# Sex differences in inter-individual gene expression variability across human tissues

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**Abstract**

Understanding phenotypic sex differences has long been a goal of biology from both a medical and evolutionary perspective. Although much attention has been paid to mean differences in phenotype between the sexes, little is known about sex differences in phenotypic variability. To gain insight into sex differences in inter-individual variability at the molecular level, we analyzed RNA-seq data from 43 tissues from the Genotype-Tissue Expression project (GTEx). Within each tissue, we identified genes that show sex differences in gene expression variability. We found that these sex-differentially variable (SDV) genes are associated with various important biological functions, including sex hormone response, immune response, and other signaling pathways. By analyzing single-cell RNA sequencing data collected from breast epithelial cells, we found that genes with sex differences in gene expression variability in breast tissue tend to be expressed in a cell-type-specific manner. We looked for an association between SDV expression and Graves' disease, a well-known heavily female-biased disease, and found a significant enrichment of Graves' associated genes among genes with higher variability in females in thyroid tissue. This suggests a possible role for SDV expression in the context of sex-biased disease. We

then examined the evolutionary constraints acting on genes with sex differences in variability and found that they exhibit evidence of increased selective constraint. Through analysis of sex-biased eQTL data, we found evidence that SDV expression may have a genetic basis. Finally, we propose a simple evolutionary model for the emergence of sex-differentially variable expression from sex-specific constraints.

## Introduction

Identifying, characterizing, and understanding phenotypic differences between the sexes has long been a major goal of biology. A very wide variety of traits in humans show significant sex differences including morphological, behavioral, metabolic, immune, and disease traits (Chella Krishnan et al., 2018; Choleris et al., 2018; Klein and Flanagan, 2016; Ober et al., 2008). These trait differences are likely in part due to sex differences in gene expression and regulation (Naqvi et al., 2019; Parsch and Ellegren, 2013; Williams and Carroll, 2009). As a phenotype, gene expression is both relatively straightforward to measure and highly quantitative. As a result, many studies have examined differences in mean gene expression levels between the sexes in many species (Catalán et al., 2012; Gershoni and Pietrokovski, 2017; Khodursky et al., 2020; Lopes-Ramos et al., 2020; Naqvi et al., 2019; Oliva et al., 2020; Parsch and Ellegren, 2013). Sex-biased expression (SBE) is thought to arise from sex-specific genetic architectures which are ultimately established by sex-determination systems initiated during early development (Williams and Carroll, 2009). In addition to underlying sexually dimorphic phenotypes, genes with SBE have been found to possess many interesting evolutionary and genomic properties (Parsch and Ellegren 2013). For example, SBE has been associated with increased rates of evolution at both the coding and expression levels in *Drosophila* and other animals (Khodursky et al., 2020; Meisel et al., 2012; Parsch and Ellegren, 2013; Ranz et al., 2003). In humans, genes with SBE have been associated with reduced selective constraint (Gershoni and Pietrokovski, 2017; Naqvi et al., 2019). Additionally, genes with SBE have been shown to be distributed across the genome in a non-random fashion with respect to the X chromosome – thereby linking the evolution of sex-biased genes to the unique evolutionary forces experienced by the X chromosome (Khodursky et al., 2020; Oliva et al., 2020; Parisi et al., 2003).

Relative to sex differences in phenotypic means, sex differences in phenotypic variabilities have been less thoroughly studied. In the biomedical sciences there has been a long-standing assumption that females must generally be more phenotypically variable due to the menstrual or estrous cycles; however, there has been little empirical evidence to support this assumption (Beery and Zucker, 2011). In fact, a meta-analysis of microarray studies in mice and humans revealed that males may have slightly higher expression variability (Itoh and Arnold, 2015) and a meta-analysis of various traits in mice indicated that sex differences in phenotypic variability are trait dependent (Zajitschek et al., 2020). Despite growing interest in understanding sex differences in phenotypic variability, the biological and evolutionary properties of genes with sex differences in gene expression variability have not, to our knowledge, been explored in humans. Here, we define genes with sex-differentially variable (SDV) expression to be genes which show significant differences in inter-individual gene expression variability between the sexes. From a medical standpoint, genes with SDV expression may underlie diseases for which there are sex differences in prevalence or outcome. For example, one could imagine a disease partially caused by the aberrant expression of a certain gene (akin to the liability threshold model; (Falconer, 1967)). If one sex has more variable expression of the gene in a normal healthy population, this may help explain a higher prevalence of that disease in that sex. Additionally, since more variable expression has been associated with reduced effectiveness of drug targets it is possible that SDV expression may account for sex differences in drug effectiveness, which are known to exist (Simonovsky et al., 2019; Soldin and Mattison, 2009).

Understanding the evolutionary properties of these genes may give us novel insight into the evolution of sex differences. For example, genes with sex differences in expression variability may evolve under sex-specific constraints (e.g., purifying or stabilizing selection) and be of particular functional importance for a given sex. Here we examine the biological properties and evolutionary constraints associated with SDV expression across 43 human tissues.

## Results

**Identification and characterization of genes with sex differences in gene expression variability**

We obtained RNA-seq count data for 43 tissues from the GTEx project (version 8). Since we sought to identify genes with sex differences in gene expression variability, we first compared several statistical methods using power simulations. Specifically, we compared three tests: the Fligner-Killeen test, Levene's test, and a test based on negative binomial generalized additive models for location, scale and shape (GAMLSS) ( Rigby and Stasinopoulos, 2005). Since RNA-seq count data is known to follow an approximately negative binomial distribution (Robinson and Smyth, 2007; Robinson et al., 2010), we performed power simulations by drawing random samples from negative binomial distributions with identical means but different variances by varying the overdispersion term (see methods). At each parameter combination, 1000 pairs of samples were drawn, and tests were performed at an alpha of 0.05. We found that GAMLSS had the highest power across a range of parameters, and this was especially true when testing for differences in variance between samples of unequal size (**Supplementary Figure 1A-D**). Both GAMLSS and Levene's test managed to maintain an adequate false positive rate (FPR) across a range of parameters. Beyond increased power, GAMLSS offers other advantages over the other two tests: GAMLSS negative binomial models allow for the independent parametrization of both the mean and the non-Poisson variation (overdispersion) terms as a function of variables and covariates of interest, including library size (de Jong et al., 2019; Rigby and Stasinopoulos, 2005).

When considering the inter-sample variation of gene expression, it is natural to consider the coefficient of variation: the standard deviation divided by the mean. This provides a mean-normalized statistic for variability. Notably, it is thought that the Poisson component of the coefficient of variation in RNA-seq data is due to technical variation while the overdispersion component of the coefficient of variation contains the true biological variation (McCarthy et al., 2012). The overdispersion parameter fit by GAMLSS can therefore be considered to be the square of the biological coefficient of variation (de Jong et al., 2019; McCarthy et al., 2012). Observed overdispersion values and effect sizes in GTEx data were similar to the simulated values, as determined by models fit using GAMLSS (**Supplementary Figure 1E**).

105 To determine the effect of sex on gene expression variation we fit three negative binomial
106 generalized additive models using the R library 'gamlss' for each expressed gene in each tissue (Rigby
107 and Stasinopoulos, 2005). To reduce the risk of confounding due to unequal sampling across various
108 populations, we limited our analysis to European-American individuals. Significance of the effect of sex
109 on overdispersion and mean expression level was determined through a pair of likelihood-ratio tests (see
110 methods). All models included covariates for age, RNA integrity number (RIN), ischemic time, and the
111 first five genotype principal components (to control for population structure) for both mean and
112 overdispersion terms. We initially considered genes to be sex-differentially variable (SDV) if the effect of
113 sex on overdispersion was significant at FDR<0.05. Since we observed that outliers dramatically
114 influenced estimates of variation in some genes, as a secondary test we performed randomization tests by
115 randomly permuting the sex labels on genes with initially significant coefficients for the effect of sex on
116 overdispersion and refitting the GAMLSS models (see methods) (**Figure 1A**). Coefficients for the effect
117 of sex on overdispersion were saved after each permutation. We used 1000 rounds of permutation to
118 generate empirical null distributions for the effect of sex on overdispersion. Initially significant genes
119 with FDR-adjusted empirical p-values greater than 0.05 in the permutation test were no longer considered
120 to be SDV (see methods). Results from all tested genes are included in **Supplementary Table 1**.
121 Across 43 tissues, we identified 3895 unique genes with SDV expression in at least one tissue out
122 of a total 22054 tested genes (**Figure 1A, B and Supplementary Table 1**). We were able to identify
123 SDV genes in 42 out of the 43 tested tissues (**Figure 1B**). Examples of two genes with SDV expression in
124 the pituitary gland and breast tissue are shown in **Figure 1C**. A total of 2792 unique genes showed higher
125 variability in males in at least one tissue, while 1382 genes showed higher variability in females in at least
126 one tissue. This imbalance between the sexes was largely driven by breast tissue. Surprisingly, 279 genes
127 had higher variability in males in one tissue and higher variability in females in at least one other tissue.
128 The vast majority (3347) of SDV genes only showed SDV expression in one tissue while 548 genes
129 showed SDV expression in multiple tissues.

