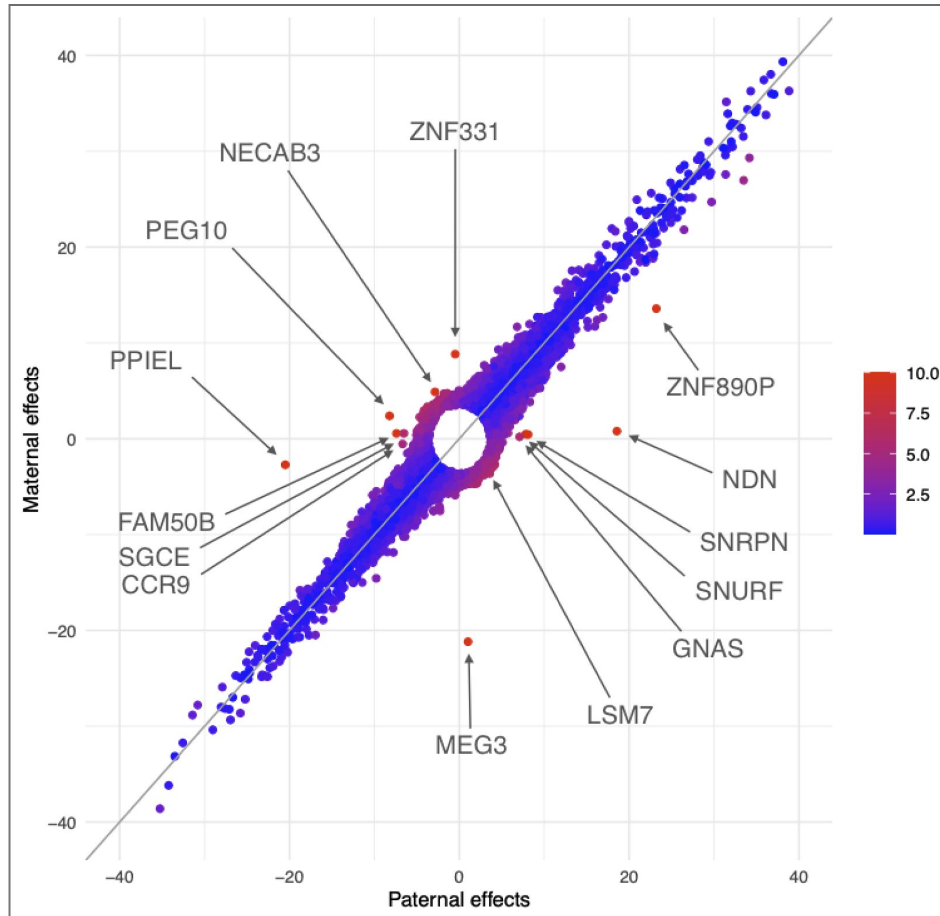

Figure 1. **Comparison of paternal effects and maternal effects of sentinel eQTL in joint analysis.**

Each point is the most prominent eQTL with $\log_{10} BF_j > 3$ of gene. The paternal effect is on x-axis and maternal effect y-axis. The coloring reflects significance of differentials between paternal effects and maternal effects, with red more significant than blue.

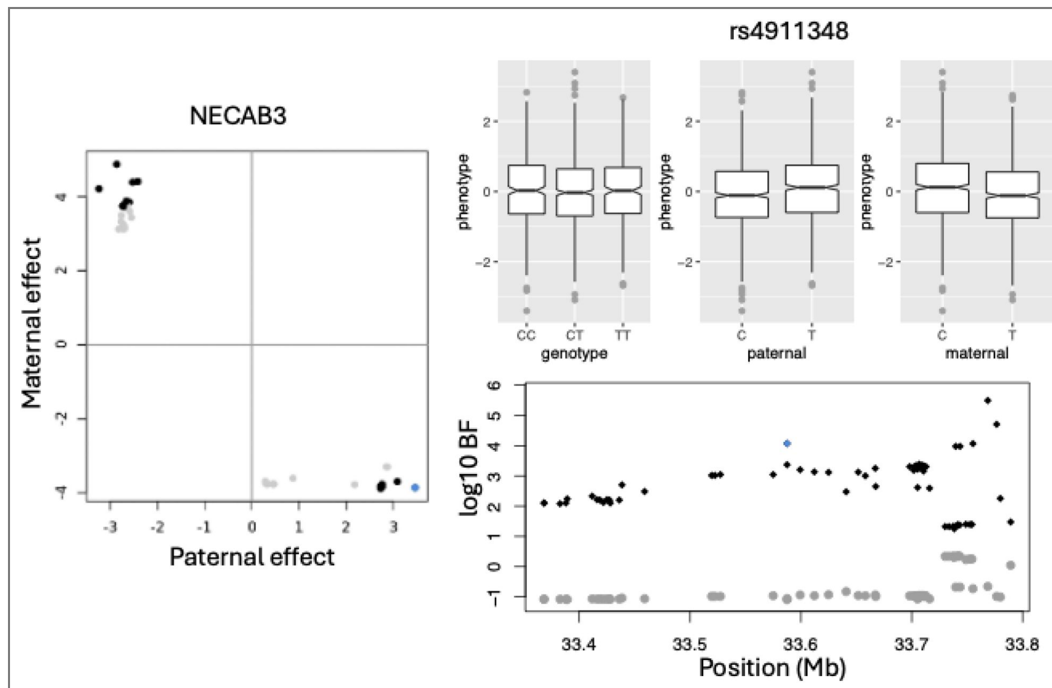

Figure 2. **An example of gene that whose paternal and maternal eQTL have opposite effect sizes.**

On the left is paternal vs maternal effect sizes normalized by their corresponding standard deviation. The effect sizes were estimated by joint analysis. The non-significant eQTL are colored in gray and significant eQTL colored in black. An eQTL marked in blue was chosen to show boxplots with gene expression (phenotype) of NECAB3. The three panels of boxplots are for genotypes, paternal alleles, and maternal alleles. For this SNP rs4911348, the genotypes has no association with phenotype, but both paternal alleles and maternal alleles are associated with the phenotype, and the paternal and maternal effects are in opposite direction. Right panel bottom show Manhattan plot of cis-eQTL of the gene NECAB3. The genotype Bayes factor were colored in gray and joint test Bayes factor were colored in black. SNP rs4911348 was highlighted in blue.

eQTL from 577, 701 SNPs and 4940 eGenes. The percent of eGenes with genotype eQTL is on par, but smaller than GTEx study (GTEx Consortium, 2020), presumably because $\log_{10} BF_g > 4$ is a more stringent threshold for eQTL analysis.

2.6 Paternal eQTL are enriched with GWAS hits

Using ANNOVAR (Wang et al., 2010), we annotated all four sets of eQTL (Table 2). We made the following observations: 1) The median distance to transcription start site (TSS) for opposing eQTL is 673.2Kb, much larger than other types of eQTL, and majority of opposing eQTL S_O are located in introns of nearby genes. 2) The percent of GWAS hits for SNPs in S_O is 0.022, significantly lower than that of SNPs in genotype eQTL S_G's 0.054 (test for proportion $P = 0.001$). This is reasonable as GWAS mainly use genotype test and are agnostic to opposing eQTL. 3) The precent of GWAS hits for SNPs in paternal eQTL S_P is higher than that of SNPs in S_G (test for proportion $P = 9 \times 10^{-11}$), and the percent of GWAS hits among SNPs in maternal eQTL S_M is similar to that of SNPs in S_G (test for proportion $P = 0.051$).

Number of eQTL per SNP (r) measures degree of pleiotropy of a set of SNPs. SNPs in S_P and SNPs in S_M have similar pleiotropy $r \approx 1.1$, SNPs in S_G have the largest $r = 1.53$, and SNPs in S_O display $r = 1.41$. In other words, SNPs are more exclusive for imprinting eQTL, less exclusive for genotype eQTL, and somewhere in between for opposing eQTL. Table 2 (bottom) compares how the degree of pleiotropy affects their annotation for SNPs in S_G, where set P_k contains SNPs in S_G that are eQTL of at least k eGenes. The most interesting observation is that the percent of GWAS hits increases with rising SNP pleiotropy. This feature is only observed in genotype eQTL.

2.7 POE eGenes are abundant

There are 1, 188 eGenes harboring paternal eQTL and 1, 209 eGenes harboring maternal eQTL. Their union contains 2, 139 eGenes harbor imprinting eQTL, and their intersection contains 258 eGenes harboring both paternal and maternal eQTL. There are 4, 940 eGenes harboring genotype eQTL, and 1, 867 eGenes harboring both genotype eQTL and imprinting eQTL. Supplementary Figure S1 showed an example of an eGene harboring both maternal and paternal eQTL: *GZMH*, and an example of an eGene harboring both genotype eQTL and imprinting eQTL: *DSE*. The most prominent feature is that paternal eQTL and maternal eQTL cluster in separate genomic regions, and genotype eQTL and imprinting eQTL also cluster in separate genomic region.

2.8 eGenes that harbor exclusively imprinting eQTL

There are 129 eGenes (set G_1) that harbor exclusively paternal eQTL, and 139 eGenes (set G_0) that harbor exclusively maternal eQTL. Naturally, G_1 contain many bona fide imprinted genes with paternal expression such as *FAM50B*, *NDN*, *SGCE*, *SNRPN*, *SNURF* and *PEG10*. Remaining genes in G_1 are candidates for imprinted genes with paternal expression, for example *CCR9* and *NCOA2*. G_0 contain bona fide imprinted genes with maternal expression such as *GRB10*, *ZNF331* and *MEG3*. The remaining in G_0 are candidates for imprinted genes with maternal expression, for example *COA8* and *ZNF888*.

The Supplementary Material provides a full list of the candidate imprinted genes from the eQTL analysis. Note however, that not all imprinted genes have eQTL, therefore this list is incomplete. Moreover, a gene on the list is considered behaving like an imprinted gene, but may not be a bona fide imprinted gene. For example, a gene that has no cis-eQTL on its own, may “acquire” a cis-eQTL through gene-gene interaction with a putative imprinted gene such that the cis-eQTL is in fact its trans-eQTL.

We used G_1 and G_0 as proxies for paternal and maternal imprinted genes, and used cytoBands these genes occupies as proxies for imprinted regions to examine to what extend paternal and maternal eQTL locate in the same cytoBands as these proxy imprinted genes. There are 811 autosome cytoBands in humans, 765 of them larger than 1 Mb, and the median size is 3.2 Mb (Cheung et al., 2001). The 129 proxy paternal imprinted genes occupy 89 cytoBands (set C_{P_I}), while 15, 576 paternal eQTL occupy 445 cytoBands (set C_{P_e}), among them 9, 947 (or 64%) were not

Set	eQTL	eGene	Len	D_{TSS}	Genic	Intron	Exon	ncRNA	SNP	GWAS
S_O	688	180	28.1	**673.2**	0.260	**0.663**	0.006	0.026	485	**0.023**
S_P	15,576	1188	23.3	122.9	0.362	0.490	0.016	0.080	14,372	**0.066**
S_M	14,783	1209	23.8	122.5	0.365	0.487	0.016	0.076	13,293	0.050
S_G	884,119	4940	24.6	84.1	0.383	0.449	0.017	0.091	577,701	0.054
P_1	884,119	4940	24.6	84.1	0.383	0.449	0.017	0.091	577,701	0.054
P_2	471,354	2855	17.7	96.8	0.400	0.403	0.019	0.112	164,936	0.071
P_3	269,546	1536	13.8	114.4	0.440	0.349	0.020	0.129	64,032	0.088
P_4	172,871	858	11.0	134.2	0.488	0.295	0.017	0.143	31,807	0.097
P_5	126,107	510	8.6	143.8	0.508	0.284	0.016	0.140	20,116	0.110
P_6	94,367	383	7.7	146.7	0.520	0.284	0.015	0.135	13,768	0.117
P_7	46,925	272	6.1	131.8	0.593	0.234	0.015	0.106	5,861	0.133
P_8	26,695	178	3.7	116.6	0.619	0.221	0.010	0.099	2,971	0.144
P_9	12,783	124	2.3	121.8	0.483	0.355	0.011	0.111	1,232	0.198

Table 2.

Top tabular: Annotation of eQTL. S_O is a set of opposing eQTL, S_P is a set of paternal eQTL, S_M is a set of maternal eQTL, and S_G is a set of genotype eQTL. The column eQTL contain counts of eQTL in each set, and column SNP contains counts of distinct SNPs of eQTL in the set. The column *eGene* is the number of genes associated with eQTL in the set. The column of Len is the median length in Kb of the eGenes. (The pattern is the same with the mean length.) The column D_{TSS} contains median distance in Kb to transcription start site. The column GWAS contains percent of GWAS hits among SNPs, with GWAS p-value threshold of 5×10^{-8}. Bottom tabular: A SNP set P_k contain SNPs in S_G that are eQTL of at least k eGenes. (Note $P_1 = S_G$.) Percent of GWAS hits for SNP set P_k increases with k.

in the cytoBands set C_{P_I}. On the other hand, the 139 proxy maternal imprinted genes occupy 96 cytoBands (set C_{M_I}), and 14, 783 maternal eQTL occupy 465 cytoBands (set C_{M_e}), among them 7, 987 (or 54%) were not in the cytoBand set C_{M_I}.

Therefore, a majority of imprinting eQTL are not in the vicinity of the proxy imprinted regions. Interestingly, C_{P_I} and C_{M_I} share only 14 cytoBands, 8% of their union of 171 cytoBands. As a comparison, C_{P_e} and C_{M_e} share 349 cytoBands, 62% of their union of 561 cytoBands.

2.9 eGenes that harbor exclusively opposing eQTL

There are 99 eGenes (set G_2) that harbor only opposing eQTL (Supplementary). Among them 58 encode phosphoproteins (Supplementary), a significant enrichment (FDR = 5.5×10^{-4}) according to DAVID, a web server for functional enrichment analysis (Sherman et al., 2022). A phosphoprotein is a protein that is posttranslationally modified by the attachment of either a single phosphate group, or a complex molecule such as 5'-phospho-DNA, through a phosphate group. Because phosphorylation often serves as an on/off switch, targeting phosphoproteins or the enzymes regulating them can restore normal cell signaling in diseases. In addition, drugs can modulate the functions of these proteins, either inhibiting or enhancing their activities. This makes phosphoprotein attractive drug targets. Indeed, according to a therapeutic target database (Zhou et al., 2024), among 58 phosphoproteins genes that harbor exclusively opposing eQTL, there are three successful drug targets *ITPR1*, *ITPR2*, and *REL*, and three additional clinical trial targets *CD46*, *NOTCH3*, and *XPO1*.

