1   **An X Chromosome Transcriptome Wide Association Study Implicates ARMCX6 in**

2   **Alzheimer's Disease**

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5   **Abstract**

6   **Background:** The X chromosome is often omitted in disease association studies despite

7   containing thousands of genes which may provide insight into well-known sex differences in the

8   risk of Alzheimer's Disease.

9   **Objective:** To model the expression of X chromosome genes and evaluate their impact on

10   Alzheimer's Disease risk in a sex-stratified manner.

11   **Methods:** Using elastic net, we evaluated multiple modeling strategies in a set of 175 whole

12   blood samples and 126 brain cortex samples, with whole genome sequencing and RNA-seq data.

13   SNPs (MAF>0.05) within the *cis*-regulatory window were used to train tissue-specific models of

14   each gene. We apply the best models in both tissues to sex-stratified summary statistics from a

15   meta-analysis of Alzheimer's disease Genetics Consortium (ADGC) studies to identify AD-

16   related genes on the X chromosome.

17   **Results:** Across different model parameters, sample sex, and tissue types, we modeled the

18   expression of 217 genes (95 genes in blood and 135 genes in brain cortex). The average model

19   R^2 was 0.12 (range from 0.03 to 0.34). We also compared sex-stratified and sex-combined

20   models on the X chromosome. We further investigated genes that escaped X chromosome

21   inactivation (XCI) to determine if their genetic regulation patterns were distinct. We found ten

22   genes associated with AD at $p < 0.05$, with only *ARMCX6* in female brain cortex ($p = 0.008$)

23   nearing the significance threshold after adjusting for multiple testing ($\alpha = 0.002$).

1   **Conclusions:** We optimized the expression prediction of X chromosome genes, applied these

2   models to sex-stratified AD GWAS summary statistics, and identified one putative AD risk gene,

3   *ARMCX6*.

4

5

6

7   **Keywords**: Alzheimer's disease, X Chromosome, Gene Expression, Bioinformatics,

8   Transcriptome, Sex Differences, Elastic Net Regression, Gene Prediction

9   **Running title**: An X Chromosome TWAS Implicates ARMCX6 in AD

10

1   **Introduction**

2   Nearly two-thirds of individuals with Alzheimer's Disease (AD) are women [1], as many other

3   human traits also present sex disparities. While these differences may be due to systematic

4   exposure to sex hormones or other endogenous factors, X chromosome genetic variation and its

5   expression are potential contributing factors. The human X chromosome is composed of 155

6   million base pairs and contains thousands of genes. The copy number of the X chromosome

7   defines biological sex. While females have two copies, genes on one X chromosome are

8   randomly silenced via DNA methylation in a process called X-chromosome inactivation (XCI).

9   The XCI patterns vary from person to person, with a proportion of genes escaping the silencing

10   process [2–5]. The choice of the silenced copy within a cell is random early in embryo

11   development. At the same time, X-linked genes' mosaic expression at the individual level can be

12   skewed towards the maternal or paternal copy of the gene [6,7]. Functionally, X-linked genes are

13   responsible for various processes, most notably human cognition and the development of

14   multiple tissues, including neural and bone [8].

15   Despite its potential importance, X chromosome variants are typically removed from genome-

16   wide association studies (GWAS) during quality control due to the complexity of accounting for

17   differences in chromosomal copy number between males and females. In general, methods used

18   for autosomal genotypes cannot be applied directly to X chromosome variants as they do not

19   account for the genetic imbalance or expected X-inactivation. [9–11] As a result, only one-third

20   of genome-wide association studies include evaluations of variants on the X [12]. None of the

21   recent large sample AD GWAS [13–17] included X chromosome. However, one smaller MRI-

22   based study (N = 931) by Homann et al. [18] is notable as they meta-analyzed sex-specific X

23   chromosome associations. The number of reported GWAS associations on the X chromosome is

1   smaller than that of autosomes [19], and Kukurba et al. discovered a relative depletion of *cis-*

2   eQTLs on the X chromosome using a general eQTL-mapping pipeline on both autosomes and

3   the X chromosome [20]. These differences can either result from the inefficient power of the

4   tests or the underlying different biological mechanisms and genetic architecture between

5   autosomes and the X chromosome. The exclusion of X chromosome variants also propagates

6   into the post-GWAS era. Expression QTL (quantitative trait loci) and other studies of molecular

7   phenotypes also often exclude the sex chromosomes. MetaBrain [21], which harmonized RNA-

8   seq samples from 14 studies and prioritized *cis-* and *trans*-eQTLs for brain-related traits, also did

9   not report any loci on the X chromosome. Similarly, the largest harmonized blood *cis-* and *trans-*

10   eQTLs resource, eQTLGEN [22], also excluded the X chromosome. Popular extensions of

11   GWAS, such as transcriptome-wide association studies (TWAS) [23,24], omit the X

12   chromosome due to lack of adequate models of X-linked gene expression. Tissue-specific and

13   cross-tissue TWAS on AD [25,26] did not include the X chromosome.

14   In this study, we evaluated multiple approaches to fit expression prediction models for X

15   chromosome genes and produced a set of sex-stratified X-chromosome prediction models. By

16   applying these models in a TWAS on AD GWAS summary statistics, we detected one gene,

17   *ARMCX6*, borderline associated with AD risk in the cortex in female-only samples.

18

19   **Methods**

20   **Data resources**

21   We retrieved WGS and RNA-seq expression levels data (phs000424.v7.p2) from the brain cortex

22   and whole blood sample from the GTEx data [27] to train the prediction models. We selected

23   cortex because of the availability and larger sample size of the validation dataset, in addition to

1   its direct relevance to the AD disease process. Whole blood is the most accessible tissue for

2   transcriptome evaluations and also captures immune cell expression, which is also likely relevant

3   for AD. For validation, we used (1) whole-genome imputed genotype data and whole blood

4   sample RNA-seq data from the DGN (Depression susceptibility Genes and Network) expression

5   cohort [28] and (2) WGS and temporal cortex (TCX) RNA-seq data from the MayoRNAseq

6   study [29]. Gene expression data of the MayoRNAseq cohort has already been adjusted with

7   Alzheimer's Disease (AD) diagnosis, sex, and age at death.

8   *Table 1. Overview of the study datasets for training and validating the models.*

		GTEx blood	GTEx cortex	DGN blood	MayoRNAseq cortex
Sample size		175	126	917	255
	Female	58	46	648	130
	Male	117	80	269	125
Age		56.1(10.5)	57.3(10.6)	44.7(10.9)	80.5(8.5)
	Female	57.4(9.0)	57.9(11.4)	45.0(10.5)	81.5(8.5)
	Male	55.5(11.2)	57.0(10.3)	44.1(11.7)	79.4(8.3)
Platform		WGS RNA-seq	WGS RNA-seq	Genotype RNA-seq	WGS RNA-seq
Genome Assembly		GRCh37	GRCh37	GRCh37	GRCh37
Number of expressed genes		600	766	486	587

9

10   The GTEx donors were from post-mortem autopsy or organ and tissue transplantation settings.

11   The DGN cohort reported no differences in eQTL detection between depression cases and

12   controls. [28] MayoRNAseq study included patients with AD and related dementias and health

13   control on donors from the Mayo Clinic Brain Bank and Banner Health. [29] These datasets can

14   represent typical patterns of gene expression in blood and cortex.

1   The key features of the three study populations are listed in Table 1. DGN and MayoRNAseq

2   only recruited subjects of European descent from the United States. To evaluate different

3   modeling strategies in a homogeneous study population and make generalizable

4   recommendations, we restricted the samples to the GTEx participants who were self-identified as

5   non-Hispanic white (NHW). The age data in GTEx and MayoRNAseq is the age at death of the

6   donors, while that for DGN is the age at sampling. MayoRNAseq subjects over 90 years old

7   (recorded as 90+) were treated as age 91. It is noteworthy that the three studies exhibit different

8   sex ratios, with males comprising two-thirds of the GTEx NHW cohort, females comprising two-

9   thirds of the DGN cohort, and the MayoRNAseq TCX cohort displaying gender balance.

10 Additionally, MayoRNAseq subjects are significantly older than GTEx and DGN. Human

11 subjects and ethics statements for these studies are available in their respective publications [27–

12 29].

13 **Data processing**

14 To keep the analysis concise, we dropped pseudoautosomal regions (PARs) (GRCh37, X:60,001-

15 2,699,520, and X:154,931,044-155,260,560) for this study. The PAR boundaries were retrieved

16 from https://www.ncbi.nlm.nih.gov/grc/human. Genetic variants with missing call rates

17 exceeding 5% and samples with missing call rates exceeding 10% were removed. A minor allele

18 frequency (MAF) threshold of >5% was applied to the overall dataset. In both sex-stratified and

19 sex-combined modeling, the genotypes of female subjects were encoded as 0/1/2, which assumed

20 entirely random X chromosome inactivation (XCI), and male genotypes were encoded as 0/2.

21 [30] For sex-stratified analyses, the normalized expression was adjusted for covariates, including

22 top 3 PCs, PEER factors [31], and sequencing platform. We also adjusted for sex in the sex-

23 combined analysis. We fit linear regression models of expression against the covariates and kept

7

1   the residuals as the adjusted expression. (Supplementary Figure 1) We obtained a list of 59 genes

2   (Supplementary Table 1) that escape the XCI (XCI escapees) from Navarro-Cobos et al. [3] and

3   investigated whether a different modeling strategy would improve the predictions of these

4   escaping genes.