130 Breast tissue had the largest number of SDV genes with 2049 genes showing significantly higher
131 variation in males and 125 genes showing significantly higher variation in females (**Figure 1B).** Apart
132 from a few tissues**,** SDV genes were not consistently expressed at higher or lower levels than non-SDV
133 genes across tissues (in terms of both CPM and TPM), suggesting that our discovery of SDV genes was
134 not significantly biased towards genes of high or low expression (**Supplementary Figure 2**).
135 Given that differences in variation are generally difficult to detect between groups, we performed
136 a replication analysis using data from breast tissue to help validate our statistical approach. First, we
137 randomly split individuals into discovery and replication sets – 70% of males and females were placed in
138 the discovery set while the rest were placed in the replication set. We identified genes with sex
139 differences in overdispersion in the discovery set using the combined likelihood ratio test and permutation
140 approach described above (both at FDR<0.05). These genes were then tested in the replication set: genes
141 with p-values for the overdispersion term less than 0.05 in both the likelihood ratio test and permutation
142 test were considered 'replicated' if the sex with higher variability in the discovery set was also more
143 variable in the replication set. We observed a replication rate of 21.9% (**Supplementary Figure 3**). This
144 replication rate is limited by reduced power due to the relatively small size of the replication set. To
145 determine the significance of this replication rate we generated an empirical null distribution under the
146 null hypothesis that our statistical approach was simply randomly sampling genes. Therefore, we
147 randomly sampled genes from the discovery set and calculated the replication rate for each sample. The
148 median replication rate under the null hypothesis was found to be 8.4% and out of 10,000 random
149 samples none had a replication rate higher than 11.3% (**Supplementary Figure 3**). This null replication
150 rate should not be considered as a type 1 error rate. Since we are randomly sampling genes from the set of
151 all expressed genes, we are also capturing some true SDV genes in each sample. One would expect the
152 replication rate under random sampling to be higher than 5% even if the FDR is properly controlled at
153 5%.
154 Since our GAMLSS framework allowed us to simultaneously test for sex differences in both
155 mean and overdispersion we examined the relationship between SDV genes and genes with sex-biased

156 expression (SBE). In several tissues a majority of SDV genes also displayed sex-biased expression
157 (**Supplementary Figure 4**). Although a minority of SDV genes in breast tissue, 466 genes with
158 significant sex differences in both mean and overdispersion showed discordance, with higher
159 overdispersion but lower mean expression in one sex versus the other (**Supplementary Figure 4**).
160 Specifically, 387 of those genes simultaneously had significantly higher overdispersion in males and
161 significantly higher mean expression in females.

162 Given that genes with sex-biased expression had been shown to be overrepresented on the X
163 chromosome in a variety of tissues and species (Khodursky et al., 2020; Oliva et al., 2020; Parisi et al.,
164 2003), we wondered if SDV genes were similarly enriched on the X chromosome. However, we found no
165 consistent enrichment of SDV genes on the X chromosome; the enrichment patterns were highly tissue-
166 dependent (**Supplementary Figure 5**).

167
168 To identify biological processes associated with SDV gene expression, we performed an
169 overrepresentation analysis using gene sets obtained from the hallmark gene set collection of MSigDB
170 (Liberzon et al., 2015; Subramanian et al., 2005). The hallmark gene set collection consists of non-
171 redundant and refined gene sets associated with various biological processes and states (Liberzon et al.,
172 2015). The hallmark gene set collection is ideal for our analysis since we identified few SDV genes in
173 most tissues – a larger, more redundant, and less refined collection would introduce more noise while
174 increasing the multiple testing burden for our overrepresentation analysis. We first performed an
175 enrichment analysis at the tissue level and aggregated results across tissues using an empirical approach
176 based on Fisher's combined probability method (**Figure 2A, B, C**) (see methods). Following the meta-
177 analysis, we found that genes with higher variation in males were overrepresented in sets related to
178 estrogen and androgen response, immune response, and cancer-associated pathways (**Figure 2A**). Genes
179 with increased variation in females were similarly overrepresented in sets related to immune response,
180 estrogen response, and cancer-associated pathways (**Figure 2A)**. Notably, there was overlap in multiple
181 sets between the two sexes even though the lists of SDV genes did not show much overlap. At the single-

182 tissue level, genes with higher variation in males in breast tissue were overrepresented in sets related to
183 estrogen response and immune response (**Figure 2B**). This suggests that genes of particular biological
184 importance to one sex (genes related to estrogen response in female breast tissue) may show higher
185 variation in the opposite sex. To identify transcription factors whose targets are overrepresented in the set
186 of SDV genes we downloaded the GTRD dataset from MSigDB (Liberzon et al., 2015; Subramanian et
187 al., 2005). The GTRD collection contains sets of transcription factors and their predicted binding targets
188 (within the promoter region) (Kolmykov et al., 2021). We first performed an enrichment analysis at the
189 individual tissue level, followed by a meta-analysis across tissues using the same modified Fisher's
190 combined probability approach mentioned above (see methods). Following the meta-analysis, we found
191 that genes with higher variability in females were significantly enriched for targets of MAML1 and GLI1
192 (**Figure 2C**). Genes with higher variability in males were significantly enriched for targets of KMT2D.
193 GLI1 is a transcription factor that is part of the Hedgehog signaling pathway, which is involved in
194 differentiation during development. However, in fully differentiated tissues GLI1 can act as an oncogene
195 has been implicated in a wide variety of cancers, including glioblastoma, breast cancer, and colon cancer
196 (Avery et al., 2021; Clement et al., 2007; Sun et al., 2014). MAML1 is a transcription factor involved in
197 Notch signaling and is a transcriptional co-activator of Notch receptors (Wu et al., 2000). Additionally,
198 MAML1 has been shown to interact cooperatively with GLI1 to regulate hedgehog signaling (Quaranta et
199 al., 2017). KMT2D is a broadly expressed histone methyltransferase that is believed to function as a
200 tumor suppressor and is frequently mutated in a wide variety of cancers (Rao and Dou, 2015).
201
202 **Single-cell RNA-seq data from breast epithelial cells shows that genes with SDV expression tend to**
203 **be expressed in a cell type-specific manner**
204 To get further biological insight into genes with SDV expression in breast tissue, which had the most
205 SDV genes identified out of any tissue by far, we analyzed a previously published single cell RNA-seq
206 dataset (Nguyen et al., 2018). With count data from individual 4 (female), we applied a standard single
207 cell RNA-seq pipeline using the 'R' library 'Seurat' (Hao et al., 2020)(see methods). Our analysis

208 recovered the three major cell types found in the breast epithelium: type 1 and type 2 luminal cells along
209 with basal cells (Nguyen et al., 2018)(**Supplementary Figure 6**). To determine the cell type specificity of
210 each gene we combined raw count data from each cell to generate a pseudo-bulk count matrix for each
211 cell type and calculated CPM values for each gene. We then applied a standard measure of tissue
212 specificity, $\tau$, to the log-transformed CPM values across the three cell types (see methods).
213 We found that genes exhibiting SDV expression were expressed in a significantly more cell-type-
214 specific manner than non-SDV genes (p=1.9e-41, Mann-Whitney U test)(**Figure 2D**). If there exist
215 significant sex differences in inter-individual cell-type composition variation, such cell-type-specific
216 expression could lead to sex differences in expression variability in bulk tissue samples. Overall, these
217 results suggest that SDV expression may be partly due to sex differences in inter-individual cell-type
218 composition variation.
219
220 **SDV genes in the thyroid with higher variability in females are associated with Graves' disease**
221 Given the preponderance of sex-biased diseases (Mauvais-Jarvis et al., 2020), we wondered if genes with
222 SDV expression could be associated with a classically sex-biased disease. Hence, we decided to examine
223 Graves' disease: a common autoimmune disease of the thyroid which results in hyperthyroidism (McIver
224 and Morris, 1998). The incidence of Graves' disease is almost ten-fold higher in females than in males
225 (McIver and Morris, 1998). We obtained GWAS summary data from the NHGRI-EBI GWAS catalog
226 compiled from 5 studies on the genetics of Graves' disease in various populations (Buniello et al., 2019;
227 Chu et al., 2011; Cooper et al., 2012; Nakabayashi et al., 2011; Sakaue et al., 2021; Zhao et al., 2013). Of
228 the 36 genes implicated in Graves' disease which were expressed in thyroid tissue, 6 displayed SDV
229 expression with higher variation in females. This corresponds to a significant 9-fold enrichment of
230 Graves' associated genes among SDV genes with higher variability in females (p=3.5e-5, hypergeometric
231 test) (Figure 2E). Although many of the genes implicated in Graves' disease are expressed in immune
232 cells, a characteristic of Graves' disease is the infiltration of immune cells into the thyroid (Dayan et al.,