Other notable phosphoproteins genes include *CDKN2A*, *PHIP*, and *LEP*, among others. Where *CDKN2A* produces two major proteins p16(*INK4*), which is a cyclin-depnednet kinase inhibitor, and p14(*ARF*), which binds the p53-stabilizing protein MDM2 (Serrano et al., 2000); *PHIP* is associated with Chung-Jansen syndrome, featuring behavioral problems, intellectual disability, obesity, and dysmorphia (Webster et al., 2016; Jansen et al., 2018); and *LEP* encodes leptin, a protein that plays a critical role in the regulation of body weight. Leptin is secreted by white adipocytes, and it binds to the leptin receptor in the brain, which in turn inhibits appetite and promotes energy expenditure (Maffei et al., 1995; Weigle et al., 1997).

2.10 Paternal eGenes are enriched with drug targets

We used a therapeutic target database (Zhou et al., 2024) to examine the enrichment with drug targets of the four sets of eGenes, grouped by their eQTL. Table 3 contains the enrichment of the four gene sets in either successful targets, clinical trial targets, or combined targets. The significant enrichment that (following Bonferroni correction of 12 tests) is highlighted. Both paternal eGenes and genotype eGenes are enriched in clinical trial targets and combined targets, but paternal eGenes are significantly more enriched than genotype eGenes (one-sided test for proportion P = 0.035). The enrichment of drug targets for paternal eGenes echoes the enrichment of GWAS hits for paternal eQTL (Table 2). This asymmetry between paternal and maternal eGenes perhaps finds its root in the conflicting interests of two sets of genes in relation to transfer of nutrients from the mother to her offspring (Moore and Haig, 1991; Hitchcock and Gardner, 2019). In maze, gene expression of the hybrid is regulated exclusively by the paternally transmitted alleles (Swanson-Wagner et al., 2009).

3 Discussion

We performed parent-of-origin analysis of eQTL in whole blood gene expression using 1477 trios from the Framingham Heart Study. Some highlights of our study include: 1) There are more than 2,000 genes harboring POE eQTL and majority POE eQTL are not in the vicinity of known imprinted genes; 2) Among 180 genes harboring opposing eQTL, 99 harbor exclusively opposing eQTL, and 58 of the 99 are phosphoprotein coding genes, reflecting significant enrichment; 3) Paternal eQTL are enriched with GWAS hits, and genes harboring paternal eQTL are enriched with drug targets.

	Set	eGenes	nTarget	$\hat{f}$	f_0	$P(\hat{f} > f_o)$
Successful target	G_O	180	7	0.0389	0.0220	0.098
	G_P	1188	38	0.0320	0.0220	0.012
	G_M	1209	23	0.0190	0.0220	0.728
	G_G	4940	124	0.0251	0.0220	0.075
Clinical trial target	G_O	180	7	0.0389	0.0421	0.512
	G_P	1188	69	**0.0581**	0.0421	**0.004**
	G_M	1209	67	0.0554	0.0421	0.013
	G_G	4940	254	**0.0514**	0.0421	**0.0006**
Combined target	G_O	180	14	0.0778	0.0641	0.275
	G_P	1188	107	**0.0901**	0.0641	**0.0002**
	G_M	1209	90	0.0744	0.0641	0.079
	G_G	4940	374	**0.0757**	0.0641	**0.0005**

Table 3. Enrichment of drug target genes.

G_O contains eQTL with both paternal and maternal effects but they are in opposite direction. G_P contains eQTL of paternal effects. G_M contains eQTL of maternal effects. G_G contain eQTL that have both paternal and maternal effects and they are in the same direction. Column nGene are sizes of gene sets, Column nTarget are counts of successful drug targets. The last column contains one-sided test-of-proportion p-values. The highlighted p-values are significant after Bonferroni correction of 12 tests.

Although our study lacks replications in independent cohorts, we have taken several steps to ensure the internal validity and robustness of our findings within the current study. Specifically, we used well-established, *bona fide* imprinted genes (e.g., *MEG3*, *NDN*, *SNURF*, as listed in Table 1 and Figure 1) as positive control. We used conservative calling creiteria, and explored different parameters (Supplementary Table S2) and chose the one that minimized the inclusion of false positives, ensuring that our core gene sets (e.g., G_1, G_0) are high-confidence discoveries. We developed a rigorous analytical pipeline, including traio-based phasing validated by simulation (Supplmentary Table S1), the use of linear mixed models to control for relatedness and population structure, and the application of Bayes factors which inheritenly panalize variants with low minor allele frequencies, thereby reducing spurious associations. We believe these internal consistency checks and methodological rigor provide strong confidence in our findings.

Our study demonstrated abundance of POE in gene expression, illustrated additional complexity of gene expression regulation, and provided a resource that is complementary to existing resources such as GTEx. We conclude by revisiting two previous findings in light of our work, and advising caution on conducting Mendelian randomization using gene expression as the exposure.

3.1 T2D and maternal allele of rs2237892

SNP rs2237892 in the last intron of *KCNQ1* was found in association with type 2 diabetes (T2D) in a Japanese cohort (Yasuda et al., 2008), and the association was later confirmed in a Chinese cohort (Liu et al., 2009). Three other SNPs (rs2283228, rs2237895, and rs2237897) in the same last intron of *KCNQ1* were found in association with T2D in another Japanese cohort (Unoki et al., 2008). Both rs2237895 and rs2237897 were replicated in a Singaporean cohort, a Danish cohort (Unoki et al., 2008), and a Chinese cohort (Liu et al., 2009). In these studies, *KCNQ1* is identified as T2D candidate gene.

Based on these results from the genotype test, Kong et al. (2009) demonstrate that SNP rs2237892 is maternally associated with T2D in an Iceland cohort. This maternal association was replicated with a larger odds ratio in a Pima Amerindian cohort (Hanson et al., 2013). According to data from GTEx, none of these SNPs are eQTL of *KCNQ1* and *CDKN1C* in any tissue, consistent with the fact that these are imprinted genes and GTEx study is oblivious to parent of origin. Our eQTL analysis showed that SNPs rs2237892, rs2283228, and rs2237897 are maternal eQTL of *CDKN1C*, a down-stream neighbor of *KCNQ1*, and none of these SNPs are eQTL of *KCNQ1*.

We therefore suggest that *CDKN1C*, instead of *KCNQ1*, is a T2D candidate gene, for the following reasons: 1) These GWAS SNPs connect T2D and *CDKN1C* quantitatively, but connect T2D and *KCNQ1* only geographically; 2) A study suggests that *CDKN1C* mutations may represent a novel monogenic form of diabetes (Kerns et al., 2014); 3) A boy carrying a frameshift mutation in *CDKN1C* was diabetic from week 29 (Berland et al., 2022); and 4) Targeted demethylation at the CDKN1C/p57 locus induces human β cell replication, while the loss of insulin-secreting β cell is characteristic among T1D and T2D Ou et al. (2019).

We note that gene expression in our study is from whole blood. While whole blood is a valuable and accessible tissue, replication in T2D-relevant tissues (e.g., pancreas, adipose) would be an important future direction, and our findings provide a hypothesis for such targeted investigations.

3.2 Polar overdominance and rs1802710

Heterozygous overdominance is a pattern of inheritance such that both homozygous AA and BB have the same baseline trait value, while heterozygous AB has a higher trait value. Polar overdominance (Cockett et al., 1996) introduces asymmetry into overdominance to separates AB into two groups according to parent-of-origin of A (or B), such that AB with paternal A (or B) has a high trait value, while AB with maternal A (or B) has the baseline trait value.

Wermter et al. (2008) studied trios of extremely obese offspring and identified rs1802710 in exon 5 of *DLK1*, homologous to the ovine callipyge locus (Cockett et al., 1996), whose allelic transmission pattern was consistent with polar overdominance: frequent transmission of the

paternal C allele to obese children, but the relative risk for carriers of the homozygous CC genotype was not increased compared to the reference TT genotype.

Our eQTL results show that *DLK1* has no significant eQTL, but the maternal C allele of rs1802710 reduces expression of *MEG3*, 53Kb downstream of *DLK1*. Both *MEG3* and *DLK1* are imprinted with *MEG3* maternally expressed and *DLK1* paternally expressed. The expression of *MEG3* was shown to be significantly higher in the obese group (Danesh-moghadam et al., 2021). Therefore the maternal C allele is associated with reduced risks of obesity, which balances out the increased risk conferred by paternal C allele, thus offering an explanation for the baseline risk of homozygous CC samples in (Wermter et al., 2008). Since rs1802710 is not an eQTL of any gene in any tissue according to GTEx data, our study is critical to link rs1802710 with *MEG3*, albeit not in the adipose tissue.

3.3 Mendelian randomization

Mendelian randomization (MR) refers to the random allocation of alleles at the time of gamete formation. Observational epidemiology studies use MR to infer the causal effect of an exposure on an phenotype (Smith and Ebrahim, 2003), as if the random allocation of alleles is comparable to a randomized clinical trial. An important assumption for MR, among many other important assumptions, is that the phenotype conferred by a specific genetic variant is homogeneous in the population, and exchangeable between paternally and maternally inherited alleles. POE is a direct violation of this assumption (Bochud et al., 2008).

There is a growing interest in using gene expression as an exposure to perform MR across various traits (Porcu et al., 2019; van der Graaf et al., 2020). If an exposure has eQTL with POE, ignoring the parent-of-origin will bias prediction of a subset of samples. If the bias is severe it will change the ranking of the exposure. Consequently, it either compromises the power of the MR or leads to a false conclusion of a causal relationship between the exposure and the phenotype.

Our study demonstrates the abundance of POE in gene expressions, and we therefore advise caution when conducting MR using gene expression as the exposure. We suggest checking the list of POE SNPs and eGenes we provided in the Supplementary Material and exclude those that show POE towards the exposure. The same suggestion is also applicable to computing polygenic risk scores, and imputing gene expression such as PrediXcan (Gamazon et al., 2015) and a Bayesian method motivated by it (Qi et al., 2018).

4 Method

4.1 Genotype data

We first used pedigree and availability of RNAseq data to narrow the samples down to 2955, and performed routine QC to obtain bi-allelic SNPs (Taliun et al., 2021). We then used Kindred to infer pairwise kinship for each chromosome, and computed mean and sd of the kinship. A pair of sample that is parent-offspring can be distinguished from full sibs by the sd (Supplementary Figure S2). Trios are consisted of sample A, B, and C such that $\Phi(A, B) \approx 0$ and $\Phi(A, C) \approx \Phi(B, C) \approx 0.25$ where $\Phi(\cdot, \cdot)$ is the kinship. In the end, we identified 1477 trios that have whole genome sequencing data and whose children have RNAseq data. Totally, we have 7, 752, 281 autosome bi-allelic SNPs with minor allele frequency > 0.01 (not corrected for relatedness). Across all 1477 trios, the Mendelian error is negligible 4.76 per 100, 000 SNPs per trio. To handle Mendelian errors, we assume child was correctly genotyped, and errors happened in parents, but if the child is heterozygous, we assume this is a triple heterozygous SNP. On average 1477 trios have 65 triple heterozygous genotypes per SNP. The triple heterozygous SNPs cannot be phased by Mendelian inheritance, but can be phased based on a linkage disequilibrium model (below).

4.2 Inference of parent of origin

For each trio, we first phase all markers that are not triple heterozygous using rule of Mendelian inheritance, where for the child in each trio we obtain paternal and maternal haplotypes with triple heterozygous markers whose phase are unresolved. We mark those triple heterozygous markers as missing. For markers that show Mendelian incompatibility, we assume child's genotype is correct and mark as missing if it is heterozygous. Using a hidden Markov model that was designed to model haplotype variation (Guan, 2014), we impute missing markers in each haplotypes to obtain dosage estimates (between 0 and 1). Then for each marker that are marked as missing, we compare imputed dosages between paternal and maternal haplotypes, and assign the large dosage as 1 and small dosage as 0. We thus obtain paternal and maternal haplotypes with no missing data. We simulated trios using haplotypes from 1000 Genomes project, and investigated the accuracy of the phasing by mask and imputation approach described above. The phasing is highly accurate, the error rate is less than 1 out of 200 triple heterozygous SNPs (Supplementary Table S1).

4.3 RNAseq data

The RNAseq data of 1477 chilren comes from three batches with 1381 comes from batch 1; 14 comes from batch 2, and 82 comes from batch 3. Our primary goal is to correct for GC content bias. To this end, we first remove genes whose proportion of 0 TPM values is greater than 5%, which retain 16, 969 genes out of total 58, 103 transcripts, majority of which are coding genes. Among those, 16, 824 genes have GC content. To correct for GC content bias, we took an approach used by EDAseq to regress out the GC content from log(1+*TMP*), but instead of using local linear regression as documented in EDAseq, we used local quadratic regression implemented in loess in R. Specifically, let y = log(1+*TMP*) be a gene expression and g be the GC content, we fit $ly = loess(y, g)$ and compute $\hat{y} = median(y) + ly\$residuals$. We then quantile normalize $\hat{y}$ by $qqnorm(\hat{y}, plot.it = F)\$y$ separately for each gene.

4.4 eQTL analysis

We used IDUL to perform eQTL analysis. IDUL fits linear mixed models to achieve exact optimal. It was specifically developed to analyze multi-omics data to achieve high efficiency by reusing the intermediate computations (Guan and Levy, 2024a). Linear mixed model includes a random effect with a covariance structure defined by the genetic relatedness matrix (GRM). This approach is widely regarded as more robust than including a limited number of principal components, as it accounts for both fine-scale population stratification and known relatedness simultaneously. Previous analysis showed that for Framingham heart study, the genomic inflation was well controlled using linear mixed model with kinship matrix computed by Kindred (Guan and Levy, 2024c). In this study, we also controlled age, sex, BMI, and cell compositions in peripheral blood. We now briefly describe how Bayes factors were computed.