5   **Elastic net regression**

6   We selected the elastic net regression to model gene expression levels on the X chromosome.

7   While other approaches are available, elastic net regression allows for comparisons to both the

8   autosomal models from PrediXcan [23] pipeline and published sex-stratified models of

9   autosomal genes [32]. Elastic net regression is a regularized method that combines the LASSO

10   (L_1) and ridge (L_2) penalties to select variables while fitting a linear regression model. Both

11   penalty terms shrink regression coefficients by penalizing the regression model. The difference is

12   that the LASSO penalty L_1 often reduces some regression coefficients to 0 when the ridge

13   penalty L_2 does not.

14   The estimate from elastic net regression is $\hat{\beta} = \underset{\beta}{\mathrm{argmin}}(\|y - G\beta\|^2 + \lambda[(1-\alpha)L_1 + \alpha L_2])$,

15   where y is the vector of the measured expression of one gene in all the individuals, and G is the

16   genotype matrix of all the individuals. The genotypes of female subjects were encoded as 0/1/2,

17   and male genotypes were encoded as 0/2. The shrinkage parameter λ controls the amount of

18   penalization applied to the regression. Theoretically, an elastic net model favoring the LASSO

19   penalty (mixing parameter $\alpha = 0$) will lead to a sparse model with a few SNPs with non-zero

20   effects, while favoring the ridge penalty (mixing parameter $\alpha = 1$) will lead to a polygenic

21   model. We implemented k-fold nested cross-validation to tune each gene's shrinkage parameter

22   λ at any given mixing parameter α and evaluate the model performance for how the model

23   predicts on the hold-out fold. The best shrinkage parameter λ was selected by the lowest mean

1   squared error (MSE). The model assessment metrics include the coefficient of determination R^2

2   and p-value, where the null hypothesis is that there is no correlation between predicted and

3   measured expression. We only kept models with $R > 0.1$ and p-value < 0.05, the significant

4   models, for the downstream analyses.

5   PrediXcan implements the elastic net regression model with a mixing parameter $\alpha = 0.5$, where

6   the penalty is defined as a balanced (50%-50%) combination of L_1 and L_2. This selection of

7    $\alpha = 0.5$ has been shown to be optimal by previous studies of autosomes. [23,33] Since we are

8   unclear about the underlying regulatory function on the X chromosome, we fit elastic net

9   regression models with different mixing parameters $\alpha = 0.05, 0.25, 0.5, 0.75,$ and 0.95. The

10   models were fit in both sex-stratified and sex-combined manners. (Figure 1).

1
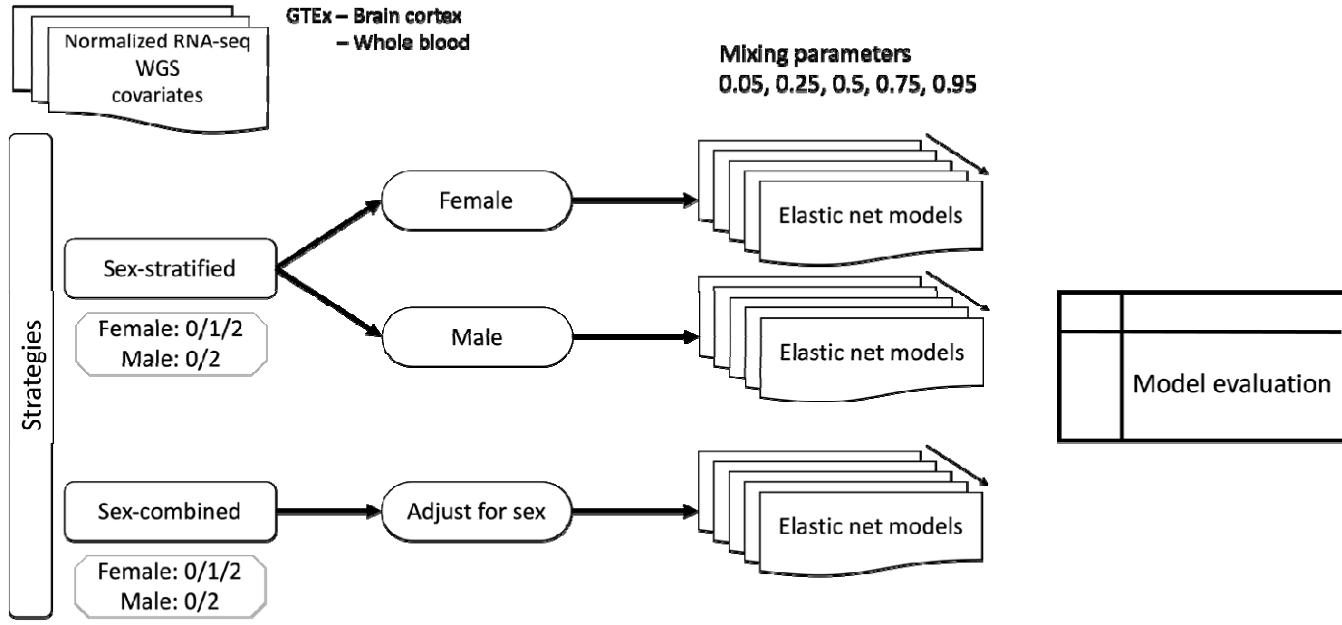
Figure 1. The working flow of model training using GTEx data. Elastic net regression models of the X chromosome

gene expressions were trained in whole blood and brain cortex with different mixing parameters, in both sex-

stratified and sex-combined manner. Genetic variants on the X chromosome were coded as 0/1/2 in female samples

and 0/1 in male samples.

Model comparisons with two-way ANOVA

We compared the performance of the fitted prediction models to determine (1) whether the

widely adopted mixing parameter   also works the best in X chromosome models; (2)

whether sex-stratified models outperform the sex-combined models. Two-way ANOVA serves

perfectly for our goal. Compared to one-way ANOVA, two-way ANOVA allows us to examine

the combined effects of modeling strategies and biological factors.

We applied a data transformation   to the model's coefficient of determination   to

meet the normality assumption of two-way ANOVA since the distribution is right skewed with a

long tail. The density plots and QQ plots of the original   and the transformed   are in

Supplementary Figure 2. Though a light tail still exists, the transformed prediction performance

metrics exhibit a more normal distribution than the original   . The transformed   shows

1   homoscedasticity (homogeneity of variance) (see Supplementary Table 2). The design of GTEx

2   and most other studies measuring human transcriptome provide independent observations of

3   tissue-specific gene expression levels in each individual. Therefore, there is no violation of the

4   assumptions of the two-way ANOVA test.

5   After transformation, we compare the model performance in different settings (mixing

6   parameters, tissue types, female- vs. male-specific models, and sex-stratified vs. sex-combined

7   analysis). We drew conclusions from the F statistics and p-values from the two-way ANOVA.

8   **Model Validation**

9   We predicted the gene expression levels in the whole blood and brain cortex by applying the sex-

10   stratified GTEx gene prediction models to DGN and MayoRNAseq data, respectively [23].

11   Genotypes of the DGN cohort were imputed to the HRC reference panel using the Michigan

12   Imputation Server. We estimated the genetically regulated expression for each gene and tested its

13   correlation with the measured expression with R^2 statistics.

14   **AD risk analysis**

15   We applied the validated significant models to sex-stratified GWAS meta-analysis summary

16   statistics from the ADGC (Alzheimer's Disease Genetics Consortium) with S-PrediXcan [34]

17   pipeline, to identify potentially causal X-linked genes for AD. S-PrediXcan is a TWAS pipeline

18   that allows summary statistics instead of individual-level data to impute the transcriptome and

19   test for disease-associated genes. Due to the need for meta-analysis of the ADGC cohorts, we

20   chose to perform this analysis using summary statistics rather than individual-level data. We

21   matched the SNPs by rsIDs, since the models were trained in GRCh37 WGS data and the ADGC

22   sex-stratified summary statistics are in GRCh38. A detailed description of the ascertainment of

23   age at AD onset along with the descriptive statistics of each cohort can be found in Kunkle et al.

1 [13]. All study participants were recruited under protocols approved by the appropriate

2 institutional review boards. Written informed consent was obtained from study participants or,

3 for those with substantial cognitive impairment, from a caregiver, legal guardian, or other proxy.

4 The sex-stratified ADGC summary statistics detailed the effect of SNPs on clinically diagnosed

5 AD in independent NHW subjects of the ADGC cohort. The summary statistics were from three

6 different models stratified by sex: model 1 – additive effect of genetic variants adjusting for the

7 first 3 PCs, model 2 – additive effect of genetic variants adjusting for the first 3 PCs and age,

8 model 3 – additive effect of genetic variants adjusting for the first 3 PCs, age and APOE e4 allele

9 count. These GWAS tests assumed no XCI. A total of 12,906 case participants (N=7,235 female

10 cases) and 14,111 cognitively normal controls (N=8,411 female controls) were analyzed across

11 26 ADGC studies. Mean age-at-onset across studies ranged from 61.2 (standard deviation = 10.5)

12 to 85.8 (SD = 5.7) and mean age-at-exam ranged from 64.0 (SD = 2.9) to 83.8 (SD = 7.5).

13 Summary demographics of all studies can be found in Supplementary Table 3. The total number

14 of SNPs analyzed for the male models ranged from 347,663 to 348,800 and for female models

15 ranged from 487,238 to 489,735.