233 1991; McIver and Morris, 1998; Totterman et al., 1979). Overall, this analysis suggests a role for SDV
234 expression in sex-biased diseases.
235
236 **Genes with sex-differentially variable expression show evidence of increased selective constraint**
237 **acting on gene expression and sequence**
238 Given that genes with sex-biased expression (SBE) have been shown to be associated with
239 relaxed selective constraint at both the sequence and expression levels, we asked whether SDV genes
240 would similarly be associated with reduced constraint (Gershoni and Pietrokovski, 2017; Naqvi et al.,
241 2019). Stabilizing selection acting on gene expression is thought to reduce *cis*-regulatory variation and
242 reduce the minor allele frequency (MAF) of *cis*-regulatory variants (Glassberg et al., 2019; Josephs et al.,
243 2015). Therefore, if SDV expression is associated with reduced selective constraint, one would expect an
244 enrichment of *cis*-regulatory variation in SDV genes. To determine whether SDV genes were enriched or
245 depleted for *cis*-regulatory variation, we obtained expression quantitative trait loci (eQTL) summary data
246 for each tissue from GTEx (v8). For each tissue with at least 20 autosomal SDV genes, we tested for
247 enrichment of genes with significant eQTLs (eGenes, q-value<0.1) within that tissue's set of SDV genes.
248 Only genes that were tested in both studies in a given tissue were considered. Contrary to expectation, we
249 observed a depletion of eGenes within the set of SDV genes in most tissues, and this depletion was
250 significant in three tissues: sun-exposed skin, breast tissue, and pituitary glands (**Figure 3A**). We also
251 tested whether the median fold enrichment of eGenes across the 20 tested tissues (median fold enrichment
252 = 0.93) was lower than expected by chance. To this end, we randomly permuted the SDV labels in each
253 tissue (genes were either SDV or not SDV in each tissue) and calculated a median fold enrichment across
254 all tissues. In total 10,000 permutations were performed and for each round of permutation, the median
255 fold enrichment of eGenes in the set of SDV genes was recorded. We found that the observed median fold
256 enrichment of eGenes among SDV genes was significantly lower than expected by chance suggesting that
257 expression levels of SDV genes are evolving under elevated constraint (p=1.3e-03, permutation test)
258 (**Figure 3B).**

259 We examined if the observed depletion of *cis*-regulatory variation in SDV genes in breast tissue
260 could be explained by their elevated cell-type specificity. For example, one could imagine that most
261 expression variation (across bulk samples) in cell-type specific genes is driven by sample-to-sample
262 differences in cell-type composition and not by *cis*-regulatory genetic factors. Therefore, one might
263 expect to observe a depletion of eQTLs in cell-type specific genes. Upon examination, we found no
264 significant relationship between cell-type specificity and the presence or absence of a significant eQTL
265 (p=0.99, Mann-Whitney *U* test)(**Supplementary Figure 7**).
266 Although our analysis of eQTL data provided some evidence that expression levels of SDV genes
267 are evolving under increased constraint, a depletion of *cis*-regulatory variation might also be expected if
268 the gene expression variation observed in SDV genes was due to environmental factors and/or *trans*-
269 regulatory genetic variation to a larger extent than in non-SDV genes. We therefore sought to quantify
270 selective constraint using a complementary—and more direct—approach. To this end, we used 20-species
271 phastCons scores to look for further evidence that the *cis*-regulatory regions of SDV genes were
272 evolutionary highly constrained (Siepel et al., 2005). In particular, we examined the sequence
273 conservation in the proximal promoter regions of genes –100 bp upstream from the transcription start
274 sites (TSSs). The phastCons score of a nucleotide can be interpreted as the probability that the nucleotide
275 resides in a conserved element (Siepel et al., 2005). We found that genes with SDV expression in at least
276 one tissue showed significantly elevated phastCons scores compared to genes which did not exhibit SDV
277 expression in any tissues (p=4.1e-05, Mann-Whitney U test) (**Figure 3C**). Only genes expressed in all
278 tissues with at least 20 SDV genes were considered to remove bias, i.e., a gene is more likely to be SDV
279 if it's expressed in more tissues and genes expressed in many tissues tend to be more evolutionarily
280 constrained (Karczewski et al., 2020) .
281 Similarly, we also examined the LINSIGHT scores of SDV genes. LINSIGHT scores combine
282 information from intraspecific genetic polymorphism, interspecific genetic divergence, and functional
283 genomic data to provide a probability that a mutation at a given base-pair will have deleterious fitness
284 consequences (Huang et al., 2017). We found that genes with SDV expression in at least one tissue

285 showed significantly higher mean LINSIGHT scores within their proximal promoter regions than genes
286 without SDV expression (p=1.8e-02, Mann-Whitney U test).
287 Finally, we asked whether the increased constraint acting on the expression levels of SDV genes
288 would be reflected in other metrics of dosage sensitivity. To do this, we compared LOEUF scores in SDV
289 genes and non SDV genes. LOEUF (loss-of-function observed/expected upper bound fraction) is a score
290 that reflects the tolerance of a protein to loss-of-function mutations (Karczewski et al., 2020). The scores
291 range from 0 to 2, with higher scores indicating higher tolerance to loss-of-function mutations and
292 therefore reduced selective constraint. We only considered genes expressed in all tissues with at least 20
293 SDV genes and found that genes with SDV in at least one tissue had significantly reduced LOEUF scores
294 compared to genes without SDV expression in any tissue (p=4.2e-10, Mann-Whitney U test) (**Figure 3E**),
295 corroborating that SDV genes tend to be more constrained at expression level.
296
297 **SDV genes in breast tissue show evidence of sex-specific genetic regulation**
298 To determine if SDV genes showed evidence of sex-specific genetic regulation, we downloaded sex-
299 biased eQTL (sbQTL) data from GTEx (Oliva et al., 2020). We restricted our analysis to breast tissue
300 given that it was found to have both the largest number of SDV genes (in our study) and by far the largest
301 number of sbQTLs of any tissue (Oliva et al., 2020). To test if genes with sbQTLs were enriched for
302 SDV genes we performed a gene set enrichment analysis (GSEA). By only considering genes tested in
303 both analyses and ranking genes according to their corrected -$\log_{10}$(p-values) in the sbQTL analysis we
304 found that SDV genes were significantly enriched at lower p-values (p=1.2e-2, permutation test)
305 (**Supplementary Figure 8**). Additionally, we found that all 13 SDV genes with higher variation in males
306 and significant sbQTLs (FDR<0.25, the threshold used in Oliva et al., 2020) had eQTLs with larger effect
307 sizes in males than females. This represents a significant enrichment of genes with larger eQTL effect
308 sizes in males relative to the set of all genes with significant sbQTLs (p=7.4e-4, hypergeometric test).
309 These results indicate that SDV genes are enriched for sex-specific genetic architectures regulating gene
310 expression and suggest that SDV expression may have an underlying genetic component.