Consider a model

$$\begin{aligned} \mathbf{y} &= \mathbf{W}\mathbf{a} + \mathbf{x}\beta + \mathbf{Z}\mathbf{u} + \mathbf{e} \\ \mathbf{u} &\sim \mathrm{MVN}_n\left(0, \tau^{-1}\eta\mathbf{K}\right) \\ \mathbf{e} &\sim \mathrm{MVN}_n\left(0, \tau^{-1}\mathbf{I}_n\right) \end{aligned} \quad (1)$$

where $\mathbf{W}$ contains conventional covariates such as age and sex, including a column of 1, $\mathbf{x}$ contains genetic variant(s) to be tested for association, $\mathbf{u}$ is the random effect with $\mathbf{Z}$ as its loading matrix and twice of kinship $\mathbf{K}$ as its covariance (both $\mathbf{Z}$ and $\mathbf{K}$ are known), MVN_n denotes an n-dimensional multivariate normal distribution, $\mathbf{I}_n$ is n-dimensional identity matrix. In our anlaysis $\mathbf{Z}$ is the identity matrix. Denote $\mathbf{X} = (\mathbf{W}, \mathbf{x})$ and $\mathbf{b} = (\mathbf{a}, \beta)$, then $\mathbf{Xb}$ is the fixed effect, and we assume $\mathbf{X}$ has a full rank c. In genetic association studies, the random effect $\mathbf{Zu}$ is a nuisance term that absorbs part of the phenotype $\mathbf{y}$ that is attributable to population stratification and relatedness. The maximum likelihood estimate (MLE) of η can

be efficiently obtained (Guan and Levy, 2024a), plug $\hat{\eta}$ back into (1), and specify the following conjugate prior

$$\begin{aligned} \mathbf{a} &\sim N\left(0, \tau^{-1} V_{\mathbf{a}}\right) \\ \beta &\sim N\left(0, \tau^{-1} \sigma^{2}\right) \\ \tau &\sim \Gamma\left(\kappa_1/2, \kappa_2/2\right) \end{aligned} \quad (2)$$

and let $V_{\mathbf{a}} \to \infty$, $\kappa_1 \to 0$ and $\kappa_2 \to 0$, Bayes factor can be evaluated efficiently in a closed form. This study we used $\sigma = 0.5$, following prior work of Bayes factors for linear models (Servin and Stephens, 2007). Complete details on computing Bayes factors for linear mixed model can be found in (Guan and Levy, 2024b).

4.5 P-value for difference between paternal maternal effects

For joint analysis, we have

$$\mathbf{y} = \mathbf{W}\mathbf{a} + \mathbf{x}_1\beta_1 + \mathbf{x}_2\beta_2 + \mathbf{Z}\mathbf{u} + \mathbf{e} \quad (3)$$

with the prior for β_1 and β_2 as

$$\begin{aligned} \beta_1 &\sim N\left(0, \tau^{-1} \sigma^{2}\right) \\ \beta_2 &\sim N\left(0, \tau^{-1} \sigma^{2}\right) \end{aligned} \quad (4)$$

The Bayes factor can be similarly computed in a closed form. We obtain the posterior estimates of $\hat{\beta}_1$ and $\hat{\beta}_2$, their variances s_1^2 and s_2^2 and covariance s_{12}. To test null hypothesis $\hat{\beta}_1 = \hat{\beta}_2$, we compute test statistics $t = \frac{\left(\hat{\beta}_1 - \hat{\beta}_2\right)^2}{s_1^2 + s_2^2 - 2s_{12}}$. Since under the null t following χ^2 distribution with 1 degree of freedom, we can compute a p-value.

4.6 Threshold for eQTL sets and gene sets

For paternal eQTL set S_P, we require $\log_{10} BF_1 > 4$ and $\log_{10} BF_0 < \theta$; For maternal eQTL set S_M, we require $\log_{10} BF_0 > 4$ and $\log_{10} BF_1 < \theta$; For opposing eQTL set S_O, we require $\log_{10} BF_j - \log_{10} BF_g > 4$ and $\log_{10} BF_1 > \theta$ and $\log_{10} BF_0 > \theta$, and $\beta_1 * \beta_0 < 0$; For genotype eQTL set S_G, we require $\log_{10} BF_g > 4$ and $\log_{10} BF_1 > \theta$ and $\log_{10} BF_0 > \theta$, and $\beta_1 * \beta_0 > 0$. It's easy to see for a particular type of eQTL, different θ produce nested eQTL sets. We tried $\theta = 0$, $\log_{10} 2$, and $\log_{10} 3$ to obtain eQTL sets (Supplementary Table S2), from which we obtain gene sets harboring those eQTL G_P, G_M, G_A and G_G, from which we obtain genes harbor exclusively paternal eQTL $G_1 = G_P \setminus (G_M \cup G_A \cup G_G)$, genes harbor exclusive maternal eQTL $G_0 = G_M \setminus (G_P \cup G_A \cup G_G)$, and genes harbor exclusively opposing eQTL $G_0 = G_A \setminus (G_P \cup G_M \cup G_G)$. We compare known imprinted genes with paternal expression with G_1, and known imprinted genes with maternal expression with G_0. $\theta = \log_{10} 2$ produced minimum G_1 and G_0 that contain known imprinted genes.

Supplementary

1 Simulation study of phasing trios

There are 503 European (EUR), 504 East Asian, and 661 African (AFR) phased samples in 1000 Genomes project. Our simulation was conducted separately for each population. In each population, we randomly selected two samples without replacement and assign the first as father and the second as mother, until exhausted all samples or had only one sample left. Using Chromosome 22, we selected one haplotype from the father and one from the mother to simulate the child. We didn't simulate genotyping error, as in real data the Mendelian error is negligibly small. We then ran the process of phasing by rule of Mendelian inheritance for each trio, masked triple heterozygous SNPs for each child, jointly fit an LD model using all chidren's haplotypes to

impute masked SNPs, and finally based on imputed allele dosage to assign alleles (reference or alternative) to maternal and paternal haplotypes. We tallied all counts and put in Table S1, and results show our approach of phasing is highly accurate.

2 Examples of eGenes harboring different set of eQTL

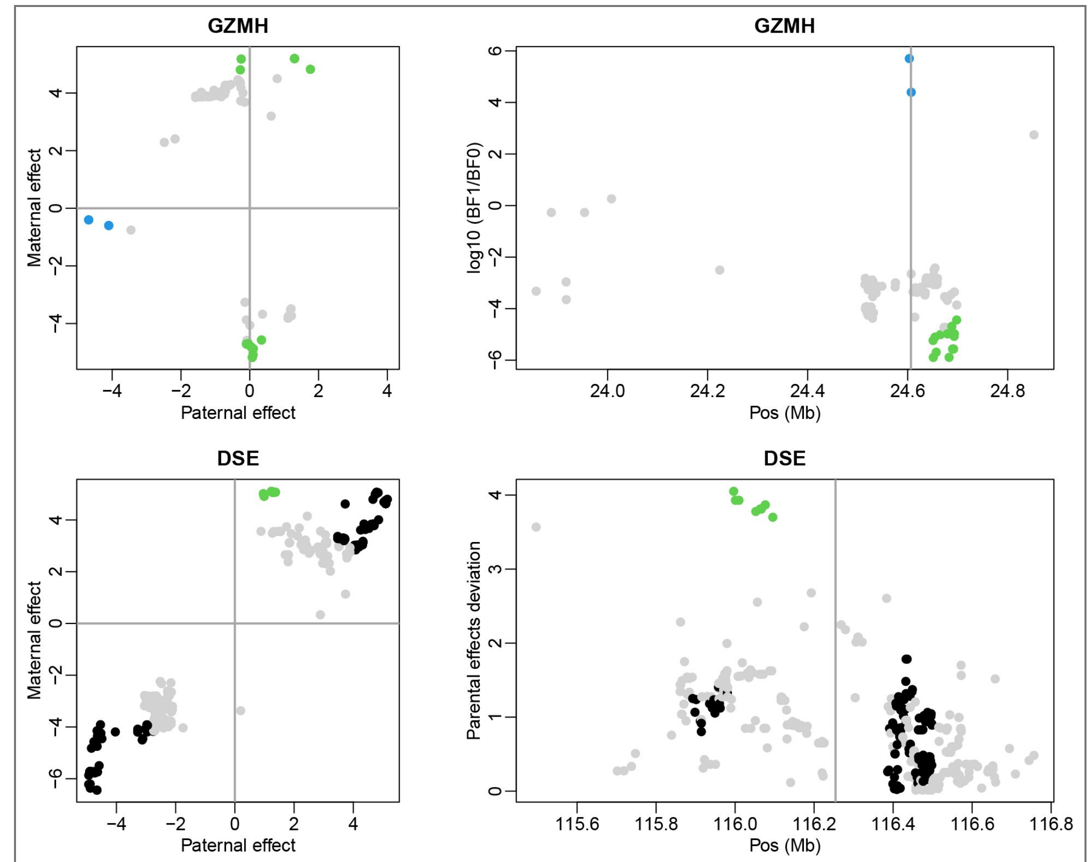

Figure S1. Examples of eGenes harboring different sets of eQTL: *GZMH* and *DSE*. Each gene has two plots: a square plot showing normalized paternal effect (x-axis) vs normalized maternal effect (y-axis), and a rectangle plot showing test statistics along chromosome position. In each plot, gray dots are insignificant eQTL, black dots are significant genotype eQTL, blue dots are significant paternal eQTL, and green dots are significant maternal eQTL. The vertical line in the right panels mark the transcription start site.

	EUR	EAS	AFR
N_I	251	252	330
N_S	30,501	30,551	30,551
N_{3H}	882,076	837,090	1,179,930
N_C	878,711	833,192	1,175,340
R	0.996	0.995	0.996

Table S1. Statistics and accuracy of phasing triple heterozygous sites via mask and imputation.

N_i: number of simulated samples. N_s number of biallelic SNPs used in simulation. N_{3H} : number of triple heterozygous SNPs across N_i samples. N_T : number of triple heterozygous SNPs that being correctly phased. R: ratio of the correctly phased triple heterozygous SNPs. EUR: European samples. EAS: East Asian samples. AFR: African samples.

3 Kinship estimates

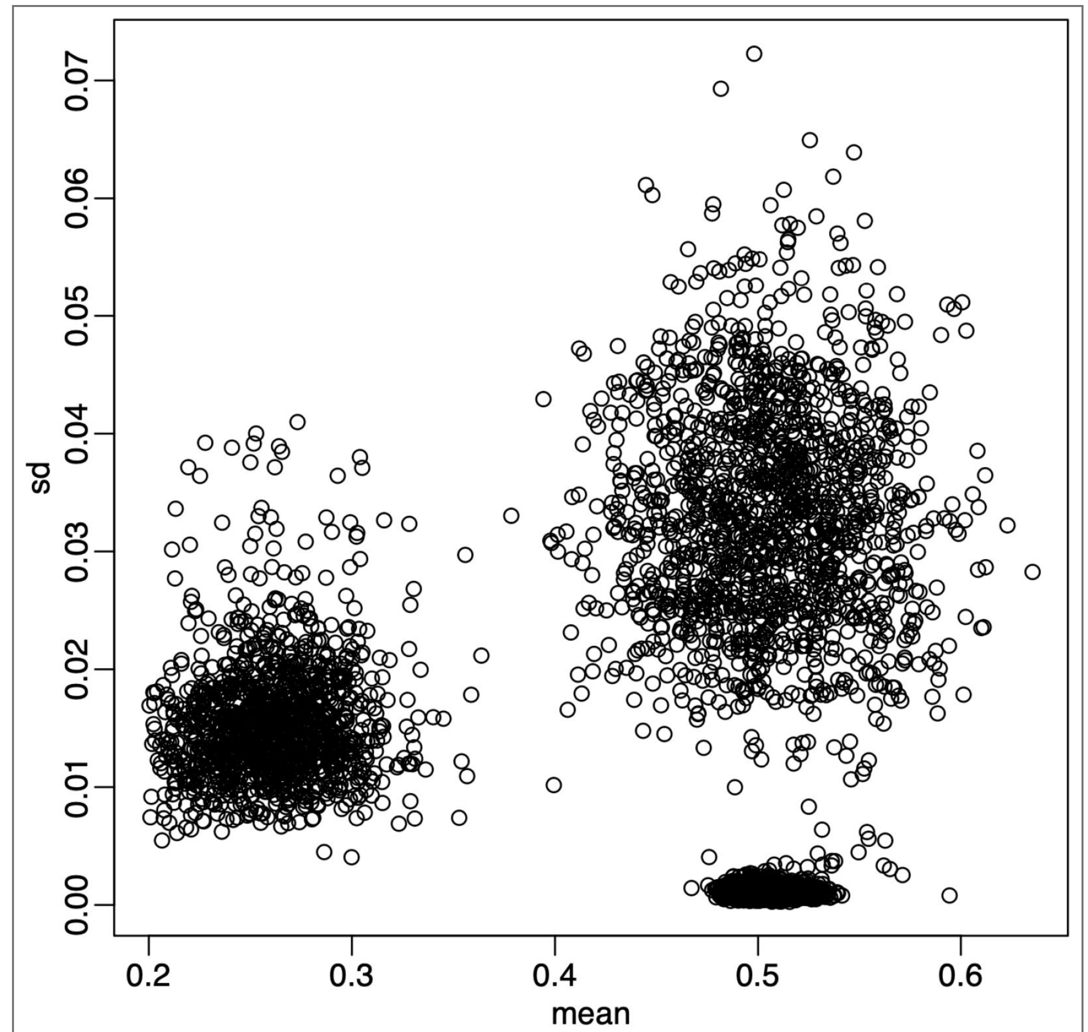

Figure S2. Kinship and sd. The x-axis is twice of kinship averaged over 22 estimates, one for each autosome. The y-axis is standard deviation of those 22 estimates. Plot only show relevant portion of the kinship.