16

17 **Results**

18 Given that there are numerous possible approaches to the analysis of X chromosome variants, we

19 evaluated multiple options to determine the best modeling strategy for estimating the genetically

20 regulated component of expression for genes on the X chromosome. We assessed whether the

21 model parameters may differ by sex, and also attempted sex-combined and sex-stratified

22 analyses. Given the unique biological features of the X chromosome, we also examined possible

23 differences in the modeling of genes based on their X chromosome inactivation status. We

12

1   further applied the significant models to AD summary statistics for a TWAS to identify AD risk

2   genes on the X chromosome.

3   **Evaluation of mixing parameters within sex-stratified models**

4   We trained sex-stratified models with GTEx data using five different mixing parameters:

5    $\alpha = 0.05, 0.25, 0.5, 0.75$, and 0.95. We used a nested cross-validation approach (similar to

6   PrediXcan: https://github.com/hakyimlab/PredictDB-Tutorial) that tunes the shrinkage parameter

7   λ in an inner loop while model performance was evaluated in an outer loop. The models were fit

8   in four tissue-sex sets: female-cortex, female-blood, male-cortex, and male-blood. We dropped

9   intercept-only models since we are only interested in models that included genetic variants, and

10   all the models will have at least one cis-eQTL with non-zero effect size on gene expression

11   trait. As described in Methods, models with $R < 0.1$ and $p > 0.05$ were removed for

12   downstream analyses.

13   Across different α, sex, and tissue types, we trained 499 significant gene models for 95 genes in

14   whole blood and 135 genes in brain cortex. The average number of SNPs in each model is 21.3,

15   and the average $R^2 = 0.12$ (range from 0.03 to 0.54) (Table 2).

16   *Table 2. Summary of sex-specified models in blood and cortex with different mixing parameters. Only models with*

17   *$R > 0.1$ and $p < 0.05$ were kept.*

Sex	α	Whole blood (600 genes)				Brain Cortex (766 genes)			
		Models number	Average SNPs	Average R^2	R^2 range	Models number	Average SNPs	Average R^2	R^2 range
Female	0.05	18	52.3	0.15	(0.08, 0.37)	26	66.0	0.18	(0.09, 0.35)
	0.25	21	29.0	0.14	(0.08, 0.39)	33	30.3	0.21	(0.09, 0.54)
	0.5	30	16.2	0.11	(0.06, 0.21)	25	17.2	0.18	(0.10, 0.34)
	0.75	22	23.1	0.16	(0.07, 0.49)	25	15.2	0.21	(0.11, 0.38)
	0.95	17	15.5	0.16	(0.06, 0.38)	27	10.7	0.19	(0.11, 0.37)
Male	0.05	22	36.2	0.06	(0.03, 0.16)	33	35.6	0.08	(0.05, 0.15)
	0.25	23	12.6	0.07	(0.04, 0.13)	31	14.3	0.08	(0.05,0.17)
	0.5	22	11.3	0.07	(0.03, 0.24)	26	8.3	0.09	(0.06, 0.17)

0.75	20	10.3	0.07	(0.03, 0.24)	28	8.6	0.09	(0.05, 0.24)
0.95	21	7.1	0.07	(0.04, 0.17)	29	5.9	0.08	(0.05, 0.15)

1

2   In both whole blood and brain cortex, the transformed R^2 of female-only models present a wider

3   spread and higher central tendency than male-only models (Supplementary Figure 3). We

4   checked the distributions of the original R^2 in the sex-stratified models. For each tissue-sex set,

5   we compared the original R^2 of the significant gene models with the R^2 of the same genes in the

6   other tissue-sex set. (See Figure 2 for the comparison of $\alpha = 0.5$ and Supplementary Figure 4-7

7   for the other mixing parameters)
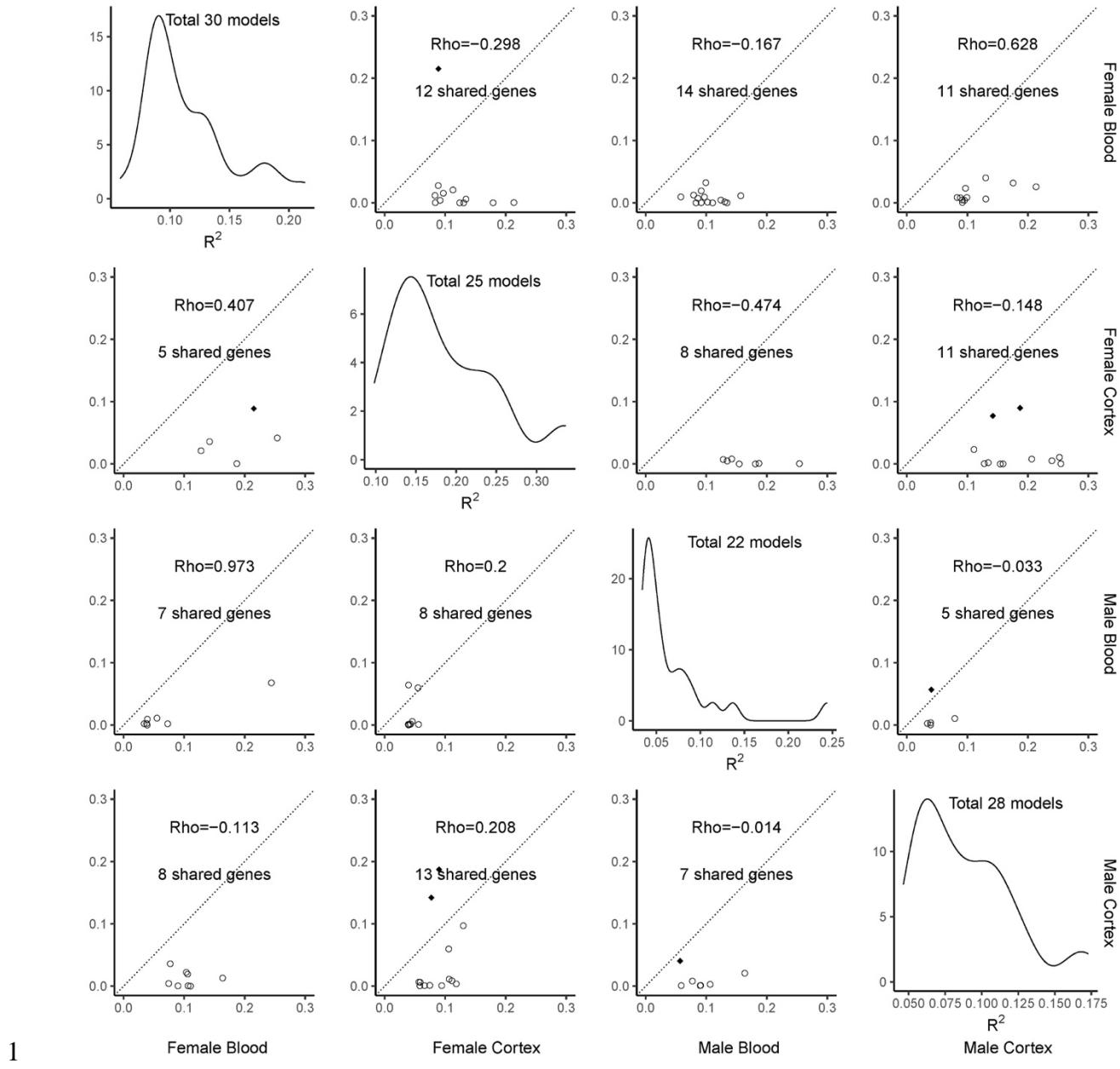
Figure 2. Comparison matrix of the balanced sex-stratified elastic net models trained in GTEx whole blood and brain cortex data ($\alpha = 0.5$). The four diagonal panels are the distributions of model R^2. The number of genes being modeled in each tissue-sex set is listed in the distribution plot. The off-diagonal panels compared the R^2 of the models in pairs of tissue-sex sets. The four rows are for the female-blood, female-cortex, male-blood, and male-cortex. For each row, the genes of the significant models in the tissue-sex set were matched in the other sets for the matching R^2. The diamonds represent genes with a significant model ($R < 0.1$ and $p > 0.05$) in another tissue-sex

1   *set, while the circles represent genes with a non-significant model in another tissue-sex set. The number of gene*

2   *models and the Pearson correlation coefficients were listed in the comparison plots.*

3   We performed a two-way ANOVA under the null hypothesis that there are no differences in the

4   transformed R^2 among sex-stratified elastic net models with different mixing parameters

5     $\alpha = 0.05, 0.25, 0.5, 0.75$, and 0.95. In both cortex and whole blood models, we found no

6   significant differences in the average R^2 among different α's in sex-stratified models ($p = 0.21$

7   for blood, $p = 0.55$ for cortex), but did observe significant differences in the average R^2 by the

8   sample sex ($p = 1.58 \times 10^{-26}$ for blood, $p = 1.83 \times 10^{-55}$ for cortex). This result is driven by

9   lower average R^2 in male-only models conditioned on tissue and α, as shown in Table 2. Sex by

10   α interaction was also non-significant ($p = 0.69$ for blood, $p = 0.55$ for cortex). This finding

11   indicates that different mixing parameters generally do not impact the performance of gene

12   expression prediction models. In contrast, the models trained in female- and male-only samples

13   have different coefficients of determination. While our elastic net models capture more genes

14   with $\alpha = 0.25$, to keep consistent with the literature and existing PredictDB autosomal models,

15   we used a mixing parameter of $\alpha = 0.5$ (balanced penalty) for all subsequent analyses.