311 **Discussion**

312 In this work, we examined a largely unappreciated aspect of transcriptomic sex differences: genes with
313 sex differences in inter-individual expression variation. In particular, we focused on genes that showed
314 sex differences in inter-individual expression overdispersion. We termed these genes sex-differentially
315 variable (SDV) genes. Across 43 tested tissues, we found that roughly 18% of expressed genes showed
316 SDV patterns of expression in at least one tissue (**Figure 1B**). Our results were undoubtedly affected by
317 the unequal number of samples in different tissues – we had more power to detect SDV genes in some
318 tissues than others. Nevertheless, we identified more SDV genes in breast tissue than in any other tissue.
319 Issues of sample size aside, from a physiological perspective this seems logical since the breast is the only
320 tissue tested known to have functional significance in only one sex: the production of milk is essentially
321 female-specific. Other studies have found evidence that low inter-individual transcriptional variation in a
322 gene is indicative of increased constraint acting on the gene (Fair et al., 2020). Since the vast majority
323 (94%) of SDV genes identified in breast tissue showed higher expression variability in males than in
324 females, this is consistent with the idea that expression levels should be more functionally constrained in
325 female breast tissue than in male breast tissue.

326 To gain biological insight into the nature of SDV genes we performed a gene set
327 overrepresentation analysis followed by meta-analysis. We found that SDV genes were enriched for genes
328 from sets related to sex hormone response, immune response, and various signaling and cancer-associated
329 pathways (**Figure 2A, B**). Additionally, we found that SDV genes were enriched for the targets of three
330 transcription factors implicated in cancer (**Figure 2C**). The overrepresentation of SDV genes in gene sets
331 related to various biological pathways and transcription factors indicates that SDV expression may be
332 caused by SDV activity at the pathway level. In breast tissue, the overrepresentation of genes with high
333 variability in males in sets related to estrogen response suggests that genes of particular biological
334 importance to one sex may show higher variation in the opposite sex.

335 In order to further examine the biological significance of SDV genes in breast tissue we analyzed
336 previously published single-cell RNA sequencing data of breast epithelial tissue. We found that SDV

genes (identified in the GTEx data) were expressed in a more cell-type specific manner (**Figure 2D**). These results indicate that SDV genes may be more likely to be involved in cell-type specific functions. Additionally, this suggests that sex differences in gene expression variability may be partially driven by sex differences in cellular composition variability across individuals.

To examine a possible link between SDV expression and disease, we analyzed summary GWAS data from a classically female-biased disease: Graves' disease. We found a significant enrichment of Graves' associated genes in the set of SDV genes with higher variability in females in healthy thyroid tissue (**Figure 2E**). In the future, the role of SDV expression in sex-biased diseases should be thoroughly investigated by examining expression in both healthy and diseased tissue.

We found that SDV genes tended to be depleted for *cis*-regulatory variation overall (**Figure 3A. B**). Additionally, we showed that SDV genes show significantly increased constraint in both proximal promoter and protein-coding sequences. These results are consistent with increased constraint acting on the expression levels of SDV genes and suggest that SDV genes may be evolving under increased sex-specific constraint acting on gene expression.

We propose that SDV expression can arise through sex-specific increases in stabilizing selection acting on gene expression. To test this, we performed a forward Monte Carlo simulation of the evolution of a gene evolving under sex-specific stabilizing selection (**Figure 4**). Our model assumes that the expression depends on the additive contributions of a shared genetic locus (this locus contributes equally to both sexes), two sex-specific loci (each locus only contributes to expression in one sex), and noise (to model environmental effects) (see methods). Therefore, an assumption of the model is some degree of sex-specific genetic architecture for SDV genes, which is supported by empirical evidence (**Supplementary Figure 8**). The three loci were in linkage equilibrium. Increased stabilizing selection in one sex results in a difference in inter-individual phenotypic variances between the two sexes – with the sex experiencing increased selection having relatively reduced variation (**Figure 4A, B**). Additionally, increased selection in one sex results in reduced genetic variation in the set of shared genetic loci – consistent with our observations of reduced *cis*-regulatory variation in SDV genes (**Figure 4C**). These

results are qualitatively consistent with predictions from quantitative genetics theory. Within a single generation, stronger stabilizing selection in one sex – for example, females – would lead to reduced additive genetic variance in females compared to males. Assuming that phenotypic variance is the sum of additive genetic and environmental variances, and that environmental variances are the same in both sexes, it implies that phenotypic variance will be lower in females than in males. Additionally, increased stabilizing selection in females will decrease the additive genetic covariance between the sexes to a larger extent than identical stabilizing selection in both sexes. This corresponds to our observation of reduction in the variance of the shared genetic locus under conditions of increased selection in females (**Figure 4C**). A more detailed description of the theoretical predictions is presented in the **Appendix**.

Although sex-specific selection resulting in decreased expression variance in the sex with increased selection is one possible explanation for SDV expression, it is not the only possibility. Alternatively, SDV expression, in particular genes with higher variability in females, could be a result of hormonal fluctuations as part of the menstrual cycle. The overrepresentation of estrogen response genes among female SDV genes supports this as a possible explanation (**Figure 2A**). These genes may also evolve under increased selective constraint in females. Both explanations for SDV expression suggest sex-specific or sex-biased functions for SDV genes, which could help explain the observation that a majority of SDV genes also exhibit SBE expression in a few tissues (**Supplementary Figure 4**).

Overall, our results suggest that genes with sex differences in inter-individual gene expression variability are possibly involved in sex-specific biological functions and may be evolving under sex-specific constraint acting on gene expression. These results highlight the possible importance of SDV genes in a functional and evolutionary sense, but future work needs to be done to properly understand the role of SDV genes in human health.

## Methods

### Power Simulations

Random samples were generated from negative binomial distributions using the 'R' function 'rnegbin' from the library 'MASS'. To simulate the effect of 'sex' on the overdispersion parameter, samples were drawn from two negative binomial distributions with equal mean ($\mu$) parameters and different overdispersion ($\sigma$, or equivalently $\frac{1}{\theta}$ in the 'MASS' documentation) parameters. Similarly, to determine false positive rates samples were drawn from identical distributions. To restrict the search space, $\mu$ was fixed at 1500 for all simulations and the size of both samples combined was fixed at 400. These parameters were typical of the gene-expression levels and sample sizes found in GTEx data. Moreover, varying the mean parameter only marginally affected power calculations. For the unbalanced group sizes simulations, the size of one group was 320 while the size of the other was 80. Both the baseline overdispersion parameter ($\sigma$) and the difference in overdispersion between groups ($\Delta\sigma$) were varied over the course of the simulation experiments. The variances of the two samples were compared using the Fligner-Killeen test ('fk.test' function from the 'R' library 'npsm'), Levene's test ('levene.test' function from the 'R' library 'lawstat'), and a likelihood-ratio test implemented using negative binomial GAMLSS models. The more complex GAMLSS model parametrized the mean ($\mu$) and the overdispersion ($\sigma$) parameters as functions of sex, $\sigma \sim sex$ and $\mu \sim sex$, while the simplified model parametrized only the mean in terms of sex with the overdispersion being modeled as constant. The p-value for the significance of the sex overdispersion term was saved. At each parameter combination 1000 sample pairs were drawn and p-values from each of the three tests were saved. Power was calculated as the fraction of 1000 simulations where the p-value fell below an alpha of 0.05. False positive rate was calculated as the faction of 1000 simulations where the p-value fell below and alpha of 0.05 when the two underlying distributions being sampled for a sample pair were identical. It was observed that power for Levene's test markedly improved when log-transforming the sampled count data, while the Fligner-Killeen test showed largely unaltered power: hence samples were transformed as ln(counts + 1) before testing with Levene's test and the Fligner-Killeen test. Raw samples were used for the GAMLSS likelihood-ratio test.

413 **Identification of SDV genes**

414 For each tissue, SDV genes were identified separately using RNA-seq count data downloaded from
415 GTEx-v8 (dbGAP accession number # phs000424.v8.p2). To reduce confounding due to population
416 structure, only European Americans were considered. First, counts per million (CPM) values were
417 calculated using edgeR and genes with no expression (CPM=0) in at least one individual were removed.
418 To identify individuals that were global expression outliers, dimensionality reduction was then performed
419 on $\log_2$ transformed CPM values using principal component analysis (PCA) with the 'R' function
420 'prcomp'. Since outliers can greatly affect estimates of variation, we removed individuals whose global
421 expression profiles deviated significantly from other individuals in a given tissue. Individuals whose
422 global expression patterns were more than 1.5 interquartile ranges higher than the third quartile or lower
423 than the first quartile along any principal component explaining more than 5% of the total variance in the
424 dataset were considered outliers and removed from further analysis.