4 GC content bias correction

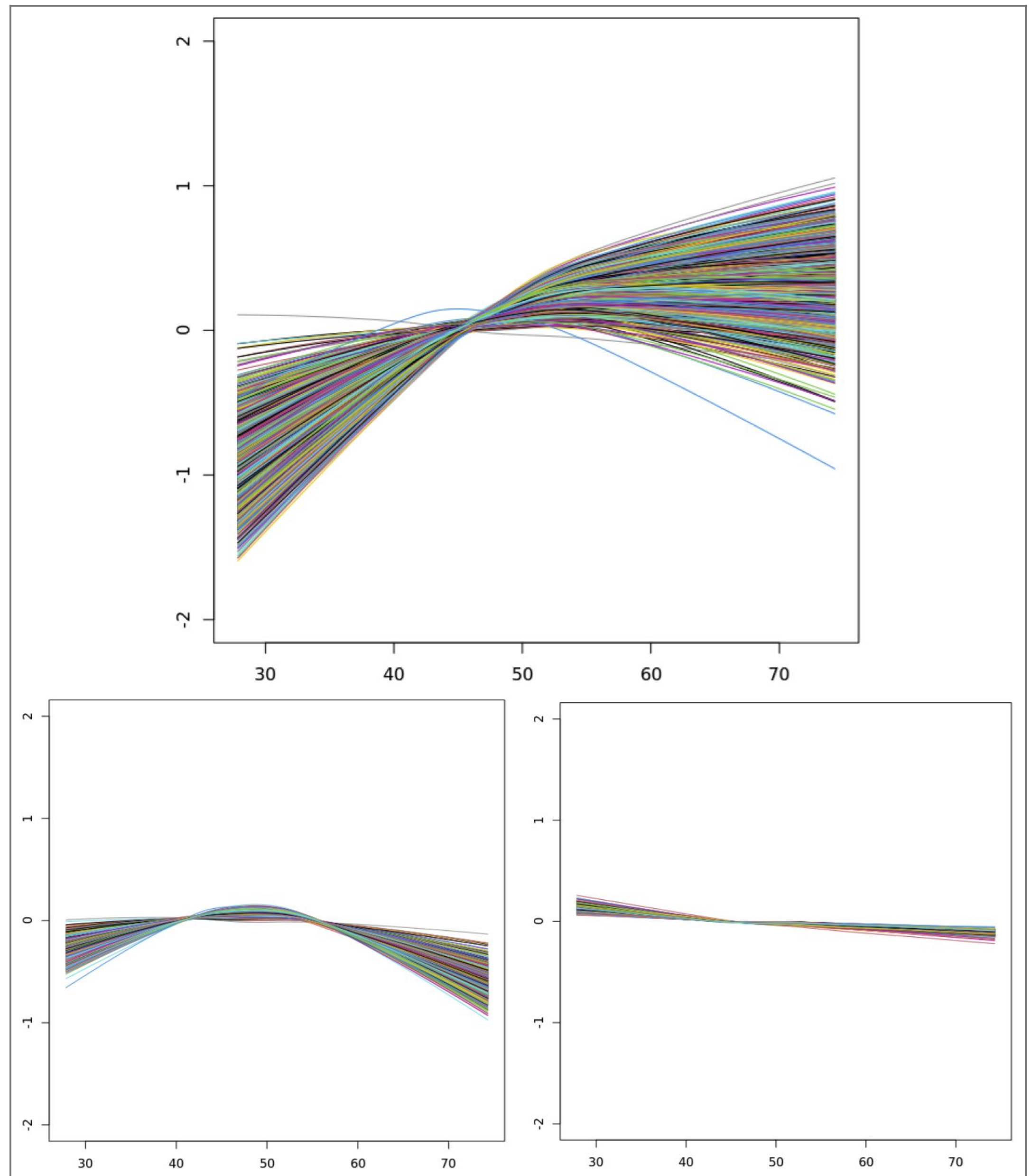

Figure S3. GC content bias correction. Top panel is before correction; Bottom left is after correction with local linear regression; Bottom right is after correction with local quadratic regression (loess in R). Each line represents fitted values of one sample.

5 Gene sets for different θ

Set	eQTL	D_{TSS}	Genic	Intron	Exon	ncRNA	SNP	GWAS
S_O	713	**666.9**	0.293	**0.630**	0.006	0.020	505	**0.022**
S_P	10,113	115.3	0.354	0.490	0.015	0.088	9,276	**0.069**
S_M	9,816	135.3	0.373	0.481	0.013	0.075	8,666	0.050
S_G	895,880	84.4	0.366	0.482	0.016	0.077	584,449	0.054
S_O	688	**673.2**	0.260	**0.663**	0.006	0.026	485	**0.023**
S_P	15,576	122.9	0.362	0.490	0.016	0.080	14,372	**0.066**
S_M	14,783	122.5	0.365	0.487	0.016	0.076	13,293	0.050
S_G	884,119	84.1	0.383	0.449	0.017	0.091	577,701	0.054
S_O	664	**671.7**	0.255	**0.670**	0.005	0.026	474	**0.023**
S_P	19,288	120.1	0.371	0.483	0.016	0.079	17,701	**0.063**
S_M	18,311	119.5	0.376	0.475	0.016	0.075	16,544	0.050
S_G	872,406	83.8	0.382	0.448	0.017	0.091	571,184	0.054

Table S2. Annotation of eQTL for different threshold. Top tabular: $\theta = 0$. Middle tabular $\theta = \log_{10} 2$. Bottom tabular: threshold= $\theta = \log_{10} 3$. S_O is a set of opposing eQTL, S_P is a set of paternal eQTL, S_M is a set of maternal eQTL, and S_G is a set of genotype eQTL. The column eQTL contain counts of eQTL in each set, and column SNP contains counts of distinct SNPs of eQTL in the set. The column *eGene* is the number of genes associated with eQTL in the set. The column of Len is the median length in Kb of the eGenes. (The pattern is the same with the mean length.) The column D_{TSS} contains median distance in Kb to transcription start site. The column GWAS contains percent of GWAS hits among SNPs, with GWAS p-value threshold of 5×10^{-8}.

6 Gene sets

6.1 Genes harbor exclusively paternal eQTL G_1

ANGEL1, ANKRD11, ANKS6, AQP3, ARNILA, ASB3, BAG3, BCL2L12, C21orf58, C2orf49-DT, CA11, CAMSAP2, CASP9, CCDC96, CCR9, CDKL3, CEBPZ, CFH, CHKA, CLIP4, CPNE5, CRK, CRY2, DBP, DCANP1, DDX51, DISP2, DLX4, EEF1E1, EHMT1, EPB41L2, FAM136A, FAM220A, FAM50B, FLT3, GEMIN4, GMCL1, GNA15-DT, GRAMD1A, HCLS1, HINFP, HPS6, HSDL1, KPNA2, LCDR, LINC00656, LINC01145, LINC01624, LINC01694, LINC03034, LRIG1, LTBR, MAST2, MBD1, MCEE, MED24, MEF2C-AS1, MICAL2, MIF, MIR22HG, MOCS2, MPHOSPH10, MPP7, MRPL39, NCEH1, NCOA2, NDN, NOPCHAP1, NOTCH2NLC, NPM2, NR1H2, PABPC1, PARP15, PCMTD1-DT, PEG10, PER3, PIAS3, PLPP6, POLD1, PPP1CB-DT, PPP1R14BP3, PRR12, PTGS2, PTK2, RBKS, RNF115, RPL5P1, RRM2, SAGSIN1, SCAF1, SCFD1, SEC14L1P1, SGCE, SLC7A5, SLC9A7P1, SMG1P2, SNRPN, SNURF, SQSTM1, TAF6, TANGO2, TARM1, TCAP, TCEA1P2, TIA1, TIMM23B, TINCR, TMEM129, TMEM30A, TMOD1, TRMT2A, TXNIP, UBR5-DT, UROD, VASH1-AS1, VPS9D1-AS1, WAPL-DT, YWHAZ, ZKSCAN5, ZNF276, ZNF34, ZNF407AS1, ZNF658B, ZNF706, ZNF778-DT, ZNNT1, ZRANB3, ZSCAN16, ZSCAN25

6.2 Genes harbor exclusively maternal eQTL G_0

ACTBP11, AIP, AKAP8, AMDHD2, ANKRA2, ANKUB1, ANO10, ASAP3, ASCC3, ATG12, BBIP1, BISPR, BLOC1S4, CABP4, CAMKMT, CASS4, CBR3-AS1, CC2D1A, CCDC117, CCDC30, CCT6P1, CDK2AP2, CDKN2AIPNL, CEPT1, CES4A, CMTM3, COA8, CTBP1-DT, CYP2U1, CYSTM1, DDX39A, EIF4B, ELOCP19, EMB, EPS15, EZR, FASTKD3, FEM1A, FUT8-AS1, GALNT4, GLUL, GNL2, GPR108, GRB10, GRK2, GTF2F1, GUCY1B1, GUSB, HAVCR1, HEATR1, HELZ2, HNRNPA1P21, HSPA1A, IGHG4, IGKV2-28, IL23A, ING1, INPP5A, KCTD15, KDM2A, KHSRP, KIR2DL4, LINC00310, LINC00467, LINC02908, LONP1, LRRC8C-DT, LTC4S, LYSMD2, MAN2B2, MAPK8, MCM4, MEG3, METTL15, METTL9, MRFAP1L1, MRPL14 NCF2, NEURL1, NIPSNAP1, NOC4L, NTNG2, NUDT22, OSBPL9, PCM1, PF4, PGGT1B, PI4KAP1, PITPNM1, PLIN3, PMPCB, POC1B-AS1, PPP4R2, PRR5L, PSMB6, PSPN, RANBP3, RASL10A, RCE1, RFX1, RNF43, RPL37AP1, RPS6KB2, RTL10, S1PR2, SCARNA16, SEC23B, SH2D2A,

SMIM19, *SNHG22*, *SPRYD7*, *STAM-DT*, *STPG3-AS1*, *SWSAP1*, *TAGAP*, *TDP2*, *TMEM106C*, *TMEM134*, *TMEM218*, *TRAJ37*, *TRAJ39*, *TRBV15*, *UBE2B*, *UHRF1*, *USP47*, *VAV1*, *VDR*, *ZBED3-AS1*, *ZFP37*, *ZNF286A*, *ZNF326*, *ZNF331*, *ZNF468*, *ZNF561*, *ZNF714*, *ZNF781*, *ZNF879*, *ZNF888*, *ZSWIM4*

6.3 Genes harbor exclusively opposing eQTL G_2

ADAMTSL4-AS1, *AFF1*, *AKR7A2*, *ANLN*, *ARID4A*, *BICD2*, *BNIP3*, *BORCS6*, *BROX*, *C18orf25*, *C1orf56*, *CACNA2D3*, *CCP110*, *CD300LF*, *CD46*, *CD82*, *CDK5RAP3*, *CDKN2A*, *CENPM*, *CHD3*, *CNTROB*, *COQ7*, *CPEB4*, *CPPED1*, *CXXC5*, *DNAJB6P1*, *DPH5-DT*, *EBLN2*, *ECSIT*, *EXOC5*, *FAM192BP*, *FBXW7*, *FGFR1OP2*, *FXR2*, *GABARAP*, *GNB4*, *GUCY2D*, *HEBP1*, *HSCB*, *IDI1*, *IQCK*, *ITPR1*, *ITPR2*, *KDM4A*, *KDM7A*, *KIAA0586*, *LEP*, *LINC01786*, *LRRC8D-DT*, *LZIC*, *MALINC1*, *MED21*, *MIR29B2CHG*, *MPHOSPH8*, *MRPS14*, *MZB1*, *MZT1*, *NECAB3*, *NFIA*, *NNT-AS1*, *NOTCH3*, *OPN3*, *PALLD*, *PAPOLG*, *PARPBP*, *PEX13*, *PHIP*, *PIBF1*, *PIK3CA*, *PLSCR3*, *PODNL1*, *PPP1R3E*, *PSMA3-AS1*, *PSMD9*, *PSTPIP2*, *PTRHD1*, *RARA-AS1*, *RBL2*, *REL*, *RNPC3*, *RPGRIP1L*, *RPL23P2*, *RPS28P7*, *RYBP*, *SEC61G*, *SIGLEC15*, *SLC15A4*, *STAM*, *SYNGR2*, *TMEM182*, *TMEM40*, *TOMM6*, *TPRG1L*, *UNC45A*, *WASL*, *WRAP53*, *XPO1*, *ZBTB4*, *ZDHHC20*

6.4 58 phosphoprotein genes

CCP110, *ARID4A*, *WASL*, *CHD3*, *BICD2*, *AFF1*, *PSMD9*, *AKR7A2*, *XPO1*, *PSTPIP2*, *SEC61G*, *PAPOLG*, *FGFR1OP2*, *BORCS6*, *CDK5RAP3*, *UNC45A*, *FBXW7*, *CACNA2D3*, *TMEM40*, *RBL2*, *PLSCR3*, *PALLD*, *PPP1R3E*, *PHIP*, *EXOC5*, *CD46*, *KDM7A*, *NOTCH3*, *NECAB3*, *ZDHHC20*, *TPRG1L*, *C1ORF56*, *ITPR1*, *ITPR2*, *RNPC3*, *CXXC5*, *ZBTB4*, *FXR2*, *SYNGR2*, *CD300LF*, *SLC15A4*, *KDM4A*, *OPN3*, *CDKN2A*, *BNIP3*, *STAM*, *PEX13*, *CPPED1*, *ANLN*, *RYBP*, *NFIA*, *KIAA0586*, *REL*, *GNB4*, *CNTROB*, *MPHOSPH8*, *CPEB4*, *WRAP53*

7 Sensitivity Analysis

Set	$\log_{10} BF = 4$		$\log_{10} BF = 4.5$		$\log_{10} BF = 5$	
	eQTL	eGene	eQTL	eGene	eQTL	eGene
S_O	688	180	156	52	38	12
S_P	15,576	1,188	9,797	762	6,263	504
S_M	14,783	1,209	9,341	768	6,025	509

Table S3. Counts of eQTL and eGenes for different Bayes factor threshold. As the threshold increases, type I error rate reduces, power reduces as a consequence, so the numbers of positive eQTL and eGene reduce.