16   **Differences in the tissue-specific sex-stratified models**

17   For 103 models with a mixing parameter of $\alpha = 0.5$, the average number of SNPs in each model

18   is 13.4, and the average $R^2 = 0.116$ (range from 0.03 to 0.34). There are 30, 22, 25, and 26

19   significant models for female-only blood, male-only blood, female-only cortex, and male-only

20   cortex samples, respectively (see Figure 2). Among all the genes modeled, no blood-specific

21   expression was modeled both in males and females, while two cortex-specific expressions

22   (ENSG00000005893 *LAMP2*, ENSG00000101955 *SRPX*) were modeled in both sexes, and one

16

1   gene was modeled in both tissues in male- or female-only data (ENSG00000197582 *GPX1P1*,

2   ENSG00000013563 *DNASE1L1*).

3   We further compared the differences between models trained with data from different tissue

4   types. We found a statistically significant difference in the model's average R^2 by sex ($p =$

5   1.93×10^{-13}). As expected, we observed a statistically significant difference between whole

6   blood and brain cortex ($p = 5.33 \times 10^{-8}$). As shown in Table 2, the average R^2 of blood-

7   specific models is lower than cortex-specific models, and the average R^2 of male-only models is

8   lower than female-only models. Notably, there were no significant differences noted between

9   sex-stratified models of autosomal genes from the same dataset (reported in Table 1 of Mahoney

10   et al. [32]), regardless of sample size differences between males and females. The interaction

11   between sex and tissue, however, was not significant ($p = 0.59$). We highlight that sex

12   differences are just as crucial as tissue differences for X chromosome gene expression.

13   **Sex-combined models with a balanced penalty**

14   The tissue-specific sex-combined models were trained in blood and cortex, respectively, with a

15   balanced mixing parameter $\alpha = 0.5$. After removing intercept-only models and models with

16   $R < 0.1$ and $p > 0.05$, we have 45 significant sex-combined models in whole blood and cortex.

17   The average number of SNPs in the sex-combined models across different tissues is 10, while the

18   average $R^2 = 0.052$ (range from 0.023 to 0.11). (Table 3) The average R^2 of the sex-combined

19   models is smaller than that of sex-stratified models (single-sided t-test $p < 2.2 \times 10^{-16}$).

20

1 *Table 3. Summary of sex-combine, balanced penalty models in blood and cortex. Models with $R < 0.1$ and $p > 0.05$*

2 *were removed. The models are summarized conditioned on the XCI escaping status. None of the genes escaping XCI*

3 *were significant in blood.*

Tissue	Escape XCI	Models number	Average SNPs	Average R^2	R^2 range
Blood	×	23	8.96	0.045	(0.023, 0.087)
Cortex	×	20	11	0.059	(0.03, 0.11)
	√	2	11.5	0.051	(0.034, 0.067)

4 We compared different modeling strategies (stratified-female, stratified-male, and sex-combined

5 models) to determine whether the sex-combined analysis would improve the overall fit for X

6 chromosome genes by increasing the sample size. Considering the potential different underlying

7 regulatory effects, we tested the genes escaping X chromosome inactivation (XCI escapees) and

8 the other genes separately. Besides, there are some XCI escapees in the sex-stratified balanced

9 penalty models (3 for female-blood, 2 for male-blood, and 1 for male-cortex).

10 For the models of XCI escapees (n=8), we found no significant differences in the average R^2 in

11 the modeling strategies ($p = 0.15$) nor tissue types ($p = 0.55$). Contrarily, in both blood and

12 cortex, genes not escaping from the XCI show significantly different R^2 between any pair of

13 modeling strategies (one-way ANOVA $p = 3.4 \times 10^{-11}$ for blood and $p = 1.01 \times 10^{-15}$ for

14 cortex).

15 **Model Validation**

16 The sex-stratified GTEx gene prediction models in blood and cortex were applied to DGN and

17 MayoRNAseq validation datasets, respectively. 65 SNPs from GTEx whole blood models were

18 not available in DGN whole-genome imputed genotype data, and 15 SNPs from GTEx brain

19 cortex models were not available in MayoRNAseq WGS data (Figure 3 A and B). In DGN whole

20 blood validation, 20 and 15 genes were predicted by stratified-female and stratified-male

1   significant models, respectively. There is no gene predicted in both sexes. In MayoRNAseq TCX

2   validation, we predicted 19 and 18 genes with stratified-female and stratified-male significant

3   models, respectively, among which two genes were predicted in both females and males. (Figure

4   3 C and D). In addition, two sex-combined significant models were applied to genes that escape

5   from XCI in MayoRNAseq data.

6
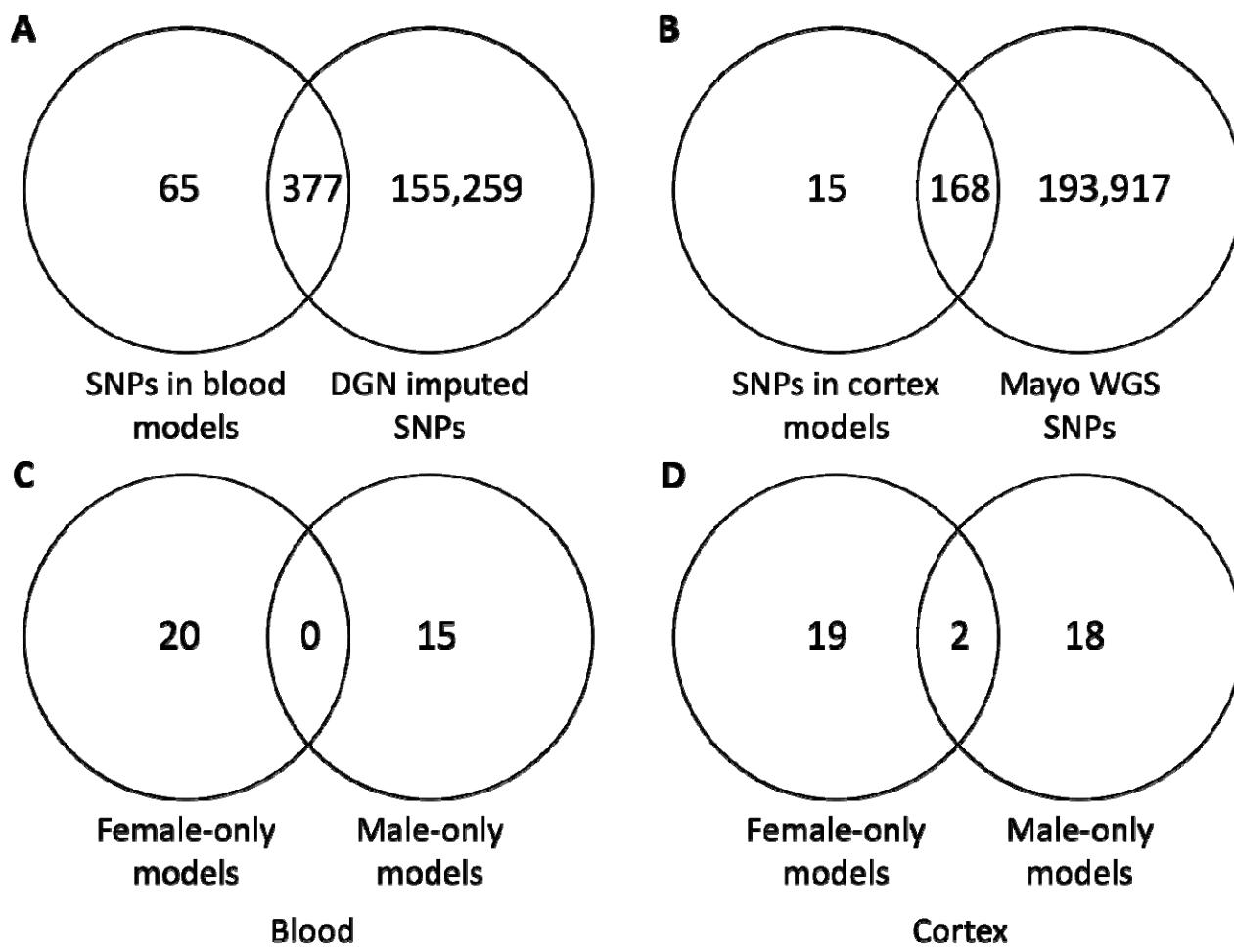
1   *Figure 3. The number of SNPs in the validation datasets and the number of genes predicted. A and B: Venn*

2   *diagrams representing the number of SNPs in the tissue-matching validation datasets and our sex-stratified*

3   *prediction models. C and D: Venn diagrams illustrating the number of genes predicted by the sex of the models and*

4   *validation samples.*

5   We calculated prediction R^2 by regressing the estimated expressions against the measured

6   expression via RNA-seq from the validation datasets (range from 9.94×10^{-5} to 0.091). Table 4

7   lists the prediction R^2 of the sex-stratified and sex-combined models in each tissue. Comparisons

8   of the expected and observed distribution of prediction R^2 in blood samples (DGN) and cortex

9   samples (MayoRNAseq) for all models (Supplementary Figure 8) show a deviation from the

10   expected null distribution, demonstrating that models generally explain variability in X

11   chromosome gene expression.