425 Following global outlier removal three negative binomial models were fit to the count data (one
426 gene at a time) using gamlss. The first and most complex model (model 1) parametrized the mean and
427 non-Poisson variation terms of the negative binomial distribution ( $\mu$ and $\sigma$, respectively) as $\mu \sim sex +$
428 $age + ischemic\ time + RIN + PC1 + PC2 + PC3 + PC4 + PC5 + \ offset(offset)$ and $\sigma \sim sex +$
429 $age + ischemic\ time + RIN + PC1 + PC2 + PC3 + PC4 + PC5$ , where $RIN$ represents RNA integrity
430 number and the 5 PCs represent genotype principal components obtained from GTEx . The offset term, to
431 account for library size was calculated as $\log(library\ size\ * normalization\ factors)$ , where the
432 normalization factors were calculated using the 'calcNormFactors' function from edgeR using the
433 'upperquartile' method. Model 2 had the same form as model 1 for $\mu$ , however for the formula for the
434 non-Poisson variation was $\sigma \sim age + ischemic\ time + RIN + 5PCs$. Model 3 had the same formula as
435 model 1 for $\sigma$ however the formula for the mean was $\mu \sim age + ischemic\ time + RIN + 5PCs +$
436 $offset(offset)$ . To test for the significance of the effect of sex on non-Poisson variation ($p_{\sigma,sex}$ ) a
437 likelihood ratio test was performed comparing model 1 and model 2. To test for the significance of the

438 effect of sex on mean expression ($p_{\mu,sex}$ ) a likelihood ratio test was performed comparing model 1 and
439 model 3. The coefficients corresponding to the sex terms for both $\mu$ and $\sigma$ ($\beta_{\mu,sex}$ and $\beta_{\sigma,sex}$ ) from model
440 1 were recorded for each gene. After every gene was tested in a given tissue p-values for $\mu$ and $\sigma$ were
441 corrected independently using the Benjamini-Hochberg (BH) procedure using the 'R' function 'p.adjust'.
442 Unsurprisingly we noticed that individual expression outliers at the gene level dramatically
443 affected our estimates of $p_{\sigma,sex}$ and $\beta_{\sigma,sex}$. To account for this and to generally validate the robustness of
444 our models we performed randomization tests for all genes with a BH corrected $p_{\sigma,sex}$ <0.05. For each
445 gene the sex labels were randomly permuted, model 1 was fit to the randomized data, and $\beta_{\sigma,sex}$ values
446 were recorded. We performed 1000 permutations for every gene to generate an empirical null distribution
447 for $\beta_{\sigma,sex}$. An empirical p-value was calculated as the fraction of random permutations where
448 $abs(\beta_{\sigma,sex,perm}) > abs(\beta_{\sigma,sex,obs})$. These empirical p-values were then corrected using the BH
449 procedure. In the end genes were considered SDV if the original BH corrected p-values ($p_{\sigma,sex}$) were less
450 than 0.05 and the BH corrected empirical p-values were less than 0.05.
451
452 **Hallmark enrichment analysis**
453 Gene set annotations for collection 'H' (hallmark gene sets) were downloaded from MSigDB using the
454 'R' function 'msigdbr' from the package 'msigdbr'. Only tissues with at least 20 male or female high
455 variability genes were considered. Enrichment of gene sets for male high variability and female high
456 variability genes was calculated separately. Enrichment p-values for each gene set in each tissue were
457 calculated using a hypergeometric test (using the 'R' function 'phyper'). For each gene set a meta-
458 analysis was performed across all tested tissues using a variation of Fisher's method. This approach was
459 implemented as follows. For each gene set a test statistic was calculated by taking -2 times the sum of the
460 log p-values in each tested tissue. In an ordinary implementation of Fisher's method this test statistic
461 would have a chi-squared distribution under the null hypothesis. However, given that the underlying p-
462 values are based on a discrete test (the hypergeometric test) a core assumption of Fisher's method is

463 violated: namely that for an individual tissue p-values should be uniformly distributed under the null
464 hypothesis. To overcome this, we generated empirical null distributions for the test statistic described
465 above using a permutation approach: within each tissue we permuted the labels of each gene (not SDV,
466 female high variability, and male high variability) and calculated a p-value for the label of interest
467 (female high variability or male high variability) using a hypergeometric test. This permutation procedure
468 was done for every tissue tested – finally resulting in a null value for the sum of log p-values test statistic
469 described above. For each gene set 10,000 such permutations were performed to generate an empirical
470 null distribution for the test statistic. The empirical p-values for each gene set were calculated by finding
471 the fraction of permutations resulting in a test statistic larger than the one observed. These combined p-
472 values across gene sets were corrected using the BH procedure and gene sets with corrected p-
473 values<0.05 were considered significant. Gene sets that were highly tissue/developmentally specific were
474 not tested in the meta-analysis. The 7 removed hallmark gene sets were pancreas beta cells, adipogenesis,
475 myogenesis, spermatogenesis, bile acid metabolism, heme metabolism, and xenobiotic metabolism.
476
477 **Transcription factor enrichment analysis**
478 The analysis was performed in a fashion identical to the hallmark enrichment analysis except that the
479 gene sets from collection 'C3' subcollection 'GTRD' were used.
480
481 **Single-cell RNA sequencing analysis**
482 The count matrix for individual 4 was downloaded from GSE113197 (Nguyen et al., 2018). All
483 clustering and marker identification analysis was performed using the 'R' library 'Seurat' version 3.2.3.
484 Cells which had fewer than 500 and more than 6000 expressed features were removed. Cells with more
485 than 10% of their transcripts coming from mitochondrial genes were removed. The counts were
486 normalized using the function 'SCTransform'. Both dimensionality reduction (using the function
487 'RunUMAP') and clustering (function: 'FindClusters') were performed using the first 10 principal
488 components of the normalized counts. L1, L2, and basal cells were identified based on their expression of

489 markers “KRT18”, “SPLI”, and “KRT14”. Clusters expressing “KRT18” and “SPLI” were deemed to be
490 L1 cells, clusters only expressing “KRT18” were deemed to be L2 cells, and clusters expressing
491 “KRT14” were taken to be basal cells.

492 To calculate the cell-type specificity of each gene raw counts were aggregated within each of the
493 three cell types, generated 3 pseudo-bulk count vectors (one for each cell type). For each cell-type CPMs
494 were calculated by multiplying the pseudo-bulk count vector by 1 million and dividing by the sum of the
495 vector. The CPM values were then $\log_2$ transformed. Cell-type specificity was calculated using the
496 standard metric for tissue specificity $\tau = \frac{sum(1 - x_{hat})}{length(x_{hat}) - 1}$, where $x_{hat}$ is the vector representing a given
497 gene’s expression across the 3 cell-types divided by the maximum expression of that gene in any of the
498 three cell-types.
499

500 **Graves’ disease analysis**

501 Summary GWAS statistics were obtained from the NHGRI-EBI GWAS catalog for study EFO_0004237,
502 downloaded on 08/04/2022 (Buniello et al., 2019).
503

504 **eQTL analysis**

505 Summary statistics for single-tissue eQTL mapping performed in European-American individuals were
506 downloaded from the GTEx-v8 website. Genes with at least one eQTL with q-value<0.1 were considered
507 to be eGenes. A two-sided Fisher’s exact test, as implemented in the ‘fisher.test’ function, was used to
508 test for the enrichment/depletion of eGenes among SDV genes in each tissue. Only tissues with at least 20
509 autosomal SDV genes were considered. Only autosomal genes with expression high enough to be tested
510 in both the original eQTL study and our study were considered. To test if the median fold enrichment of
511 eGenes across tissues was significant we randomly permuted the SDV labels in each tissue and calculated
512 a median fold enrichment across all tissues. In total 10,000 permutations were performed and for each
513 round of permutation the median fold enrichment of eGenes across the 20 tissues was recorded. The