8 Allele Frequencies

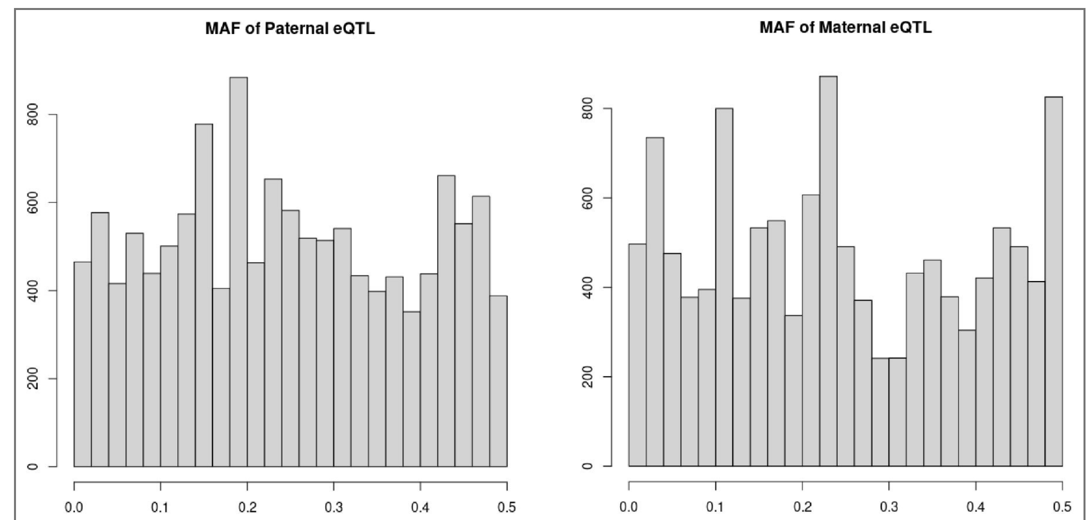

Figure S4. Allele frequecy distribution for parental eQTL and maternal eQTL. Here we used allele frequencies of European descent from 1000 Genomes project.

9 P-values, Bayes factors, and FDR

The p-value and its limitations

By definition, a p-value is the tail probability under the null hypothesis. Formally,

$$p = \mathbb{P}\left(T \geq t \mid H_0\right), \tag{1}$$

where T is a test statistic and $t = T(D)$ is the value computed from the observed data D, and H_0 denotes the null hypothesis. It is crucial to emphasize that the p-value is *not* the probability that H_0 is true; it is calculated under the assumption that H_0 holds. In hypothesis testing, we are often interested in the posterior probabilities of the null and alternative hypotheses, $\mathbb{P}(H_0 \mid D)$ and $\mathbb{P}(H_1 \mid D)$, about which the p-value alone says nothing.

From p-values to posterior probabilities

Let $R(t) = \{D : T(D) \geq t\}$ denote the set of all datasets that would yield a test statistic at least as extreme as the observed value t. This *rejection region* extends the observed data to include more extreme unobserved outcomes. Then, by Bayes' theorem,

$$\begin{aligned} \mathbb{P}\left(H_0 \mid R(t)\right) &= \frac{\mathbb{P}\left(R(t) \mid H_0\right)\mathbb{P}\left(H_0\right)}{\mathbb{P}\left(R(t) \mid H_0\right)\mathbb{P}\left(H_0\right) + \mathbb{P}\left(R(t) \mid H_1\right)\mathbb{P}\left(H_1\right)} \\ &= \frac{1}{1 + \frac{\mathbb{P}(R(t)\mid H_1)}{\mathbb{P}(R(t)\mid H_0)} \cdot \frac{\mathbb{P}(H_1)}{\mathbb{P}(H_0)}}. \end{aligned} \tag{2}$$

Here, $\mathbb{P}(R(t) \mid H_0)$ is precisely the p-value, while $\mathbb{P}(R(t) \mid H_1)$ is the *power* of the test against the alternative hypothesis. The ratio $\mathbb{P}(H_1)/\mathbb{P}(H_0)$ represents the prior odds. Thus, equation (2) reveals that

$$\frac{\mathbb{P}\left(R(t) \mid H_1\right)}{\mathbb{P}\left(R(t) \mid H_0\right)} = \frac{\text{Power}}{p}. \tag{3}$$

Although a p-value alone conveys no information about posterior probabilities, when combined with power and prior odds, it becomes a useful component for assessing evidence.

Bayes factors and their relationship to p-values

By definition, the Bayes factor is the ratio of marginal likelihoods under the alternative and null hypotheses:

$$\mathrm{BF} = \frac{\mathbb{P}\left(D \mid H_1\right)}{\mathbb{P}\left(D \mid H_0\right)}. \tag{4}$$

To compute posterior probabilities, one must specify prior odds $\mathbb{P}(H_1)/\mathbb{P}(H_0)$. The posterior odds are then BF × Prior odds, yielding

$$\mathbb{P}\left(H_0 \mid D\right) = \frac{1}{1 + \mathrm{BF} \times \text{ Prior odds}} = \frac{1}{1 + \frac{\mathbb{P}(D|H_1)}{m'|H'|\tau} \cdot \frac{\mathbb{P}(H_1)}{\frac{1}{N/'IT}}}. \tag{5}$$

Comparing (2), (3), and (5) suggests an approximate relationship:

$$\mathrm{BF} \approx \frac{\text{Power}}{p}. \tag{6}$$

Remark 1.

The approximation in (6) *is conceptual rather than computational. Power is typically defined at a specific point alternative, whereas* $\mathbb{P}(R(t) \mid H_1)$ *in* (2) *should properly be integrated over the prior distribution of effect sizes under* H_1*. This distinction lies at the heart of the Bayesian approach. Nevertheless, this relationship illuminates several important principles:*

- *Interpreting p-values requires consideration of study power*.
- *P-values are not directly comparable across studies with different power*.
- *Underpowered studies require more stringent p-value thresholds to achieve comparable evidence*.

Local false discovery rate

The local false discovery rate (lfdr) is a Bayesian-inspired measure that assigns a probability of being a false positive to each hypothesis test, conditional on the observed test statistic:

$$\mathrm{lfdr}(t) = \mathbb{P}\left(H_0 \mid T = t\right) = \frac{\pi_0 f_0(t)}{f(t)}, \tag{7}$$

where $f_0(t)$ is the density under the null, $f_1(t)$ is the density under the alternative, and $f(t) = \pi_0 f_0(t) + (1 - \pi_0) f_1(t)$ is the mixture density. Here, $\pi_0 = \mathbb{P}(H_0)$ is the prior probability of the null. Rearranging,

$$\mathrm{lfdr}(t) = \frac{1}{1 + \frac{f_1(t)}{f_0(t)} \cdot \frac{1-\pi_0}{\pi_0}}, \tag{8}$$

where $f_1(t)/f_0(t)$ is a Bayes factor (comparing pointwise densities), and $(1 - \pi_0)/\pi_0$ is the prior odds.

From local fdr to FDR

Let $F_0(t) = \mathbb{P}(T \geq t \mid H_0)$ be the null tail probability, and $F_1(t) = \mathbb{P}(T \geq t \mid H_1)$ be the alternative tail probability. The (tail area) false discovery rate at threshold t is

$$\mathrm{FDR}(t)=\mathbb{P}\left(H_0 \mid T \geq t\right)=\frac{\pi_0 F_0(t)}{F(t)} \tag{9}$$

where $F(t) = \pi_0 F_0(t) + (1 - \pi_0)F_1(t)$ is the marginal tail probability. A fundamental connection between local fdr and FDR is:

$$\mathrm{FDR}(t)=\mathbb{E}[\operatorname{lfdr}(T) \mid T \geq t], \tag{10}$$

meaning that FDR at threshold t is the average local fdr among all tests exceeding that threshold. This relationship illuminates the different philosophical choices: Bayesians often prefer local fdr for its interpretation as an exact conditional probability, while frequentists favor FDR for its reliance on familiar tail probabilities and the convenience of estimating cumulative distribution functions rather than densities.

The Benjamini-Hochberg procedure as empirical Bayes

The Benjamini-Hochberg (BH) procedure emerges naturally from an empirical Bayes formulation. Let $p_{(1)} \leq p_{(2)} \leq \cdots \leq p_{(m)}$ be the ordered p-values from m hypothesis tests, and let $H_{(i)}$ denote the null hypothesis corresponding to $p_{(i)}$. The BH procedure rejects $H_{(1)}, \ldots, H_{(k)}$, where k is the largest index for which $p_{(i)} \leq i\alpha/m$.

To see the connection, consider the empirical estimates of the marginal and null cumulative distribution functions at $p_{(k)}$:

$$\hat{F}\left(p_{(k)}\right)=\frac{k}{m}, \tag{11}$$

$$\hat{F}_0\left(p_{(k)}\right)=\tfrac{m p_{(k)}}{m}=p_{(k)} \quad \text{(assuming p-values are uniform under the null).} \tag{12}$$

The BH procedure can be derived as rejecting when the estimated FDR falls below α:

$$\frac{\hat{\pi}_0 \hat{F}_0\left(p_{(k)}\right)}{\hat{F}\left(p_{(k)}\right)}<\alpha, \tag{13}$$

where $\hat{\pi}_0$ is an estimate of the proportion of true nulls. The original BH procedure implicitly takes $\hat{\pi}_0 = 1$ (a conservative choice), yielding $p_{(k)} < \alpha k/m$ as the rejection criterion. More sophisticated procedures estimate π_0 from the data to increase power.

> ***Remark 2.***
>
> *This derivation reveals that the BH procedure is essentially an empirical Bayes method: it estimates the marginal distribution F empirically through the order statistics, assumes the null distribution F_0 is known (uniform), and rejects tests when the estimated FDR is acceptably low. For cis-eQTL analysis, the uniform null assumption is likely to fail due to LD.*

Conclusion

P-values, Bayes factors, and FDR-based measures form a coherent framework for understanding statistical evidence. P-values answer a specific probability question under the null, but require context (power and prior odds) to inform posterior beliefs. Its caluation relies on unobserved data, which voilates the likelihood principle. Bayes factors respect the likelihood principle, and provide a direct update of prior odds to posterior odds. The approximate relationship between Bayes factor and p-value in light of its power reflects the advantage of Bayes factor in eQTL analysis. Local fdr and FDR are empirical Bayes procedure in the multiple testing setting, with the BH

procedure emerging naturally from an empirical Bayes perspective. Local fdr has an intimate connection with Bayes factor. Understanding these connections helps practitioners avoid common pitfalls in interpreting p-values and choose appropriate methods for their scientific questions.

Data availability

The data used in the study is obtainned from Framingham Heart Study, which can be obtained via dbGaP Study Accession: phs000007.v34.p15.

Additional files

Supplementary

Note

This reviewed preprint has been updated to correct the corresponding author's email address.

Additional information

Funding

Funder	Grant reference number	Author
HHS | NIH | National Heart, Lung, and Blood Institute (NHLBI)	Intramural	Daniel Levy

Author ORCID iDs

Yongtao Guan: https://orcid.org/0000-0002-8055-2192

References

Berland S, Haukanes BI, Juliusson PB, Houge G (2022) Deep exploration of a CDKN1C mutation causing a mixture of Beckwith-Wiedemann and IMAGe syndromes revealed a novel transcript associated with developmental delay. *J. Med. Genet* **59**:155-164 https://doi.org/10.1136/jmedgenet-2020-107401 | PubMed

Bochud M, Chiolero A, Elston RC, Paccaud F (2008) A cautionary note on the use of mendelian randomization to infer causation in observational epidemiology. *Int. J. Epidemiol* **37**:414-6 https://doi.org/10.1093/ije/dym186 | PubMed

Cheung VG, Nowak N, Jang W, Kirsch IR, Zhao S, Chen XN, Furey TS, Kim UJ, Kuo WL, Olivier M, *et al.* (2001) Integration of cytogenetic landmarks into the draft sequence of the human genome. *Nature* **409**:953-958 https://doi.org/10.1038/35057192 | PubMed

Cockett NE, Jackson SP, Shay TL, Farnir F, Berghmans S, Snowder GD, Nielsen DM, Georges M (1996) Polar overdominance at the ovine callipyge locus. *Science* **273**:236-238 https://doi.org/10.1126/science.273.5272.236 | PubMed

GTEx Consortium (2020) The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* **369**:1318-1330 https://doi.org/10.1126/science.aaz1776 | PubMed

Daneshmoghadam J, Omidifar A, Dilmaghani N Akbari, Karimi Z, Emamgholipour S, Shanaki M (2021) The gene expression of long non-coding RNAs (lncRNAs): MEG3 and H19 in adipose tissues from obese women and its association with insulin resistance and obesity indices. *J. Clin. Lab. Anal* **35**:e23741 https://doi.org/10.1002/jcla.23741 | PubMed

DeChiara TM, Robertson EJ, Efstratiadis A (1991) Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64**:849-859 https://doi.org/10.1016/0092-8674(91)90513-x | PubMed

Deng S, Hardin J, Amos CI, Xiao F (2020) Joint modeling of eQTLs and parent-of-origin effects using an orthogonal framework with RNA-seq data. *Hum. Genet* **139**:1107-1117 https://doi.org/10.1007/s00439-020-02162-2 | PubMed

Gamazon ER, Wheeler HE, Shah KP, Mozaffari SV, Aquino-Michaels K, Carroll RJ, Eyler AE, Denny JC, GTEx Consortium, Nicolae DL, *et al.* (2015) A gene-based association method for mapping traits using reference transcriptome data. *Nat. Genet* **47**:1091-1098 https://doi.org/10.1038/ng.3367 | PubMed

Garfield AS, Cowley M, Smith FM, Moorwood K, Stewart-Cox JE, Gilroy K, Baker S, Xia J, Dalley JW, Hurst LD, *et al.* (2011) Distinct physiological and behavioural functions for parental alleles of imprinted grb10. *Nature* **469**:534-538 https://doi.org/10.1038/nature09651 | PubMed

Guan Y (2014) Detecting structure of haplotypes and local ancestry. *Genetics* **196**:625-642 https://doi.org/10.1534/genetics.113.160697 | PubMed

Guan Y, Levy D (2024a) Asymptotically exact fit for linear mixed model. *bioRxiv* https://doi.org/10.1101/2024.05.28.596229

Guan Y, Levy D (2024b) Bayes factor for linear mixed model in genetic association studies. *bioRxiv* https://doi.org/10.1101/2024.05.28.596229

Guan Y, Levy D (2024c) Estimation of inbreeding and kinship coefficients via latent identity-by-descent states. *Bioinformatics* **40**:btae082 https://doi.org/10.1093/bioinformatics/btae082 | PubMed