12   *Table 4. Prediction R^2 in validation datasets. Note that there is no significant blood-specific sex-combined models*

13   *for XCI escapees.*

	Blood – DGN			Cortex – MayoRNAseq		
	Models Number	Average R^2	R^2 range	Models Number	Average R^2	R^2 range
Female-only model	20	0.014	$(9.99 \times 10^{-6}, 0.074)$	21	0.0097	$(9.94 \times 10^{-5}, 0.091)$
Male-only model	15	0.034	$(0, 0.44)$	20	0.010	$(1.05 \times 10^{-4}, 0.036)$
Sex-combined model (Only XCI escapees)	-	-	-	2	0.0010	$(1.28 \times 10^{-4}, 0.0020)$

14

15   **Identify AD risk genes on X chromosome**

16   We applied sex-stratified gene prediction models with a balanced penalty to the ADGC sex-

17   stratified GWAS summary statistics on the X chromosome for TWAS analysis, using the S-

18   PrediXcan [34] pipeline. In this sex-stratified analysis of X-linked AD risk genes, 25 and 26

19   genes were tested in cortex in females and males respectively, while 30 and 22 genes were tested

20   in blood in females and males respectively. We found a marginally significant gene *ARMCX6* in

1 cortex in female-only data ($p = 0.0082$, while the Bonferroni correction significance threshold

2 is $\frac{0.05}{25} = 0.002$). Other associations we found from this TWAS are presented in Table 5.

3 *Table 5. S-PrediXcan results (p<0.05) on sex-stratified AD summary statistics.*

Sex	GWAS model	Tissue	Gene Name	Z score	p-value	Number of SNPs used
Female	Additive + Age + APOE	Cortex	ARMCX6	2.64	0.0082	23
Male	Additive + Age + APOE	Cortex	DIAPH2	-2.31	0.0211	15
Female	Additive	Blood	LL0XNC01-116E7.2	2.3	0.0217	17
Female	Additive + Age	Blood	TENM1	-2.26	0.0238	52
Male	Additive	Blood	SUV39H1	-2.16	0.0308	2
Female	Additive	Blood	TENM1	-2.15	0.0316	52
Male	Additive + Age	Blood	SUV39H1	-2.05	0.0407	2
Female	Additive + Age	Cortex	ARMCX6	2.03	0.0426	23
Female	Additive + Age	Cortex	LAMP2	2.02	0.0435	20
Female	Additive + Age	Blood	LL0XNC01-116E7.2	2.01	0.0444	17
Female	Additive	Cortex	UBQLN2	2.01	0.0446	5
Male	Additive + Age	Cortex	TMSB4X	-2.01	0.0447	2
Female	Additive	Cortex	ARMCX6	2	0.0454	23
Female	Additive	Cortex	LAMP2	2	0.0456	20
Male	Additive + Age + APOE	Blood	TBC1D25	1.99	0.0465	1
Female	Additive	Cortex	DNASE1L1	-1.96	0.0494	8

4

5 The prediction model of *ARMCX6* in brain cortex, trained with female-only data, selected 23

6 SNPs from the 164 SNPs in the *cis*-regulatory region. The shrinkage parameter λ of the model is

7 0.25. From 5-fold cross-validation, the correlation between predicted and observed expression

8 levels is $R^2 = 0.333$ ($p = 1.3 \times 10^{-4}$). This gene model was also trained in male-only cortex

9 data, but it is not significant ($R^2 = 0.01$, $p = 0.39$), while no model of *ARMCX6* from blood

10 data exists. Based on our TWAS results, increases in the genetically predicted expression of

11 *ARMCX6* in cortex will increase the AD risk of a female subject ($p = 0.0082$).

12 We visualized the putative AD-risk gene, *ARMCX6* in Figure 4. All the SNPs were plotted in the

13 GRCh37 positions to make them comparable across panels. The positions and weights of the 23

21

1   SNPs included in the *ARMCX6* female-only penalized prediction model are presented in Figure

2   4A. Only 2 SNPs are located upstream of the *ARMCX6* gene. The remaining 21 SNPs form

3   multiple clusters localized to 1,000 bp windows downstream of the *ARMCX6* gene. Figure 4B

4   and Figure 4C show the p-values of SNPs within the same region (as Figure 4A) from female-

5   only eQTL mapping and female-only GWAS on AD (adjusted for age and APOE), respectively.

6   The SNPs in the prediction model are in red. None of the selected SNPs are of the most extreme

7   eQTL or GWAS p-values. The female-only eQTL p-values were calculated with GTEx NHW

8   female subjects with brain cortex samples (N=46). The distributions of *ARMCX6* gene

9   expression in both sexes in brain cortex and blood from GTEx are in Supplementary Figure 9.

10   One-way ANOVA suggests the expression levels vary among the four tissue-sex sets ($p =$

11   0.014), while two-way ANOVA indicates the differences come from tissue ($p = 0.0062$) but

12   not sex ($p = 0.18$).

13
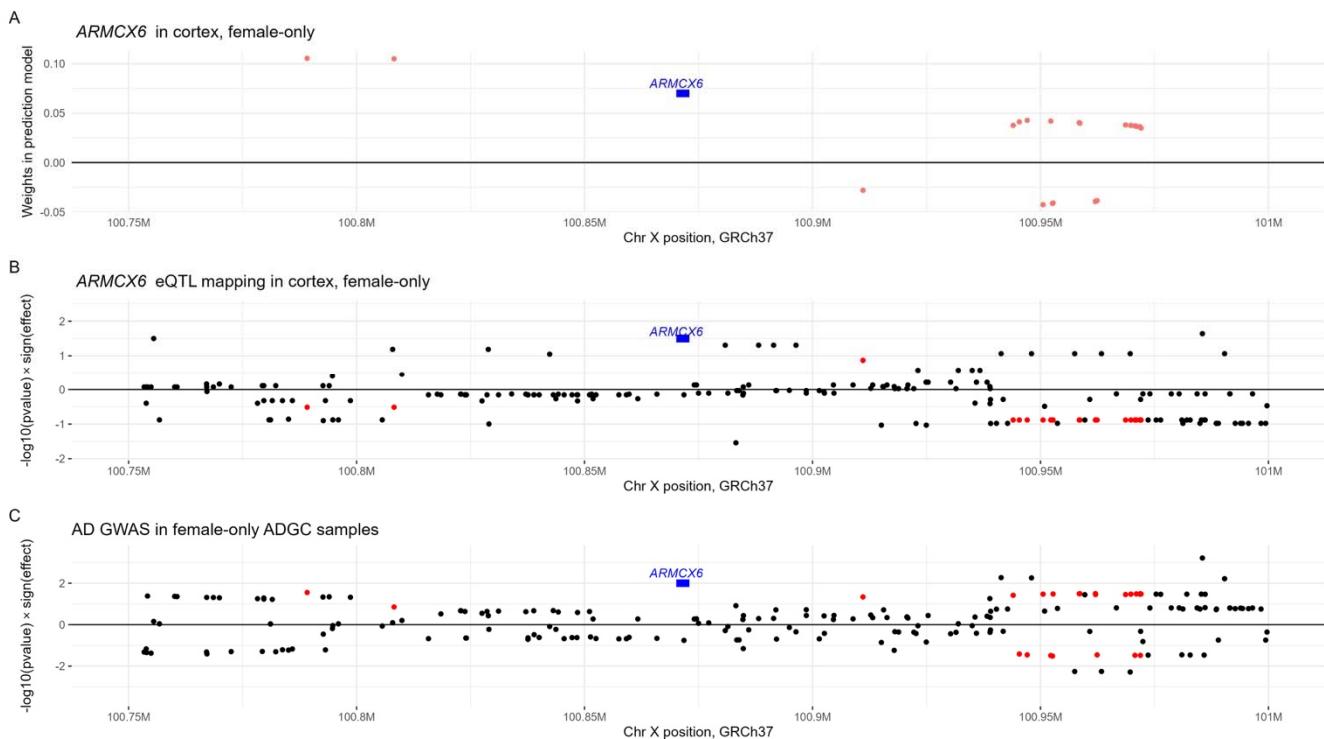
1   *Figure 4. Genetic architecture of TWAS significant hit, ARMCX6. All three panels show the same region. A: The*

2   *positions and effect sizes of the 23 selected SNPs in the cortex-specific expression prediction model trained with*

3   *female-only samples. B: Hudson plot of eQTLs of ARMCX6 in female-only GTEx cortex samples. C: Hudson plot of*

4   *ADGC meta-analysis GWAS in female-only samples. The red dots in B and C represent the SNPs in the expression*

5   *prediction model.*

6

7   **Discussion**

8   In this work, we examined multiple modeling strategies for generating X chromosome gene

9   expression prediction models. Based on our cross-validation results from analyses of the GTEx

10   data for whole blood and brain cortex, we concluded that sex-stratified models (with $\alpha = 0.5$)

11   provided the optimal R^2. We validated these models in two external tissue-matched datasets and

12   found an average prediction performance of 0.012, which deviates from the expected distribution

13   under a null hypothesis. With these models, we implemented a TWAS and tested the association

14   between AD risk and 20 genes for females and 15 genes for males on the X chromosome in

15   whole blood, 21 genes for females and 20 genes for males on the X chromosome in brain cortex.