514 empirical p-value was calculated as the fraction of all permutations where the fold enrichment was at least
515 as low as the observed fold enrichment.
516
517 **PhastCons analysis**
518 Genome-wide phastCons scores (in hg38 coordinates) generated from a 20 species alignment were
519 downloaded from http://hgdownload.cse.ucsc.edu/goldenPath/hg38/phastCons20way/. Transcription start
520 sites (TSS) for all annotated genes were downloaded from BioMart using the 'R' package biomaRt
521 (Durinck et al., 2005, 2009). The most distal TSS was used for each gene. Only genes expressed in all
522 tissues containing at least 20 SDV genes were tested (20 tissues overall). A gene was considered 'SDV' if
523 it displayed 'SDV' expression in at least 1 of the 20 tested tissues, otherwise it was considered to be 'not
524 SDV'. Requiring genes to be expressed in all 20 tissues was done to remove bias, i.e., a gene is more
525 likely to be SDV if it's expressed in more tissues and genes expressed in many tissues tend to be more
526 evolutionarily constrained (Karczewski et al., 2020). Further increasing the number of tissues (by
527 decreasing the minimum number of SDV genes allowed in each tissue) reduces power by decreasing the
528 intersection of genes expressed across all tissues. Mean phastCons scores were obtained for each gene in
529 a region 100 bp upstream from their TSSs.
530
531 **LINSIGHT analysis**
532 Data was downloaded from the Siepel Laboratory website: https://siepellab.labsites.cshl.edu/software/ .
533 The data was processed and analyzed in the same manner as the phastCons data following conversion to
534 hg38 coordinates.
535
536 **Loss of function analysis**
537 Loss of function metrics were downloaded from https://gnomad.broadinstitute.org/downloads . In
538 particular the v2.1.1 loss of function metrics by gene were used. The analysis was similar to the analysis

539 for PhastCons score: only genes expressed across all tissues with at least 20 SDV genes were considered.
540 LOEUF scores were compared between 'SDV' and 'not SDV' genes.
541
542 **Sex-specific stabilizing selection simulation**
543 The simulation assumed a diploid, diecious, and randomly mating population of 500 individuals. In each
544 individual, the phenotype (expression) was modelled as the additive contribution of 3 genetic loci and
545 environmental noise. One of the genetic loci had equal effect in both sexes while each of the two other
546 genetic loci only had effect in one sex or the other. The allelic effects of each genetic locus in each
547 individual were initialized as draws from a standard normal distribution (mean=0 and standard
548 deviation=1). The environmental component was also taken to be a random number drawn from a
549 standard normal distribution. Similarly, mutation resulted in the addition of a number drawn from a
550 standard normal distribution to the allelic effect and the mutation rate was set to 5e-3 per generation at
551 each genetic locus in each individual. The fitness of each individual was taken to be $w = e^{-s(G+E)^2}$,
552 where '$s$' represented the strength of selection, '$G$' was the total sum of genetic allelic effects (from the
553 shared and sex-specific locus), and '$E$' was the environmental effect. For the simulations with equal
554 selection in both sexes '$s$' was 0.005, while in simulation with sex-specific selection '$s$' was 0.005 in
555 males and 0.05 in females. Selection only affected viability and had no impact on fecundity. An
556 individual survived to reproduce if a random draw from a uniform distribution on [0, 1] was less
557 than or equal to the individual's fitness; therefore, the fitness represents the probability of an
558 individual surviving to reproduce. In each generation 100 males and 100 females were chosen and
559 paired at random with replacement. Each pair produced 5 offspring and the sex of each offspring was
560 determined randomly. The simulations were run for a total of 2000 generations. Every 10 generations the
561 variance of the phenotype (across individuals in the population) was recorded along with the variance of
562 the allelic effect of the shared genetic component. For both the equal selection and sex-specific selection
563 models 30 independent simulations were run.

564

565 **GSEA analysis of sbQTL data**

566 Sex-biased QTL (sbQTL) data from breast tissue was downloaded from GTEx v8. Genes were only
567 considered if they were tested in our analysis and in the sbQTL analysis. Genes were ranked according to
568 corrected -$\log_{10}$(p-values) where the (Bonferroni) correction was made to account for multiple SNPs
569 tested per gene. GSEA analysis was performed using the 'R' function 'fgsea' from the library 'fgsea'
570 (Korotkevich et al., 2021).

571

572 **Appendix**

573 **Theory behind sex-specific stabilizing selection**

574 The evolution of a single quantitative trait in males and females can be predicted from the 2-by-2 additive
575 genetic variance-covariance matrix:

576 $$\boldsymbol{G} = \begin{bmatrix} G_m & B \\ B & G_f \end{bmatrix}. \qquad (1)$$

577 Where the diagonal terms represent the additive genetic variances in males and females, while the
578 identical off-diagonal terms represent the additive genetic covariance between the sexes (Barker et al.,
579 2010; Lande, 1980). In the absence of directional selection, the change in the genetic variance-covariance
580 matrix due to stabilizing selection over the course of a generation is (Barker et al., 2010; Phillips and
581 Arnold, 1989):

582 $$\Delta\boldsymbol{G} = \boldsymbol{G\gamma G}. \qquad (2)$$

583 Where $\boldsymbol{\gamma}$ is a matrix describing the strength of stabilizing selection within each sex:

584 $$\boldsymbol{\gamma} = \begin{bmatrix} \gamma_m & 0 \\ 0 & \gamma_f \end{bmatrix}. \qquad (3)$$

585 For stabilizing selection to occur the diagonal terms must be negative with decreasing values indicating
586 stronger stabilizing selection. Substituting (3) and (1) into (2) yields:

587 $$\Delta\boldsymbol{G} = \begin{bmatrix} \Delta G_m & \Delta B \\ \Delta B & \Delta G_f \end{bmatrix} = \begin{bmatrix} \gamma_m G_m^2 + \gamma_f B^2 & B(\gamma_m G_m + \gamma_f G_f) \\ B(\gamma_m G_m + \gamma_f G_f) & \gamma_f G_f^2 + \gamma_m B^2 \end{bmatrix}. \qquad (4)$$

By assuming that initially the trait has identical genetic variance in males and females (which is assumed at the start of our simulation): we can use the simplification $G_m = G_f = G$. This leads to the following equations for $\Delta G_m$ and $\Delta G_f$ :

$$\Delta G_m = \gamma_m G^2 + \gamma_f B^2 \qquad (5a)$$

$$\Delta G_f = \gamma_f G^2 + \gamma_m B^2 \ . \qquad (5b)$$

In the case of stronger stabilizing selection in females: $\gamma_f < \gamma_m < 0$. Additionally, theoretical and empirical evidence suggests that generally the magnitude of intra-sex genetic variance is larger than the magnitude of inter-sex genetic covariance: $G > B$ (Barker et al., 2010). Therefore, $(5a, b)$ yields: $\Delta G_f < \Delta G_m < 0$. Following the assumptions made in our simulation (namely that the phenotypic variance in each sex is just the sum of the environmental variances and the additive genetic variances ($G$), and that the environmental variance is the same in both sexes) leads to the result that phenotypic variance will decrease to a larger extent in females than in males over the course of a generation. This is what we observed (however, over multiple generations) in our simulation (**Figure 4A, B**).

To further relate our simulation results to the theory described above we can formulate the phenotypes and their variances from the simulation as follows:

$$P_m = g_1 + g_2 + \varepsilon \qquad (6a)$$

$$P_f = g_1 + g_3 + \varepsilon \ . \qquad (6b)$$

$P_m$ and $P_f$ are the phenotypes in males and females respectively. $g_1$, $g_2$, and $g_3$ are the genetic contributions of the shared genetic locus, the male specific genetic locus, and the female specific genetic locus. The environmental contribution to the phenotypes is $\varepsilon$ . From $(6a, b)$, since all the phenotypic components are independent (so their covariances are 0), the phenotypic variances are:

$$Var(P_m) = Var(g_1) + Var(g_2) + Var(\varepsilon) \qquad (7a)$$

$$Var(P_f) = Var(g_1) + Var(g_3) + Var(\varepsilon) \qquad (7b)$$

613 and the phenotypic covariances are:

614 $$Cov(P_m, P_f) = Var(g_1) + Var(\varepsilon) . \qquad (7c)$$

615

616 From $(7a, b, c)$ it becomes apparent that $G_m$ corresponds to $var(g_1) + var(g_2)$ , $G_f$ corresponds to

617 $var(g_1) + var(g_3)$ , and $B$ corresponds to $var(g_1)$. From the off-diagonal terms in equation (4) it is

618 evident that increasing the strength of selection in females (decreasing $\gamma_f$) makes $\Delta B$ more negative –

619 which is consistent with the observed relative reduction in the genetic variance in the shared locus

620 ($var(g_1)$) (**Figure 4C**).