Guan Y, Stephens M (2011) Bayesian variable selection regression for genome-wide association studies and other large-scale problems. *The Annals of Applied Statistics* **5**:1780-1815 https://doi.org/10.48550/arxiv.1110.6019

Hager R, Cheverud JM, Wolf JB (2008) Maternal effects as the cause of parent-of-origin effects that mimic genomic imprinting. *Genetics* **178**:1755-1762 https://doi.org/10.1534/genetics.107.080697 | PubMed

Hanson RL, Guo T, Muller YL, Fleming J, Knowler WC, Kobes S, Bogardus C, Baier LJ (2013) Strong parent-of-origin effects in the association of KCNQ1 variants with type 2 diabetes in american indians. *Diabetes* **62**:2984-2991 https://doi.org/10.2337/db12-1767 | PubMed

Hitchcock TJ, Gardner A (2019) Parent-of-origin specific gene expression and dispersal. *Current Opinion in Behavioral Sciences* **25**:36-43 https://doi.org/10.1016/j.cobeha.2018.06.007

Hofmeister RJ, Cavinato T, Karimi R, van der Graaf A, Pajuste F.-D, Kronberg J, Taba N, Estonian Biobank research team, Mägi R, Vaudel M, *et al.* (2025) Parent-of-origin effects on complex traits in up to 236,781 individuals. *Nature* **646**:647-656 https://doi.org/10.1038/s41586-025-09357-5 | PubMed

Hofmeister RJ, Rubinacci S, Ribeiro DM, Buil A, Kutalik Z, Delaneau O (2022) Parent-of-Origin inference for biobanks. *Nat. Commun* **13**:6668 https://doi.org/10.1038/s41467-022-34383-6 | PubMed

Jadhav B, Monajemi R, Gagalova KK, Ho D, Draisma HHM, van de Wiel MA, Franke L, Heijmans BT, van Meurs J, Jansen R, *et al.* (2019) RNA-Seq in 296 phased trios provides a high-resolution map of genomic imprinting. *BMC Biol* **17**:50 https://doi.org/10.1186/s12915-019-0674-0 | PubMed

Jansen S, Hoischen A, Coe BP, Carvill GL, Van Esch H, Bosch DGM, Andersen UA, Baker C, Bauters M, Bernier RA, *et al.* (2018) A genotype-first approach identifies an intellectual disability-overweight syndrome caused by PHIP haploinsufficiency. *Eur. J. Hum. Genet* **26**:54-63 https://doi.org/10.1038/s41431-017-0039-5 | PubMed

Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP (1979) An investigation of coronary heart disease in families. the framingham off-spring study. *Am. J. Epidemiol* **110**:281-290 https://doi.org/10.1093/oxfordjournals.aje.a112813 | PubMed

Kerns SL, Guevara-Aguirre J, Andrew S, Geng J, Guevara C, Guevara-Aguirre M, Guo M, Oddoux C, Shen Y, Zurita A, *et al.* (2014) A novel variant in CDKN1C is associated with intrauterine growth restriction, short stature, and early-adulthood-onset diabetes. *J. Clin. Endocrinol. Metab* **99**:E2117-22 https://doi.org/10.1210/jc.2014-1949 | PubMed

Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, Jonasdottir A, Sigurdsson A, Kristinsson KT, Jonasdottir A, *et al.* (2009) Parental origin of sequence variants associated with complex diseases. *Nature* **462**:868-874 https://doi.org/10.1038/nature08625 | PubMed

Lawson HA, Cheverud JM, Wolf JB (2013) Genomic imprinting and parent-of-origin effects on complex traits. *Nat. Rev. Genet* **14**:609-617 https://doi.org/10.1038/nrg3543 | PubMed

Li C, Ge M, Long K, Han Z, Li M, Zhang Z, Huang L (2025) Mechanism of parent-of-origin effects revealed by multi-omic data in euro-chinese hybrid pigs. *Nat. Commun* **16**:7542 https://doi.org/10.1038/s41467-025-62243-6 | PubMed

Liu Y, Zhou DZ, Zhang D, Chen Z, Zhao T, Zhang Z, Ning M, Hu X, Yang YF, Zhang ZF, *et al.* (2009) Variants in KCNQ1 are associated with susceptibility to type 2 diabetes in the population of mainland china. *Diabetologia* **52**:1315-1321 https://doi.org/10.1007/s00125-009-1375-y | PubMed

Macias-Velasco JF, St Pierre CL, Wayhart JP, Yin L, Spears L, Miranda MA, Carson C, Funai K, Cheverud JM, Semenkovich CF, *et al.* (2022) Parent-of-origin effects propagate through networks to shape metabolic traits. *eLife* **11** https://doi.org/10.7554/elife.72989 | PubMed

Maffei M, Fei H, Lee GH, Dani C, Leroy P, Zhang Y, Proenca R, Negrel R, Ailhaud G, Friedman JM (1995) Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. *Proc. Natl. Acad. Sci. U. S. A* **92**:6957-6960 https://doi.org/10.1073/pnas.92.15.6957 | PubMed

Moore T, Haig D (1991) Genomic imprinting in mammalian development: a parental tug-of-war. *Trends in Genetics* **7**:45-49 https://doi.org/10.1016/0168-9525(91)90230-n | PubMed

Mott R, Yuan W, Kaisaki P, Gan X, Cleak J, Edwards A, Baud A, Flint J (2014) The architecture of parent-of-origin effects in mice. *Cell* **156**:332-342 https://doi.org/10.1016/j.cell.2013.11.043 | PubMed

Mozaffari SV, DeCara JM, Shah SJ, Sidore C, Fiorillo E, Cucca F, Lang RM, Nicolae DL, Ober C (2019) Parent-of-origin effects on quantitative phenotypes in a large hutterite pedigree. *Commun. Biol* **2**:28 https://doi.org/10.1038/s42003-018-0267-4 | PubMed

Ou K, Yu M, Moss NG, Wang YJ, Wang AW, Nguyen SC, Jiang C, Feleke E, Kameswaran V, Joyce EF, *et al.* (2019) Targeted demethylation at the cdkn1c/p57 locus induces human beta cell replication. *The Journal of Clinical Investigation* **129**:209-214 https://doi.org/10.1172/JCI99170 | PubMed

Porcu E, Rüeger S, Lepik K, eQTLGen Consortium, BIOS Consortium, Santoni FA, Reymond A, Kutalik Z (2019) Mendelian randomization integrating GWAS and eQTL data reveals genetic determinants of complex and clinical traits. *Nat. Commun* **10**:3300 https://doi.org/10.1038/s41467-019-10936-0 | PubMed

Qi Z, Xie S, Chen R, Aisa HA, Hon GC, Guan Y (2018) Tissue-specific gene expression prediction associates vitiligo with suox through an active enhancer. *bioRxiv* https://doi.org/10.1101/337196

Reik W, Walter J (2001) Genomic imprinting: parental influence on the genome. *Nat. Rev. Genet* **2**:21-32 https://doi.org/10.1038/35047554 | PubMed

Risso D, Schwartz K, Sherlock G, Dudoit S (2011) GC-content normalization for RNA-Seq data. *BMC Bioinformatics* **12**:480 https://doi.org/10.1186/1471-2105-12-480 | PubMed

Serrano J, Goebel SU, Peghini PL, Lubensky IA, Gibril F, Jensen RT (2000) Alterations in the p16INK4a/CDKN2A tumor suppressor gene in gastrinomas. *J. Clin. Endocrinol. Metab* **85**:4146-4156 https://doi.org/10.1210/jcem.85.11.6970 | PubMed

Servin B, Stephens M (2007) Imputation-based analysis of association studies: Candidate regions and quantitative traits. *PLOS Genetics* **3**:1-13 https://doi.org/10.1371/journal.pgen.0030114 | PubMed

Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W (2022) DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res* **50**:W216-W221 https://doi.org/10.1093/nar/gkac194 | PubMed

Smith GD, Ebrahim S (2003) 'mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease?. *Int. J. Epidemiol* **32**:1-22 https://doi.org/10.1093/ije/dyg070 | PubMed

Soloff JA, Xiang D, Fithian W (2024) The edge of discovery: Controlling the local false discovery rate at the margin. *The Annals of Statistics* **52**:580-601 https://doi.org/10.1214/24-aos2359

Stephens M, Balding DJ (2009) Bayesian statistical methods for genetic association studies. *Nat. Rev. Genet* **10**:681-690 https://doi.org/10.1038/nrg2615 | PubMed

Swanson-Wagner RA, DeCook R, Jia Y, Bancroft T, Ji T, Zhao X, Nettleton D, Schnable PS (2009) Paternal dominance of trans-eQTL influences gene expression patterns in maize hybrids. *Science* **326**:1118-1120 https://doi.org/10.1126/science.1178294 | PubMed

Taliun D, Harris DN, Kessler MD, Carlson J, Szpiech ZA, Torres R, et al. (2021) Sequencing of 53,831 diverse genomes from the NHLBI TOPMed program. *Nature* **590**:290-299 https://doi.org/10.1038/s41586-021-03205-y | PubMed

Tomé S, Panigrahi GB, Castel A López, Foiry L, Melton DW, Gourdon G, Pearson CE (2011) Maternal germline-specific effect of DNA ligase I on CTG/CAG instability. *Hum. Mol. Genet* **20**:2131-2143 https://doi.org/10.1093/hmg/ddr099 | PubMed

Unoki H, Takahashi A, Kawaguchi T, Hara K, Horikoshi M, Andersen G, Ng DPK, Holmkvist J, Borch-Johnsen K, Jørgensen T, *et al.* (2008) SNPs in KCNQ1 are associated with susceptibility to type 2 diabetes in east asian and european populations. *Nat. Genet* **40**:1098-1102 https://doi.org/10.1038/ng.208 | PubMed

van der Graaf A, Claringbould A, Rimbert A, BIOS Consortium, Westra H.-J, Li Y, Wijmenga C, Sanna S (2020) Mendelian randomization while jointly modeling cis genetics identifies causal relationships between gene expression and lipids. *Nat. Commun* **11**:4930 https://doi.org/10.1038/s41467-020-18716-x | PubMed

Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* **38**:e164 https://doi.org/10.1093/nar/gkq603 | PubMed

Webster E, Cho MT, Alexander N, Desai S, Naidu S, Bekheirnia MR, Lewis A, Retterer K, Juusola J, Chung WK (2016) De novo PHIP-predicted deleterious variants are associated with developmental delay, intellectual disability, obesity, and dysmorphic features. *Cold Spring Harb Mol Case Stud* **2**:a001172 https://doi.org/10.1101/mcs.a001172 | PubMed

Weigle DS, Ganter SL, Kuijper JL, Leonetti DL, Boyko EJ, Fujimoto WY (1997) Effect of regional fat distribution and Prader-Willi syndrome on plasma leptin levels. *J. Clin. Endocrinol. Metab* **82**:566-570 https://doi.org/10.1210/jcem.82.2.3761 | PubMed

Wermter A.-K, Scherag A, Meyre D, Reichwald K, Durand E, Nguyen TT, Koberwitz K, Lichtner P, Meitinger T, Schäfer H, *et al.* (2008) Preferential reciprocal transfer of paternal/maternal DLK1 alleles to obese children: first evidence of polar overdominance in humans. *Eur. J. Hum. Genet* **16**:1126-1134 https://doi.org/10.1038/ejhg.2008.64 | PubMed

Yasuda K, Miyake K, Horikawa Y, Hara K, Osawa H, Furuta H, Hirota Y, Mori H, Jonsson A, Sato Y, *et al.* (2008) Variants in KCNQ1 are associated with susceptibility to type 2 diabetes mellitus. *Nat. Genet* **40**:1092-1097 https://doi.org/10.1038/ng.207 | PubMed

Zhabotynsky V, Inoue K, Magnuson T, Calabrese J Mauro, Sun W (2019) A statistical method for joint estimation of cis-eQTLs and parent-of-origin effects under family trio design. *Biometrics* **75**:864-874 https://doi.org/10.1111/biom.13026 | PubMed

Zhou Q, Guan Y (2018) On the null distribution of bayes factors in linear regression. *Journal of the American Statistical Association* **113**:1362-1371 https://doi.org/10.1080/01621459.2017.1328361 | PubMed

Zhou Y, Zhang Y, Zhao D, Yu X, Shen X, Zhou Y, Wang S, Qiu Y, Chen Y, Zhu (2024) TTD: Therapeutic target database describing target druggability information. *Nucleic Acids Res* **52**:D1465-D1477 https://doi.org/10.1093/nar/gkad751 | PubMed

Zink F, Magnusdottir DN, Magnusson OT, Walker NJ, Morris TJ, Sigurdsson A, Halldorsson H, Gudjonsson SA, Melsted P, Ingimundardottir H, *et al.* (2018) Insights into imprinting from parent-of-origin phased methylomes and transcriptomes. *Nat. Genet* **50**:1542-1552 https://doi.org/10.1038/s41588-018-0232-7 | PubMed

Peer reviews

Reviewer #2 (Public review):

Summary:

The authors have used 1477 sequenced trios with available gene expression data in the offsprings to discover eQTLs that act in a parent-of-origin specific manner. The classified their associated SNPs are tested for enrichment for GWAS hits, drug target genes, etc.

Strengths:

The manuscript presents an impressive analysis of a very rich data set of parent-of-origin eQTLs. To my knowledge, it is one of the largest studies of its kind and most analyses are sound and the results are of interest to many in the field and potentially beyond. The different ideas of follow-up analyses are useful and make sense.

Weaknesses:

While in general the analyses are well-conducted, I noticed a major issue with the POE eQTL classification, which puts into question most of the downstream analysis. In the light of this problem, all claims of individual discoveries (apart from those in Table 1) should be removed. The enrichment analyses remain valid and are useful.

https://doi.org/10.7554/eLife.107920.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Public Reviews:***
>
> ***Reviewer 1 (Public review):***
>
> *Summary:*
>
> *This study presents a systematic investigation of parent-of-origin effects on gene expression using trio-based data from the Framingham Heart Study, which is notable for its relatively large number of trios. By combining whole-genome and RNA sequencing data, the authors examined the extent to which gene expression is influenced by whether genetic variants are inherited maternally or paternally.*
>
> *The authors report that parent-of-origin eQTLs are widespread, identifying 15,893 eQTLs from 14,733 variants and 1,824 genes that were significant in paternal, maternal, or joint tests but not detected by traditional eQTL approaches. They further classified these associations based on the relative strength and direction of paternal and maternal effects, highlighting a subset with opposing directions. The study also highlighted eGenes linked to known imprinted genes as well as those with opposing parent-specific effects, and observed that paternal eGenes are enriched for drug targets. Finally, the work revisits previous findings in which eQTL studies were used to interpret disease-associated loci, emphasizing that conventional eQTL analyses without testing the parent-of-origin may mislead gene prioritization efforts. The study recommends that future downstream*

analyses, such as Mendelian randomization, take into account the provided lists of SNPs and eGenes and exclude those with strong parent-of-origin effects when linking genetic regulation to disease risk.