16   *ARMCX6* is our most compelling finding, as we did not find any gene significant after strict

17   Bonferroni correction.

18   DNA methylation silences one copy of X chromosome genes in females via X inactivation, and

19   there are noted non-random patterns associated with this phenomenon, particularly skewed [6,7]

20   and escaped [2,3] X inactivation. We were surprised to observe that modeling genes escaping

21   XCI with different strategies produced no substantial differences compared to non-escaped genes.

22   In our sex-stratified models, we noted that our modeling approach assumes equal impact of each

23   allele, which for the X chromosome would also assume totally random X chromosome

1   inactivation. Accounting for skewed X-inactivation may be possible with availability of DNA

2   methylation data or allele-specific expression analyses.

3   We note that the heterogeneity in gene expression prediction between males and females was

4   substantial, and that these differences were not overcome by the power gain from increasing the

5   sample size when combining males and females. Our X chromosome gene models fit within

6   males and females are remarkably different from prior reports of similar sex-stratified analyses

7   of the autosomes, also of the GTEx dataset [32]. This finding provides support that differences

8   between male and female models are not driven by sample size differences and may also point to

9   biological differences in the evolution of the X chromosome. For example, prior reports have

10   shown depletion of regulatory variants on the X chromosome [20], etc.

11   Our strategy of selecting *statistically significant* expression prediction models only is

12   conservative, and the general distribution of prediction R^2 suggests that models not meeting our

13   statistical significance criteria may still be useful in TWAS predictions. We also chose an

14   $\alpha = 0.5$ for all subsequent analyses as the R^2 distribution was not significantly different from

15   our ANOVA analyses; however, we note that in cortex specifically, slightly more genes are

16   modeled using an $\alpha = 0.25$. Therefore, for some TWAS applications, a lower alpha threshold

17   may be preferred to capture additional genes.

18   Even though we only reported the elastic net penalized models in brain cortex and blood, the

19   modeling strategy can be applied to all the other tissues to train the tissue-specific gene

20   expression prediction models. It will then be possible to detect putative sex-specific disease risk

21   genes on the X chromosome with individual level genetic data or GWAS summary statistics.

22   *ARMCX6*, as our putative AD risk gene, also has nominally significant p-values (p<0.05) when

23   the GWAS model did not adjust for APOE ($p = 0.0426$) or age ($p = 0.0454$). Due to the

24

1   correlation among gene expression models by tissue and sex, a Bonferroni correction for all

2   statistical tests is overly conservative; we, therefore, corrected our hypothesis tests for 25 tests

3   (in female-only cortex TWAS) to establish an $\alpha = 0.002$.

4   *ARMCX6* was reported to be associated with mitochondrial dynamics in neurons [35]. *ARMCX6*

5   and other genes in the *Armcx* cluster are highly expressed in the human nervous system. These

6   genes encode a family of mitochondrial proteins regulating mitochondrial trafficking, which may

7   increase the risk of neurodegenerative diseases. A human brain proteomics study [36] suggests

8   that *ARMCX6* has sex-differentiated expression at the mRNA level.

9   There are a few SNPs near *ARMCX6* that were reported to be GWAS hits from the GWAS

10   Catalog[37]. None of these variants were selected in our modeling process; some variants were

11   not sequenced in GTEx, while others were removed by the MAF threshold. One GWAS hit,

12   rs5951278, 25,389 base pairs upstream of *ARMCX6* is associated with educational acquisition

13   [38]. This association on the X chromosome can either represent education as a potential

14   confounder of the protection from AD or suggest a potentially shared pathway between neural

15   development/degeneration and educational attainment. In addition, rs148260947, 271,216 base

16   pairs upstream of *ARMCX6*, is reported to be associated with cognitive function measured by

17   mini-mental state examination (MMSE) at baseline [18].

18   The TWAS Atlas [39] reports two X chromosome genes associated to AD, *ZC3H12B* [40] and

19   *ARHGAP4* [41]. We could not generate reliable sex-stratified TWAS models for either of these

20   genes in our analyses. Notably, these prior studies applied TWAS pipelines to the X

21   chromosome without addressing genetic differences due to sex. We have demonstrated from our

22   models that gene expression on the X chromosome should be studied in a sex-stratified manner.

1 There are several limitations to our study. First, we have a limited sample size; while the sex-

2 combined eQTL sample sizes are generally substantially smaller than other types of genetic

3 studies due to the expected larger effect sizes, our sex-stratified analyses are effectively halving

4 the total sample. This extremely small sample size on the X chromosome limits the power for

5 both eQTL mapping and prediction modeling. We also restricted analyses to whole blood and

6 cortex tissues in this study. This was due to the availability of validation datasets and their

7 relevance for AD. Many AD GWAS datasets and eQTL datasets of brain samples have excluded

8 genetic variation on the X chromosome, a practice which persists despite attempts by funding

9 agencies to promote sex chromosome studies. Our small X chromosome eQTL sample size

10 limited the power to detect AD risk genes with TWAS pipelines. Another potential approach to

11 allow for sex-specific effects while conserving statistical power is meta-analysis [21,22].

12 Therefore, we explored the possibility of performing a meta-analysis across sex-stratified models

13 in each tissue using the LASSOSum approach [42]; however, these models were difficult to fit

14 due to model convergence issues and were not included in our evaluations.

15 Despite these limitations, this work provides a set of prediction models to enable X chromosome

16 TWAS analyses of blood and cortex. Based on our results, we recommend using sex-stratified

17 models for all X chromosome genes. We also detect a putative risk gene, *ARMCX6*, for AD on

18 the X chromosome, which highlights the potential role of X chromosome variation in

19 Alzheimer's disease.

20

1   Mews for the sex-stratified meta-analysis with LASSOSUM, as well as Yousef Mustafa's insight

2   into visualizing TWAS results.

3   The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the

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6   from dbGaP accession number phs000424.v7.p2 on 04/03/2018.

7   The results published here are in part based on the MayoRNAseq data obtained from the AD

8   Knowledge Portal (https://adknowledgeportal.org). The data available in the AD Knowledge

9   Portal would not be possible without the participation of research volunteers and the contribution

10   of data by collaborating researchers.

15   **Conflict of Interest**

16   The authors have no conflict of interest to report.

17   **Availability statement**

18   The GTEx data is openly available via from dbGaP. The MayoRNAseq data is openly available

19   at the AD Knowledge Portal (https://adknowledgeportal.org). The sex-stratified predictive

20   models of X chromosome genes in whole blood and brain cortex are available at

21   https://github.com/bushlab-genomics/ChrX_predixcan.

22

1

1 References

2 [1] Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky
3   KJ, György B, Breakefield XO, Tanzi RE, Moir RD (2018) Alzheimer's Disease-Associated β-
4   Amyloid Is Rapidly Seeded by Herpesviridae to Protect against Brain Infection. *Neuron* **99**, 56-
5   63.e3.

6 [2] Wainer Katsir K, Linial M (2019) Human genes escaping X-inactivation revealed by single cell
7   expression data. *BMC Genomics* **20**, 201.

8 [3] Navarro□Cobos MJ, Balaton BP, Brown CJ (2020) Genes that escape from X□chromosome
9   inactivation: Potential contributors to Klinefelter syndrome. *Am J Med Genet* **184**, 226–238.

10 [4] Jégu T, Aeby E, Lee JT (2017) The X chromosome in space. *Nat Rev Genet* **18**, 377–389.

11 [5] Carrel L, Willard HF (2005) X-inactivation profile reveals extensive variability in X-linked gene
12   expression in females. *Nature* **434**, 400–404.

13 [6] Plenge RM, Stevenson RA, Lubs HA, Schwartz CE, Willard HF (2002) Skewed X-Chromosome
14   Inactivation Is a Common Feature of X-Linked Mental Retardation Disorders. *The American
15   Journal of Human Genetics* **71**, 168–173.

16 [7] Minks J, Robinson WP, Brown CJ (2008) A skewed view of X chromosome inactivation. *J Clin
17   Invest* **118**, 20–23.

18 [8] Schwartz CE (2013) X Chromosome. In *Brenner's Encyclopedia of Genetics* Elsevier, pp. 352–354.

19 [9] Clayton DG (2009) Sex chromosomes and genetic association studies. *Genome Medicine* **1**, 110.

20 [10] Zhu Z, Liang Z, Liany H, Yang C, Wen L, Lin Z, Sheng Y, Lin Y, Ye L, Cheng Y, Chang Y, Liu L,
21   Yang L, Shi Y, Shen C, Zhou F, Zheng X, Zhu J, Liang B, Ding Y, Zhou Y, Yin X, Tang H, Zuo
22   X, Sun L, Bei J-X, Liu J, Yang S, Yang W, Cui Y, Zhang X (2015) Discovery of a novel genetic
23   susceptibility locus on X chromosome for systemic lupus erythematosus. *Arthritis Research &
24   Therapy* **17**, 349.

25 [11] Wang J, Talluri R, Shete S (2017) Selection of X-chromosome Inactivation Model. *Cancer Inform*
26   **16**, 1176935117747272.

27 [12] Wise AL, Gyi L, Manolio TA (2013) eXclusion: Toward Integrating the X Chromosome in
28   Genome-wide Association Analyses. *The American Journal of Human Genetics* **92**, 643–647.