621

622 **Data access and code availability**

623 The code generated for this work has been deposited on GitHub: https://github.com/LiZhaoLab/SDV.

624

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641 **Competing interest statement**

642 The authors declare that no competing interests exist.

643

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790

791 **Figure Legend**

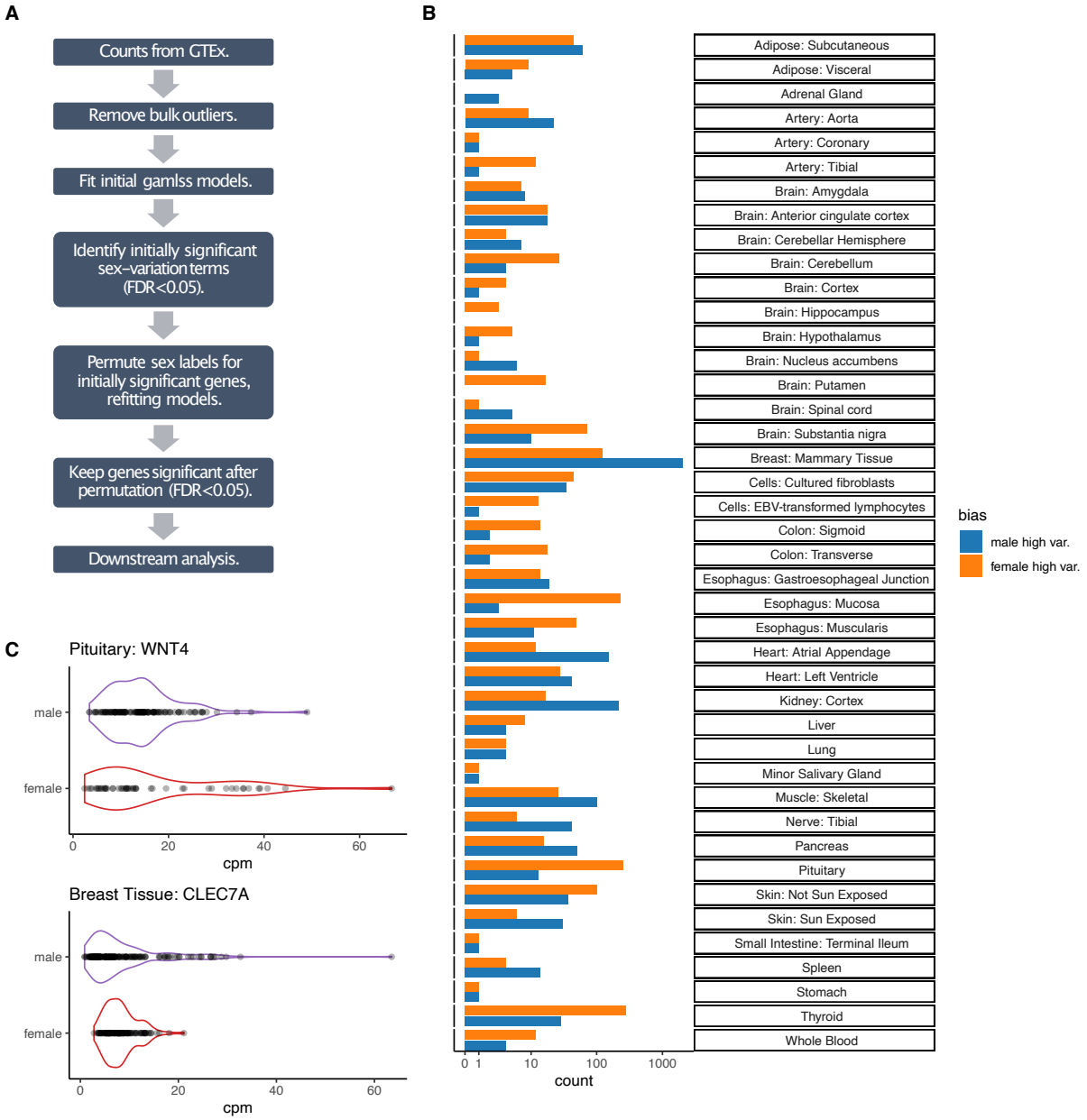


**Figure 1. The identification of genes with sex-differentially-variable gene expression.** A) Outline of pipeline to identify SDV genes. B) The number of genes with SDV expression identified in each tissue. The X-axis has a pseudo-log scale. C) Two examples of SDV genes with higher variation in females (WNT4 in the pituitary gland) and in males (CLEC7A in breast tissue), respectively.

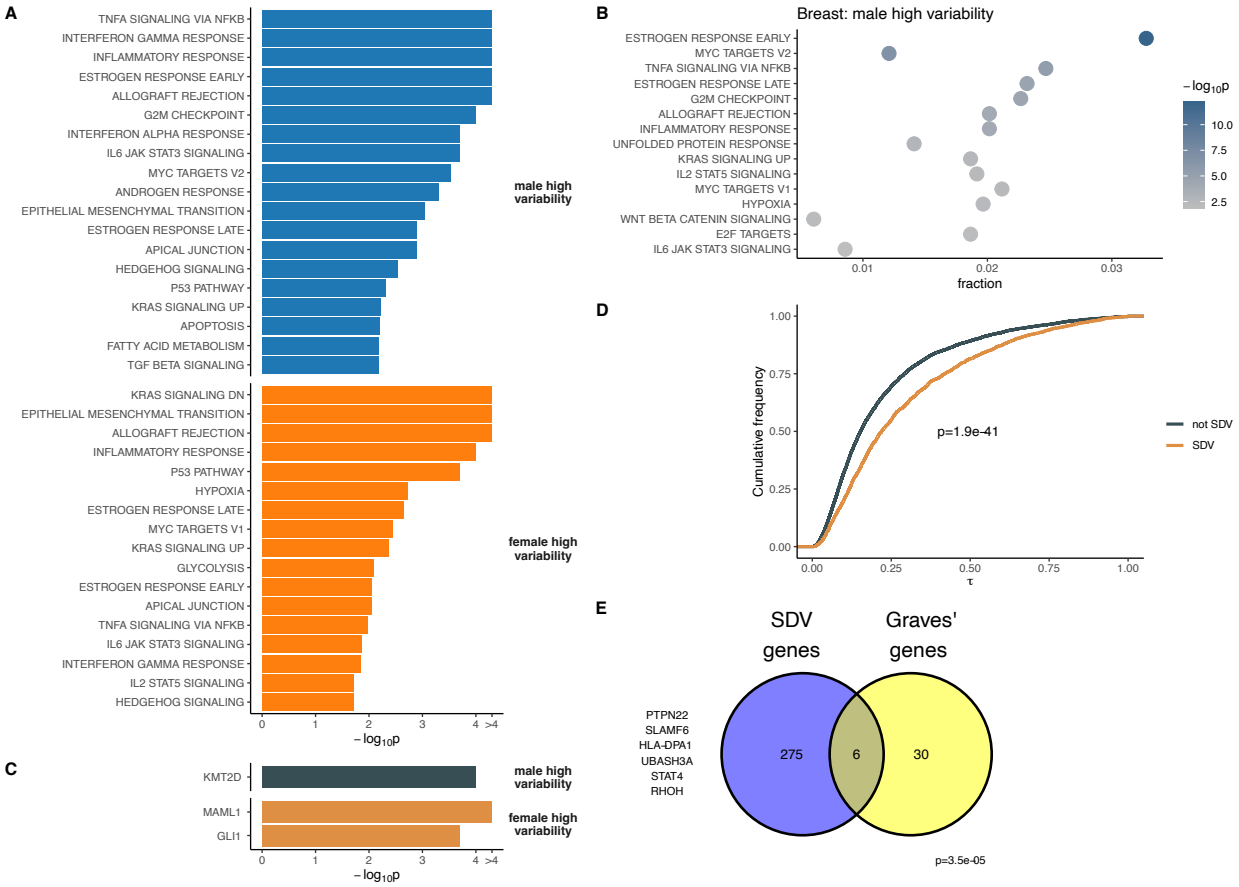


**Figure 2. Biological properties of SDV genes.** A) Following meta-analysis SDV genes with higher variation in males and females are enriched for terms related to various signaling pathways, immune response, and sex-hormone response. B) Genes with higher variability in males are enriched for terms related to estrogen and immune response in breast tissue. C) Following meta-analysis genes with higher variability in females are enriched for targets of two transcription factors. D) SDV genes identified in breast tissue show highly cell-type specific expression in breast epithelial tissue (p-value calculated using the Mann-Whitney U test) . Higher τ indicates greater cell-type specificity. E) Genes with higher variability in females in the thyroid are significantly enriched for genes associated with Graves' disease (p-value calculated using a hypergeometric test).