Strengths:

The major strength of the study lies in the scale and quality of the dataset, the trio-based design, and the systematic application of statistical tests for parent-of-origin effects. The strengths thoughtfully employed Bayes factors rather than p-values to provide stronger evidence of association, which adds rigor to their analyses. These design choices provide compelling evidence that parent-of-origin effects are widespread and that conventional eQTL analyses miss a substantial fraction of regulatory variation. The results are clearly presented and supported by robust analyses, including the identification of opposing parental effects and the enrichment of paternal eGenes for drug targets. Notably, the two examples demonstrating how these findings can reshape disease gene prioritization highlight the broader impact of the study and encourage further work in the community to incorporate parent-of-origin effects.

Weaknesses:

The main limitations of the study are threefold.

First, there is a lack of replication in independent cohorts, which is understandable given the difficulty of identifying datasets with a comparable number of trios, but replication would help establish the generalizability of the findings.

We fully agree with the reviewer that replication in an independent cohort is a crucial step for establishing generalizability. As the reviewer notes, the Framingham Heart Study, with its 1,477 trios possessing both WGS and RNA-seq data, represents a uniquely powerful and, to our knowledge, currently unmatched resource for this specific type of parent-of-origin eQTL analysis.

In the absence of an external cohort of comparable size and data richness, we have taken several steps to ensure the internal validity and robustness of our findings within the current study, which we will clarify and expand upon in the revised manuscript:

Positive Control Validation: We explicitly used well-established, bona fide imprinted genes (e.g., MEG3, NDN, SNURF, as listed in Table 1 and Figure 1) as positive controls. The fact that our analysis correctly identifies their known parent-of-origin expression patterns (e.g., maternal eQTL for MEG3, paternal eQTL for NDN) serves as a powerful internal validation of our phasing methodology, statistical models, and significance thresholds. This demonstrates that our approach has the power to detect true POE signals.

Conservative Calling Criteria: As the reviewer suggests, we prioritized specificity. Our definition of eQTL sets (Section 4.6) uses stringent thresholds (e.g., $\log_{10} BF > 4$ for primary signals and $\theta = \log_{10} 2$ for exclusivity). We explored different θ parameters (Supplementary Table S2) and chose the one that minimized the inclusion of false positives, ensuring that our core gene sets (e.g., G_1,G_0,G_2) are high-confidence discoveries.

Rigorous Analytical Pipeline: As we note in the revised text, our conclusions are supported by a robust analytical pipeline. This includes trio-based phasing validated by simulation (Supplementary Table S1), the use of linear mixed models to control for relatedness and population structure, and the application of Bayes factors which inherently penalize variants with low minor allele frequencies, thereby reducing spurious associations.

We believe these internal consistency checks and methodological rigor provide strong confidence in our findings. To further facilitate external replication, we will make the full list

of POE eQTLs and eGenes available as a comprehensive resource (as noted in the Discussion and Supplementary Materials), enabling other researchers to validate these findings as appropriate datasets become available.

> *Second, while Bayes factors are thoughtfully used to assess evidence of association, the paper does not fully explore how the chosen thresholds translate to the expected rate of false positives. For example, a minor allele frequency cutoff of 1% was applied, which seems somewhat arbitrary, and without reporting the allele frequency distribution of the identified eQTLs, it is unclear whether rare variants disproportionately contribute to the signals, potentially affecting the reliability of discoveries.*

We thank the reviewer for raising this important point regarding the calibration of our significance thresholds and the potential role of rare variants. We address this by clarifying the relationship between Bayes factors, prior odds, and false discovery rates, and by providing a more detailed characterization of the variants we identified.

Bayes Factors and False Discovery: The reviewer is correct that the connection between a Bayes factor threshold and a false positive rate is not direct as it has to take into account of prior odds. As we briefly noted, for a given prior odds of association (e.g., 1 in 100 or 1 in 1000 for a cis-eQTL), a $\log_{10} BF = 4$ corresponds to a posterior probability of association (PPA) of 0.99 or 0.90 respectively. Consequently, $1 - PPA$ can be interpreted as the local false discovery rate (lfdr), as we have now explicitly stated in Section 2.2 (citing Soloff et al., 2024). Our choice of $\log_{10} BF = 4$ was therefore chosen to ensure a very low or modest lfdr (depending on the prior odds) for our primary findings.

Minor Allele Frequency Threshold: The 1% MAF cutoff was indeed a pre-analysis filtering step. It was chosen based on the power afforded by our sample size of 1,477 trios. For variants rarer than 1%, our study is underpowered to detect associations, and any signals would be highly unstable. Importantly, the reviewer's concern about rare variants disproportionately contributing to signals is further mitigated by our use of Bayes factors. As we note in Section 2.2, the prior used in our Bayes factor computation (with $\sigma = 0.5$ in the prior for effect sizes, as described in Section 4.4) inherently penalizes variants with small minor allele frequencies. This is because for a given effect size, the evidence for association is weaker for a rare variant than a common one. Thus, the combination of a pre-analysis MAF filter and the Bayesian analysis itself guards against spurious findings driven by very rare alleles.

Allele Frequency Distribution: To directly address the reviewer's request for transparency, in the revised manuscript we include a supplementary figure (e.g., Supplementary Figure S4) showing the distribution of minor allele frequencies (1000 genomes European descents) for the SNPs identified in paternal eQTL set S_P and maternal eQTL set S_M. This empirically demonstrate that our findings are not disproportionately driven by low-frequency variants and provide a more complete picture of the genetic architecture underlying these POE signals. We also add a sentence to the Results section (Section 2.5) summarizing this distribution.

> *Third, the ancestry background of the study samples is not reported, which could be a confounding factor in the genetic analyses.*

We thank the reviewer for highlighting this omission. In the revised manuscript, we explicitly report the ancestry background of the Framingham Heart Study participants analyzed. Consistent with previous reports on this cohort, the vast majority of samples are of European descent.

Crucially, as the reviewer suggests, population stratification can be a confounder in genetic studies. To mitigate this, our analysis employed a linear mixed model (Section 4.4) that

includes a random effect with a covariance structure defined by the genetic relatedness matrix (GRM). This approach is specifically designed to control for spurious associations due to both subtle population structure and known relatedness among individuals, ensuring that our findings are robust to these potential confounders.

> ***Reviewer 2 (Public review):***
>
> *Summary:*
>
> *The authors have used 1477 sequenced trios with available gene expression data in the offspring to discover eQTLs that act in a parent-of-origin specific manner. The classified associated SNPs are tested for enrichment for GWAS hits, drug target genes, etc.*
>
> *Strengths:*
>
> *The manuscript presents an impressive analysis of a very rich data set of parent-of-origin eQTLs. To my knowledge, it is one of the largest studies of its kind, most analyses are sound, and the results are of interest to many in the field and potentially beyond. The different ideas of follow-up analyses are useful and make sense.*
>
> *Weaknesses:*
>
> *While in general the analyses are well-conducted, I noticed a major issue with the POE eQTL classification, which puts into question most of the downstream analysis. In light of this problem, most of the analysis would need to be rerun, which represents a major revision of the paper, but is straightforward to repair.*

We appreciate the reviewer's concern and take it seriously. However, we believe the issue stems from a misunderstanding of our classification framework. We clarify our reasoning below, and we are confident that no re-analysis is necessary. In fact, our Bayesian approach was specifically chosen to avoid the very problem the reviewer raises.

> *The major problem with the classification of POEs is that simply having significant maternal, but insignificant paternal effect is not an indicator of POE, this happens widely for SNPs with no POE whatsoever (it can happen by chance even when both maternal and paternal effects are the same and non-zero - the authors can see it via simulations under the null [maternal=paternal effect]).*

The reviewer raises a valid statistical concern: under the null hypothesis of equal maternal and paternal effects ($\beta_0 = \beta_1 \neq 0$), sampling variation could occasionally produce a scenario where one effect appears significant and the other does not. This is indeed a form of Type II error (failing to detect a true non-zero effect for one of the alleles).

However, this is precisely why we chose Bayes factors over p-values. A key advantage of Bayes factors is that they are not blind to power. P-values are calculated solely under the null hypothesis and do not incorporate any information about the alternative hypothesis or the study's power to detect it. Consequently, when power is low (e.g., due to minor allele frequency differences between paternal and maternal alleles), p-values can be misleading.

In contrast, Bayes factors are computed under both the null and alternative hypotheses. They inherently incorporate power through the prior specification. As we note in Section 2.2, "Bayes factors penalize genetic variants with small allele frequencies to reduce false positives." This means that a SNP where, by chance, one allele appears significant and the other does not—but where power is low due to allele frequency imbalance—will not receive a high Bayes factor, because the evidence is appropriately discounted.

> *In order to be able to talk about POE, first, a significant difference between maternal and paternal effects needs to be claimed. Therefore, none of the 4 sets of POE eQTLs are*

> *justified. To me, the only relevant criterion to pick POE SNPs is the P-value when comparing the maternal and paternal effects.*

We respectfully disagree with the reviewer's assertion that our approach to POE eQTL classification are not justified. There are multiple biologically meaningful patterns of parent-of-origin effects, and our classification scheme was designed to capture this diversity:

(1) Paternal-specific eQTL ($\beta_0 = 0$, $\beta_1 \neq 0$)

(2) Maternal-specific eQTL ($\beta_0 \neq 0$, $\beta_1 = 0$)

(3) Opposing eQTL ($\beta_0 \neq 0$, $\beta_1 \neq 0$,$\beta_0 \times \beta_1 < 0$)

(4) Genotype eQTL ($\beta_0 = \beta_1 \neq 0$)

The reviewer's proposed test (H_0: $\beta_0 = \beta_1$) collapses these distinct biological scenarios into a single binary outcome. For example: A purely paternal-specific eQTL ($\beta_0 = 0$, $\beta_1 \neq 0$) would indeed show a significant difference, and would be captured by the reviewer's test. However, a gene like ZNF890P in Table 1, where both effects are significant and in the same direction but of different magnitudes, would also show a significant difference. In the reviewer's framework, this would be classified as a POE eQTL, yet biologically it behaves more like a genotype eQTL with an allelic imbalance. Our framework correctly separates these cases.

Moreover, the reviewer's proposed test is a nested special case of our broader approach. As we note in our response, our paternal-specific test (H^0: $\beta_0 = \beta_1 = 0$ vs H_1: $\beta_0 = 0$,$\beta_1 \neq 0$) is a more constrained hypothesis that yields a subset of the SNPs that would be identified by the reviewer's difference test, were it to have sufficient power. Our approach is therefore more conservative for classifying paternal- or maternal-specific eQTLs, not less.

> *The definitions of the 4 groups are based on somewhat ad hoc priors, BF thresholds, etc. Also, in Section 4.6, the value of theta is arbitrarily chosen (along with the threshold of 4 to declare POE). In my opinion, the clean treatment of the 4 groups would start with a significant P-value (beta-maternal vs beta-paternal). Within this set, you can then use the original criteria presented in the paper, but only among these associations where there is solid evidence of different parental effects.*

We take strong issue with the characterization of our prior specifications and thresholds as "ad hoc" or "arbitrary." In Bayesian analysis, prior specification is a principled and transparent modeling choice, not an arbitrary one.

(1) Choice of $\log_{10} BF = 4$ threshold: As stated in Section 2.2, this threshold was chosen based on explicit considerations of prior odds and posterior probability of association. For a prior odds of 1:1000 (a reasonable guess for cis-eQTLs), this BF corresponds to a posterior probability of association of 0.91. If one prefers a more optimistic prior odds of 1:100, the PPA becomes 0.99. The threshold is therefore grounded in decision theory, not whim.

(2) Choice of θ in Section 4.6: We explicitly state that we explored multiple values of θ(0, $\log_{10} 2$, $\log_{10} 3$) and chose $\theta = \log_{10} 2$ because it "produced minimum G_1 and G_0 that contain known imprinted genes." This is a principled, data-driven calibration step using positive controls, not an arbitrary selection. The transparency of this process is a strength, not a weakness.

(3) Comparison to p-value thresholds: The reviewer suggests that p-value thresholds are somehow less arbitrary. However, the conventional p-value threshold of 0.05 is itself a historical convention with no universal justification. Moreover, as we note, p-values do not account for power differences across SNPs. A p-value of 5×10^{-8} from a SNP with 40% MAF is not comparable to the same p-value from a SNP with 1% MAF, because the power to detect the association differs dramatically. Bayes factors automatically adjust for this through the prior, making them more comparable across variants, not less.

In revision, we added a section in supplementary to review relationships between p-values, Bayes factors, and FDR.

> ***Recommendations for the authors:***
>
> ***Reviewer 1 (Recommendations for the authors):***
>
> *Here are some suggestions to improve the study:*
>
> *(1) Provide information about the ancestry background of participants and consider including ancestry principal components in the eQTL models, as is commonly done, to account for population structure.*

We thank the reviewer for this suggestion. In the revised manuscript, we explicitly state that the participants in the Framingham Heart Study are predominantly of European descent, consistent with previous publications from this cohort. Regarding population structure, we respectfully note that our analysis already employs a linear mixed model (Section 4.4) that includes a random effect with a covariance structure defined by the genetic relatedness matrix (GRM). This approach is widely regarded as more robust than including a limited number of principal components, as it accounts for both fine-scale population stratification and known relatedness simultaneously.