29 [13] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, Boland A, Vronskaya M, van
30   der Lee SJ, Amlie-Wolf A, Bellenguez C, Frizatti A, Chouraki V, Martin ER, Sleegers K,
31   Badarinarayan N, Jakobsdottir J, Hamilton-Nelson KL, Moreno-Grau S, Olaso R, Raybould R,
32   Chen Y, Kuzma AB, Hiltunen M, Morgan T, Ahmad S, Vardarajan BN, Epelbaum J, Hoffmann P,
33   Boada M, Beecham GW, Garnier JG, Harold D, Fitzpatrick AL, Valladares O, Moutet ML,
34   Gerrish A, Smith AVADG, Qu L, Bacq D, Denning N, Jian X, Zhao Y, Del Zompo M, Fox NC,
35   Choi SH, Mateo I, Hughes JT, Adams HH, Malamon J, Sanchez-Garcia F, Patel Y, Brody JA,
36   Dombroski BA, Naranjo MCD, Daniilidou M, Eiriksdottir G, Mukherjee S, Wallon D, Uphill J,
37   Aspelund T, Cantwell LB, Garzia F, Galimberti D, Hofer E, Butkiewicz M, Fin B, Scarpini E,
38   Sarnowski C, Bush WS, Meslage S, Kornhuber J, White CC, Song Y, Barber RC, Engelborghs S,
39   Sordon S, Voijnovic D, Adams PM, Vandenberghe R, Mayhaus M, Cupples LA, Albert MS, De
40   Deyn PP, Gu W, Himali JJ, Beekly D, Squassina A, Hartmann AM, Orellana A, Blacker D,
41   Rodriguez-Rodriguez E, Lovestone S, Garcia ME, Doody RS, Munoz-Fernadez C, Sussams R, Lin
42   H, Fairchild TJ, Benito YA, Holmes C, Karamujić-Čomić H, Frosch MP, Thonberg H, Maier W,
43   Roschupkin G, Ghetti B, Giedraitis V, Kawalia A, Li S, Huebinger RM, Kilander L, Moebus S,
44   Hernández I, Kamboh MI, Brundin RM, Turton J, Yang Q, Katz MJ, Concari L, Lord J, Beiser AS,
45   Keene CD, Helisalmi S, Kloszewska I, Kukull WA, Koivisto AM, Lynch A, Tarraga L, Larson EB,
46   Haapasalo A, Lawlor B, Mosley TH, Lipton RB, Solfrizzi V, Gill M, Longstreth WT, Montine TJ,
47   Frisardi V, Diez-Fairen M, Rivadeneira F, Petersen RC, Deramecourt V, Alvarez I, Salani F,
48   Ciaramella A, Boerwinkle E, Reiman EM, Fievet N, Rotter JI, Reisch JS, Hanon O, Cupidi C,
49   Andre Uitterlinden AG, Royall DR, Dufouil C, Maletta RG, de Rojas I, Sano M, Brice A,

Cecchetti R, George-Hyslop PS, Ritchie K, Tsolaki M, Tsuang DW, Dubois B, Craig D, Wu CK, Soininen H, Avramidou D, Albin RL, Fratiglioni L, Germanou A, Apostolova LG, Keller L, Koutroumani M, Arnold SE, Panza F, Gkatzima O, Asthana S, Hannequin D, Whitehead P, Atwood CS, Caffarra P, Hampel H, Quintela I, Carracedo Á, Lannfelt L, Rubinsztein DC, Barnes LL, Pasquier F, Frölich L, Barral S, McGuinness B, Beach TG, Johnston JA, Becker JT, Passmore P, Bigio EH, Schott JM, Bird TD, Warren JD, Boeve BF, Lupton MK, Bowen JD, Proitsi P, Boxer A, Powell JF, Burke JR, Kauwe JSK, Burns JM, Mancuso M, Buxbaum JD, Bonuccelli U, Cairns NJ, McQuillin A, Cao C, Livingston G, Carlson CS, Bass NJ, Carlsson CM, Hardy J, Carney RM, Bras J, Carrasquillo MM, Guerreiro R, Allen M, Chui HC, Fisher E, Masullo C, Crocco EA, DeCarli C, Bisceglio G, Dick M, Ma L, Duara R, Graff-Radford NR, Evans DA, Hodges A, Faber KM, Scherer M, Fallon KB, Riemenschneider M, Fardo DW, Heun R, Farlow MR, Kölsch H, Ferris S, Leber M, Foroud TM, Heuser I, Galasko DR, Giegling I, Gearing M, Hüll M, Geschwind DH, Gilbert JR, Morris JC, Green RC, Mayo K, Growdon JH, Feulner T, Hamilton RL, Harrell LE, Drichel D, Honig LS, Cushion TD, Huentelman MJ, Hollingworth P, Hulette CM, Hyman BT, Marshall R, Jarvik GP, Meggy A, Abner E, Menzies GE, Jin LW, Leonenko G, Real LM, Jun GR, Baldwin CT, Grozeva D, Karydas A, Russo G, Kaye JA, Kim R, Jessen F, Kowall NW, Vellas B, Kramer JH, Vardy E, LaFerla FM, Jöckel KH, Lah JJ, Dichgans M, Leverenz JB, Mann D, Levey AI, Pickering-Brown S, Lieberman AP, Klopp N, Lunetta KL, Wichmann HE, Lyketsos CG, Morgan K, Marson DC, Brown K, Martiniuk F, Medway C, Mash DC, Nöthen MM, Masliah E, Hooper NM, McCormick WC, Daniele A, McCurry SM, Bayer A, McDavid AN, Gallacher J, McKee AC, van den Bussche H, Mesulam M, Brayne C, Miller BL, Riedel-Heller S, Miller CA, Miller JW, Al-Chalabi A, Morris JC, Shaw CE, Myers AJ, Wiltfang J, O'Bryant S, Olichney JM, Alvarez V, Parisi JE, Singleton AB, Paulson HL, Collinge J, Perry WR, Mead S, Peskind E, Cribbs DH, Rossor M, Pierce A, Ryan NS, Poon WW, Nacmias B, Potter H, Sorbi S, Quinn JF, Sacchinelli E, Raj A, Spalletta G, Raskind M, Caltagirone C, Bossù P, Orfei MD, Reisberg B, Clarke R, Reitz C, Smith AVADG, Ringman JM, Warden D, Roberson ED, Wilcock G, Rogaeva E, Bruni AC, Rosen HJ, Gallo M, Rosenberg RN, Ben-Shlomo Y, Sager MA, Mecocci P, Saykin AJ, Pastor P, Cuccaro ML, Vance JM, Schneider JA, Schneider LS, Slifer S, Seeley WW, Smith AVADG, Sonnen JA, Spina S, Stern RA, Swerdlow RH, Tang M, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Van Eldik LJ, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Wilhelmsen KC, Williamson J, Wingo TS, Woltjer RL, Wright CB, Yu CE, Yu L, Saba Y, Pilotto A, Bullido MJ, Peters O, Crane PK, Bennett D, Bosco P, Coto E, Boccardi V, De Jager PL, Lleo A, Warner N, Lopez OL, Ingelsson M, Deloukas P, Cruchaga C, Graff C, Gwilliam R, Fornage M, Goate AM, Sanchez-Juan P, Kehoe PG, Amin N, Ertekin-Taner N, Berr C, Debette S, Love S, Launer LJ, Younkin SG, Dartigues JF, Corcoran C, Ikram MA, Dickson DW, Nicolas G, Campion D, Tschanz JA, Schmidt H, Hakonarson H, Clarimon J, Munger R, Schmidt R, Farrer LA, Van Broeckhoven C, C. O'Donovan M, DeStefano AL, Jones L, Haines JL, Deleuze JF, Owen MJ, Gudnason V, Mayeux R, Escott-Price V, Psaty BM, Ramirez A, Wang LS, Ruiz A, van Duijn CM, Holmans PA, Seshadri S, Williams J, Amouyel P, Schellenberg GD, Lambert JC, Pericak-Vance MA (2019) Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nature Genetics* **51**, 414–430.

[14] Jansen IE, Savage JE, Watanabe K, Bryois J, Williams DM, Steinberg S, Sealock J, Karlsson IK, Hägg S, Athanasiu L, Voyle N, Proitsi P, Witoelar A, Stringer S, Aarsland D, Almdahl IS, Andersen F, Bergh S, Bettella F, Bjornsson S, Brækhus A, Bråthen G, de Leeuw C, Desikan RS, Djurovic S, Dumitrescu L, Fladby T, Hohman TJ, Jonsson PV, Kiddle SJ, Rongve A, Saltvedt I, Sando SB, Selbæk G, Shoai M, Skene NG, Snaedal J, Stordal E, Ulstein ID, Wang Y, White LR, Hardy J, Hjerling-Leffler J, Sullivan PF, van der Flier WM, Dobson R, Davis LK, Stefansson H, Stefansson K, Pedersen NL, Ripke S, Andreassen OA, Posthuma D (2019) Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nature Genetics* **51**, 404–413.