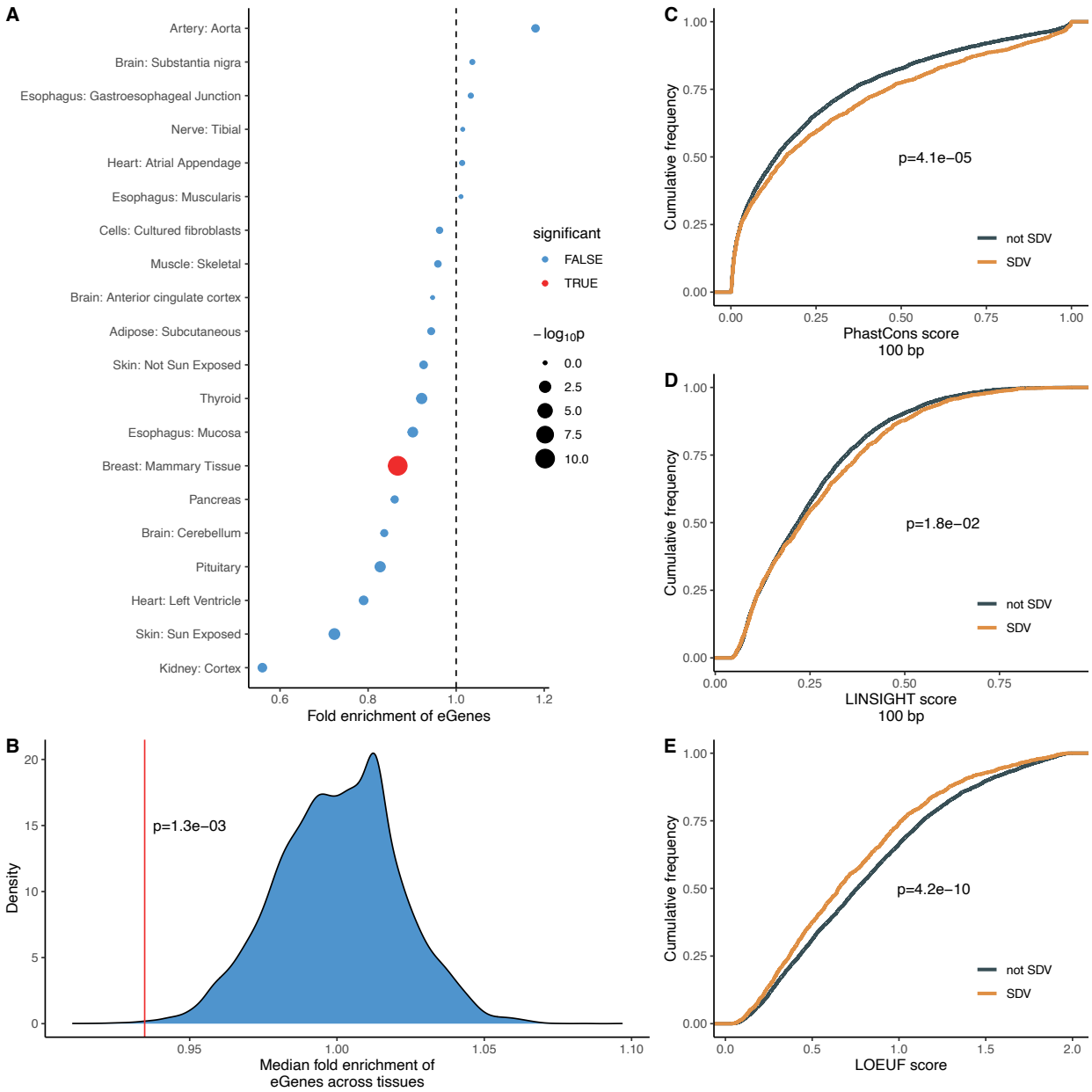


**Figure 3. SDV genes are associated with increased constraint.** A) 14 out of 20 tested tissues with at least 20 SDV genes show a depletion in eGenes among SDV genes. Significance was tested using Fisher's exact test at FDR<0.05. B) Across the 20 tested tissues the median fold enrichment of eGenes is significantly lower than expected by chance. The red line shows the observed median fold-enrichment, while the blue distribution shows the empirical null distribution. C) Genes with SDV expression in at least one tissue show significantly elevated mean phastCons scores in proximal promoters (100 bp upstream of TSS) indicating increased conservation. D) Genes with SDV expression in at least one tissue show significantly increased LINSIGHT scores, indicating greater functional importance. E) Genes with SDV expression in at least one tissue show significantly reduced LOEUF scores, indicating more intolerance to loss of function mutations. For C-E, SDV genes were considered to be SDV if they showed SDV expression in at least one tissue out of the 20 tested tissues. Only genes expressed in all 20 tested tissues were considered to remove bias. For C-E, p-values were calculated using the Mann-Whitney U test.

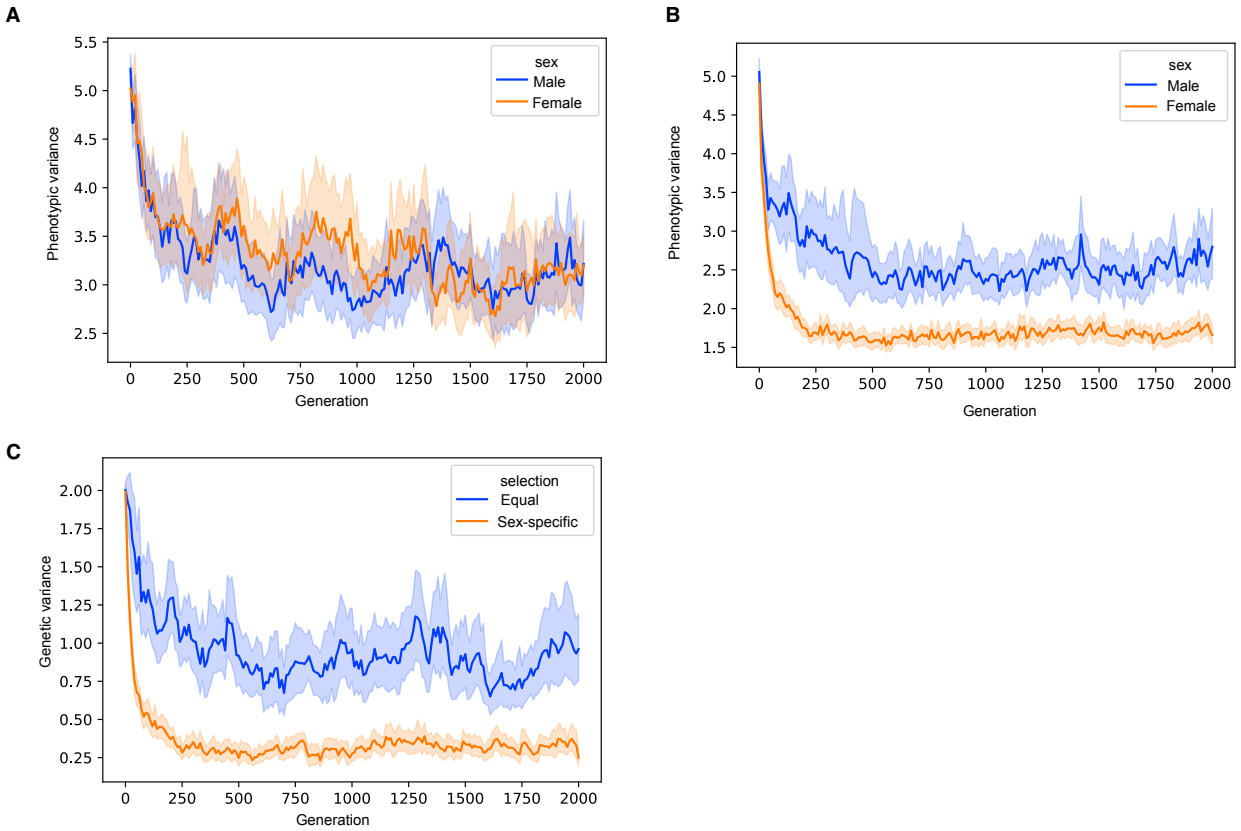


**Figure 4**. **Simulations showing that SDV expression can arise through sex-specific constraint.** Simulation results showing the output from 30 simulations in both the equal selection and sex-specific selection conditions. A) Equal constraint in both sexes results in similar phenotypic variance in both sexes. B) Increased sex-specific constraint acting in females reduces the phenotypic (expression) variation across individuals. C) Increased constraint in females results in reduced genetic variance, consistent with our observations of reduced *cis*-regulatory variation in SDV genes.