> *(2) Conduct sensitivity analyses using different Bayes factor cutoffs to assess the robustness of the findings.*

We appreciate the reviewer's concern about threshold robustness. In fact, we already conducted a form of sensitivity analysis during the classification step. As described in Section 4.6 and shown in Supplementary Table S2, we explored multiple values of θ (0, $\log_{10} 2$, and $\log_{10} 3$) and observed how they affected the composition of our gene sets. The choice of $\log_{10} BF = 4$ for significance was similarly grounded in posterior probability calculations (Section 2.2). To further address the reviewer's point, we add a Supplementary Table S3 for counts of eQTL and eGenes under different Bayes factor threshold. This demonstrates that our most significant claim, the abundance of POE eQTL, are not overly sensitive to the specific cutoff.

> *(3) In the GWAS examples for KCNQ1 and CDKN1C, the assessment of whether the SNPs act as eQTLs for the two genes is based on a single BF threshold, which may be influenced by differences in gene expression levels. The authors could compare the corresponding effect sizes of these SNPs on both genes to provide a more nuanced investigation. While the limitation of missing data from other tissues is discussed in the paper, it remains possible that KCNQ1 plays a role in tissues more relevant to T2D.*

This is an excellent suggestion for a more nuanced investigation. We re-examined the effect sizes for the SNP *rs2237892* in our published results. For gene CDKN1C, the paternal $\log_{10} BF_1 = -0.477$ and maternal $\log_{10} BF_0 = 4.94$, the normalized maternal effect in joint analysis is −4.86 vs −0.74 for paternal. Unfortunately, the published results has no eQTL for KCNQ1, which according to our selection creteria means maximum $\log_{10} BF < 3$ for all tests (genotype, paternal , maternal, joint). The concern for different gene expression level may affect BF is valid. We preempt this pitfall by quantile normalization of gene expression levels after

controlling for GC content (as documented in Method Section). We agree with the reviewer that the lack of data from pancreatic tissues is a limitation. We add a sentence in revelant section to acknowledging that while whole blood is a valuable and accessible tissue, replication in T2D-relevant tissues (e.g., pancreas, adipose) would be an important future direction, and our findings provide a hypothesis for such targeted investigations.

> ***Reviewer 2 (Recommendations for the authors):***
>
> *Major comments:*
>
> *There are some literature elements missing:*
>
> *(1) Hofmeister has a newer and larger study [https://pubmed.ncbi.nlm.nih.gov/40770099/].Please cite that too; it also has POE pQTLs, which is relevant.*
>
> *(2) POE in pigs has been explored [https://www.nature.com/articles/s41467-02562243-6], please cite it.*
>
> *(3) An insightful review covering the mechanisms of POE for gene expression (https://www.sciencedirect.com/science/article/pii/S2352154618300482) should be cited.*
>
> *(4) Further studies on POE in gene expression in social insects (https://royalsocietypublishing.org and in mice (https://www.biorxiv.org/content/10.1101/2023.08.24.554674v1.full) are also relevant.*

We thank the reviewer for bringing these important references to our attention. We incorporated the suggested citations in the revision to provide a more comprehensive context for our work, including the newer POE pQTL study by Hofmeister et al., the findings in pigs, and the mechanistic review.

> *While it's OK to report and rank SNPs by BF, it is necessary to show association P-values as well. It is not explained in the text around the Table how the P-value is obtained in the Table. And it is important to show how their priors translate to FWER control. What is the FWER when picking SNPs at a certain BF value? 1-PPA and local FDR depend on the choice of the prior, but we need a prior-independent measure of FDR/FWER.*

We appreciate the opportunity to clarify. The p-value presented in Table 1 (column "P") is indeed the frequentist p-value testing the null hypothesis of equal maternal and paternal effects ($H_0 : \beta_0 = \beta_1$), as described in Section 4.5. We included this to provide a familiar metric for readers, but our discovery framework relies on Bayes factors for the reasons outlined in Section 2.2.

Regarding error control, the reviewer is correct that 1-PPA is a local FDR that depends on the prior. We chose to control the local rate of false discoveries rather than the Family-Wise Error Rate (FWER) because FWER control (e.g., via Bonferroni) is often excessively conservative for exploratory analyses like eQTL mapping, especially given the correlation among tests due to LD.

Our Bayesian approach provides a more nuanced measure of evidence at the level of each individual test, which is precisely what is needed for prioritizing SNPs with parent-of-origin effects.

The demand for a prior-independent measure of FDR is conceptually problematic. Any probabilistic statement about a specific hypothesis being true or false necessarily requires a prior—this is a fundamental consequence of probability theory. Frequentist FDR, while prior-independent in one sense, does not provide a probability that a particular finding is false; it is

a long-run error rate over many tests. Methods like q-values, often described as "prior-free," still depend on implicit assumptions (e.g., the estimate of π_0, independence of tests, and a mixture of effect sizes).

In our specific context of cis-eQTL analysis, these assumptions are particularly questionable. LD induces correlation among nearby SNPs, violating the independence required for stable π_0 estimation. Moreover, effect sizes in a region are not randomly mixed—SNPs in high LD tend to have similar effect directions and magnitudes, which can bias the mixture model underlying q-value approaches. Our Bayesian approach, by modeling each SNP individually, avoids these cross-SNP assumptions.

Importantly, while posterior probabilities depend on the choice of prior (π_0), we have verified that our conclusions are robust across a wide range of plausible π_0 values (0.9,0.99,0.999). Given our extremely stringent Bayes factor threshold ($BF_j > 10^4$), the posterior probability for a maternal effect exceeds 0.90 for any $\pi_0 < 0.999$. Thus, the prior dependence is practically irrelevant for the SNPs we report.

In revision, we added a section in Supplementary to describe the connections between p-value, Bayes factor, and FDR. We hope this will clarify that a (seemingly) prior independent FDR has a hidden assumption that cis-eQTL analysis is likely to violate.

> *The major problem with the classification of POEs is that simply having significant maternal, but insignificant paternal effect is not an indicator of POE, this happens widely for SNPs with no POE whatsoever (it can happen by chance even when both maternal and paternal effects are the same and non-zero - the authors can see it via simulations under the null [maternal=paternal effect]). In order to be able to talk about POE, first, a significant difference between maternal and paternal effects needs to be claimed. Therefore, none of the 4 sets of POE eQTLs are justified. To me, the only relevant criterion to pick POE SNPs is the P-value when comparing the maternal and paternal effects. The definitions of the 4 groups are based on somewhat ad hoc priors, BF thresholds, etc. Also, in Section 4.6, the value of theta is arbitrarily chosen (along with the threshold of 4 to declare POE). In my opinion, the clean treatment of the 4 groups would start with a significant P-value (beta-maternal vs beta-paternal). Within this set, you can then use the original criteria presented in the paper, but only among these associations where there is solid evidence of different parental effects.*

We respectfully disagree with the reviewer's assertion that a significant difference between maternal and paternal effects is the only valid criterion for defining POE, and we maintain that our classification is statistically sound and biologically meaningful.

The Problem with the "Difference-Only" Approach: The reviewer's proposed filter (a significant p-value for $\beta_0 \neq \beta_1$) is a single hypothesis test. Our goal was to classify eQTLs into multiple, distinct biological categories (paternal-specific, maternal-specific, opposing, etc.). The "difference-only" test collapses these categories. For example, a purely paternal-specific eQTL ($\beta_0 = 0, \beta_1 \neq 0$) and a gene like ZNF890P ($\beta_0 \neq 0$, $\beta_1 \neq 0$, $\beta_0 > \beta_1$) would both show a significant difference. In the reviewer's framework, they would be lumped together, obscuring the fact that one is an imprinted gene and the other is a standard eQTL with allelic imbalance. Our framework correctly separates them.

Bayes Factors are Not "Ad Hoc": The choice of prior ($\sigma = 0.5$) follows established literature for linear model Bayes factors (Servin and Stephens, 2007). The threshold of $\log_{10} BF = 4$ was chosen based on its relationship to posterior probability (0.91-0.99 given reasonable prior odds), which is a transparent and principled decision rule. The selection of θ in Section 4.6 was calibrated using a positive control set of known imprinted genes, ensuring our definitions were conservative and accurate. This is the opposite of arbitrary.

The Suggested Procedure Has Low Power: One can run the following simple R code to verify. We simulate maternal alleles xx and maternal alleles yy, then simulate phenotype with $\beta_{xx} > 0$ and $\beta_{yy} = 0$ (maternal effect only). We fit the joint model and compute p-values for the null $\beta_{xx} = \beta_{yy}$ as suggested by reviewer. From the joint fit, we also extract p-values based on the null $\beta_{xx} = 0$ and $\beta_{yy} = 0$ respectively. The simulation was repeated 1000 times and p-values were stored in a matrix.

```
res=matrix(0,1000,3);
library(car)
for(j in c(1:1000)) {
        af = runif(1,0.05,0.5);
        xx=rbinom(2000,1,af)
        yy=rbinom(2000,1,af)
        b=runif(1,0.1,0.3);
        ph=xx*b+rnorm(2000)
        data=as.data.frame(cbind(ph,xx,yy))
        fit=lm(ph~xx+yy, data=data)
        fit2=linearHypothesis(fit, "xx=yy")
        p2=fit2[2,6];
        px= summary(fit)$coefficients[2,4]
        py= summary(fit)$coefficients[3,4]
        res[j,]=c(p2,px,py);
}
```

We call positives based on suggested procedure, and compare number of positives called using marginal p-values at two threshold of 1×10^{-5} and 1×10^{-6} to declare significance. We used threshold of 0.01 to declare insignificance.

```
> print(cbind(sum(res[,1]<1e-5), sum(res[,2]<1e-5 & res[,3]>0.01)))
     [,1] [,2]
[1,]   94  350
> print(cbind(sum(res[,1]<1e-6), sum(res[,2]<1e-6 & res[,3]>0.01)))
     [,1] [,2]
[1,]   51  253
```

The result demonstrates that the suggested procedure has a much lower power compared to the procedure based on marginal statistics.

> *For the above reasons, the follow-up enrichment analysis is somewhat questionable. Most enrichments are non-significant, and it is likely because the SP and SM groups are diluted with SG SNPs. The P1-P9 groups have nothing to do with POE, and although the observation of increased enrichment for GWAS SNPs with increased pleiotropy is interesting, it is irrelevant for POE.*

We will address the dilution concern below. We agree that P1-P9 groups are not directly related to POE. But this is an interesting observation non-theless. As we found such an observation is missing in the literature, we ask to keep it in the paper.

> *In the same way, section 2.7 is not supported; the claimed maternal and paternal POEs are heavily diluted by simple marginal associations. The same holds for sections 2.82.10. A striking example is Table 3: for clinical trial targets, paternal/maternal eQTLs behave just like simple marginal eQTLs (G_G). A similar pattern emerges for combined target enrichment.*

The reviewer's concern that our S_P and S_M sets are "diluted with S_G SNPs" is precisely the issue our Bayes factor thresholds were designed to prevent. By requiring one effect to be significant and the other to be below a low threshold (θ), we explicitly excluded SNPs where both effects are significant and in the same direction (which defines S_G).

Regarding Table 3, the reviewer's interpretation differs from ours. The fact that paternal eQTLs (GP) show significant enrichment for drug targets, while genotype eQTLs (G_G) also show enrichment, does not imply dilution. Rather, it suggests there is an overlap in the biological importance of these gene sets, which is expected. The key message of the finding is the asymmetry: G_P is significantly more enriched than G_G (p=0.035 for combined targets), a pattern that would be washed out if G_P were merely a diluted version of G_G. This asymmetry supports the interesting biological hypothesis (Moore and Haig, 1991) we discuss. The non-significance for G_M further highlights this asymmetry.

> *I'm not sure how MR would be biased by POE: MR is conducted only if there is a marginal association, i.e., the average maternal and paternal effects are significant. If the expression is causal for a trait, the POE effect is propagated to the outcome; hence, the SNP effect on the exposure will be equally biased as the SNP effect on the outcome, and these cancel out, and the causal effect remains unbiased. Can the authors propose a concrete example of maternal/paternal effects that demonstrates their claimed bias?*

We thank the reviewer for this insightful question, which allows us to clarify our point with a concrete example from our data.

Consider a scenario where one wishes to use Mendelian Randomization (MR) to test whether the expression of gene NECAB3 causally influences a particular trait (e.g., obesity). The reviewer is correct that if the causal effect is homogeneous, the average effect might still be captured. However, the bias we caution against arises in stratified analyses or in the interpretation of the genetic instrument itself.

Take the SNP rs4911348 and its effect on NECAB3 (Figure 2). The genotype model shows no marginal association. Therefore, if a researcher were conducting a standard MR study using this SNP as an instrument for NECAB3 expression, they would discard it as an invalid instrument due to the lack of a marginal association. They would miss the true underlying biology entirely. The causal effect of NECAB3 on the trait would be masked in the full population.

More subtly, even if a SNP has a marginal association, using it as an instrument while ignoring POE can lead to incorrect effect estimates in population subgroups defined by parent of origin. This is analogous to ignoring effect modification. For instance, if a treatment (exposure) has a different effect depending on which parent it came from (which is impossible, but the genetic propensity for the exposure does), failing to account for this can bias the instrumental variable estimate if the instrument's strength varies by an unmeasured factor (parental origin).

Our advice to "check the list of POE SNPs" is a practical caution: if the instrument for an exposure exhibits strong POE, the standard MR assumptions about the homogeneity of the instrument's effect may be violated, potentially leading to biased estimates or incorrect conclusions about causality.

> *Minor comments:*
>
> *(1) In Table 1, the last column header should be -log10(P), not "P".*

The column labelling is an editorial choice to prevent table overflow. This particularly labelling was explained in the caption.

> *(2) While BFg/0/1/j are explained in the text, these notations should be explained in the Table caption as well.*

Added explanation in caption.

> *(3) It should also be mentioned in the Table 1 caption how these top 10 SNPs were chosen.*

These are sentinel eQTL for each gene. We think the first paragraph of Section 2.3 explains clearly.

> *(4) "may "acquires" a cis-eQTL through" → "may "acquire" a cis-eQTL through".*

Corrected. Thank you.

> *(5) "which retained 16, 969 genes out of total 58103", I assume the 58103 are transcripts, not genes.*

You are absolutely correct. We added transcripts after 58103.

> *(6) In Equation (1), Z is not defined. In this concrete setting, isn't it simply the identity matrix?*

Yes. Z is the identitity (loading) matrix for human study. We added a sentence to clarify in revision.

https://doi.org/10.7554/eLife.107920.2.sa0