[15] Bellenguez C, Küçükali F, Jansen IE, Kleineidam L, Moreno-Grau S, Amin N, Naj AC, Campos-Martin R, Grenier-Boley B, Andrade V, Holmans PA, Boland A, Damotte V, van der Lee SJ, Costa MR, Kuulasmaa T, Yang Q, de Rojas I, Bis JC, Yaqub A, Prokic I, Chapuis J, Ahmad S, Giedraitis V, Aarsland D, Garcia-Gonzalez P, Abdelnour C, Alarcón-Martín E, Alcolea D, Alegret M, Alvarez I, Álvarez V, Armstrong NJ, Tsolaki A, Antúnez C, Appollonio I, Arcaro M, Archetti S, Pastor AA, Arosio B, Athanasiu L, Bailly H, Banaj N, Baquero M, Barral S, Beiser A, Pastor AB, Below JE, Benchek P, Benussi L, Berr C, Besse C, Bessi V, Binetti G, Bizarro A, Blesa R, Boada M, Boerwinkle E, Borroni B, Boschi S, Bossù P, Bråthen G, Bressler J, Bresner C, Brodaty H, Brookes KJ, Brusco LI, Buiza-Rueda D, Bûrger K, Burholt V, Bush WS, Calero M, Cantwell LB, Chene G, Chung J, Cuccaro ML, Carracedo Á, Cecchetti R, Cervera-Carles L, Charbonnier C, Chen H-H, Chillotti C, Ciccone S, Claassen JAHR, Clark C, Conti E, Corma-Gómez A, Costantini E, Custodero C, Daian D, Dalmasso MC, Daniele A, Dardiotis E, Dartigues J-F, de Deyn PP, de Paiva Lopes K, de Witte LD, Debette S, Deckert J, del Ser T, Denning N, DeStefano A, Dichgans M, Diehl-Schmid J, Diez-Fairen M, Rossi PD, Djurovic S, Duron E, Düzel E, Dufouil C, Eiriksdottir G, Engelborghs S, Escott-Price V, Espinosa A, Ewers M, Faber KM, Fabrizio T, Nielsen SF, Fardo DW, Farotti L, Fenoglio C, Fernández-Fuertes M, Ferrari R, Ferreira CB, Ferri E, Fin B, Fischer P, Fladby T, Fließbach K, Fongang B, Fornage M, Fortea J, Foroud TM, Fostinelli S, Fox NC, Franco-Macías E, Bullido MJ, Frank-García A, Froelich L, Fulton-Howard B, Galimberti D, García-Alberca JM, García-González P, Garcia-Madrona S, Garcia-Ribas G, Ghidoni R, Giegling I, Giorgio G, Goate AM, Goldhardt O, Gomez-Fonseca D, González-Pérez A, Graff C, Grande G, Green E, Grimmer T, Grünblatt E, Grunin M, Gudnason V, Guetta-Baranes T, Haapasalo A, Hadjigeorgiou G, Haines JL, Hamilton-Nelson KL, Hampel H, Hanon O, Hardy J, Hartmann AM, Hausner L, Harwood J, Heilmann-Heimbach S, Helisalmi S, Heneka MT, Hernández I, Herrmann MJ, Hoffmann P, Holmes C, Holstege H, Vilas RH, Hulsman M, Humphrey J, Biessels GJ, Jian X, Johansson C, Jun GR, Kastumata Y, Kauwe J, Kehoe PG, Kilander L, Ståhlbom AK, Kivipelto M, Koivisto A, Kornhuber J, Kosmidis MH, Kukull WA, Kuksa PP, Kunkle BW, Kuzma AB, Lage C, Laukka EJ, Launer L, Lauria A, Lee C-Y, Lehtisalo J, Lerch O, Lleó A, Longstreth W, Lopez O, de Munain AL, Love S, Löwemark M, Luckcuck L, Lunetta KL, Ma Y, Macías J, MacLeod CA, Maier W, Mangialasche F, Spallazzi M, Marquié M, Marshall R, Martin ER, Montes AM, Rodríguez CM, Masullo C, Mayeux R, Mead S, Mecocci P, Medina M, Meggy A, Mehrabian S, Mendoza S, Menéndez-González M, Mir P, Moebus S, Mol M, Molina-Porcel L, Montrreal L, Morelli L, Moreno F, Morgan K, Mosley T, Nöthen MM, Muchnik C, Mukherjee S, Nacmias B, Ngandu T, Nicolas G, Nordestgaard BG, Olaso R, Orellana A, Orsini M, Ortega G, Padovani A, Paolo C, Papenberg G, Parnetti L, Pasquier F, Pastor P, Peloso G, Pérez-Cordón A, Pérez-Tur J, Pericard P, Peters O, Pijnenburg YAL, Pineda JA, Piñol-Ripoll G, Pisanu C, Polak T, Popp J, Posthuma D, Priller J, Puerta R, Quenez O, Quintela I, Thomassen JQ, Rábano A, Rainero I, Rajabli F, Ramakers I, Real LM, Reinders MJT, Reitz C, Reyes-Dumeyer D, Ridge P, Riedel-Heller S, Riederer P, Roberto N, Rodriguez-Rodriguez E, Rongve A, Allende IR, Rosende-Roca M, Royo JL, Rubino E, Rujescu D, Sáez ME, Sakka P, Saltvedt I, Sanabria Á, Sánchez-Arjona MB (2022) New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nature Genetics* **54**, 412–436.

[16] Schwartzentruber J, Cooper S, Liu JZ, Barrio-Hernandez I, Bello E, Kumasaka N, Young AMH, Franklin RJM, Johnson T, Estrada K, Gaffney DJ, Beltrao P, Bassett A (2021) Genome-wide meta-analysis, fine-mapping and integrative prioritization implicate new Alzheimer's disease risk genes. *Nature Genetics* **53**, 392–402.

[17] Wightman DP, Jansen IE, Savage JE, Shadrin AA, Bahrami S, Holland D, Rongve A, Børte S, Winsvold BS, Drange OK, Martinsen AE, Skogholt AH, Willer C, Bråthen G, Bosnes I, Nielsen JB, Fritsche LG, Thomas LF, Pedersen LM, Gabrielsen ME, Johnsen MB, Meisingset TW, Zhou W, Proitsi P, Hodges A, Dobson R, Velayudhan L, Heilbron K, Auton A, Agee M, Aslibekyan S, Babalola E, Bell RK, Bielenberg J, Bryc K, Bullis E, Cameron B, Coker D, Partida GC, Dhamija

31

D, Das S, Elson SL, Filshtein T, Fletez-Brant K, Fontanillas P, Freyman W, Gandhi PM, Hicks B, Hinds DA, Huber KE, Jewett EM, Jiang Y, Kleinman A, Kukar K, Lane V, Lin K-H, Lowe M, Luff MK, McCreight JC, McIntyre MH, McManus KF, Micheletti SJ, Moreno ME, Mountain JL, Mozaffari SV, Nandakumar P, Noblin ES, O'Connell J, Petrakovitz AA, Poznik GD, Schumacher M, Shastri AJ, Shelton JF, Shi J, Shringarpure S, Tian C, Tran V, Tung JY, Wang X, Wang W, Weldon CH, Wilton P, Sealock JM, Davis LK, Pedersen NL, Reynolds CA, Karlsson IK, Magnusson S, Stefansson H, Thordardottir S, Jonsson PV, Snaedal J, Zettergren A, Skoog I, Kern S, Waern M, Zetterberg H, Blennow K, Stordal E, Hveem K, Zwart J-A, Athanasiu L, Selnes P, Saltvedt I, Sando SB, Ulstein I, Djurovic S, Fladby T, Aarsland D, Selbæk G, Ripke S, Stefansson K, Andreassen OA, Posthuma D, 23andMe Research Team (2021) A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. *Nature Genetics* **53**, 1276–1282.

[18] Homann J, Osburg T, Ohlei O, Dobricic V, Deecke L, Bos I, Vandenberghe R, Gabel S, Scheltens P, Teunissen CE (2022) Genome-wide association study of Alzheimer's disease brain imaging biomarkers and neuropsychological phenotypes in the European medical information framework for Alzheimer's disease multimodal biomarker discovery dataset. *Frontiers in Aging Neuroscience* **14**, 840651.

[19] Hay M, Kumar V, Ricaño-Ponce I (2022) The role of the X chromosome in infectious diseases. *Briefings in Functional Genomics* **21**, 143–158.

[20] Kukurba KR, Parsana P, Balliu B, Smith KS, Zappala Z, Knowles DA, Favé MJ, Davis JR, Li X, Zhu X, Potash JB, Weissman MM, Shi J, Kundaje A, Levinson DF, Awadalla P, Mostafavi S, Battle A, Montgomery SB (2016) Impact of the X chromosome and sex on regulatory variation. *Genome Research* **26**, 768–777.

[21] de Klein N, Tsai EA, Vochteloo M, Baird D, Huang Y, Chen C-Y, van Dam S, Oelen R, Deelen P, Bakker OB, El Garwany O, Ouyang Z, Marshall EE, Zavodszky MI, van Rheenen W, Bakker MK, Veldink J, Gaunt TR, Runz H, Franke L, Westra H-J (2023) Brain expression quantitative trait locus and network analyses reveal downstream effects and putative drivers for brain-related diseases. *Nature Genetics* **55**, 377–388.

[22] Võsa U, Claringbould A, Westra H-J, Bonder MJ, Deelen P, Zeng B, Kirsten H, Saha A, Kreuzhuber R, Yazar S, Brugge H, Oelen R, de Vries DH, van der Wijst MGP, Kasela S, Pervjakova N, Alves I, Favé M-J, Agbessi M, Christiansen MW, Jansen R, Seppälä I, Tong L, Teumer A, Schramm K, Hemani G, Verlouw J, Yaghootkar H, Sönmez Flitman R, Brown A, Kukushkina V, Kalnapenkis A, Rüeger S, Porcu E, Kronberg J, Kettunen J, Lee B, Zhang F, Qi T, Hernandez JA, Arindrarto W, Beutner F, 't Hoen PAC, van Meurs J, van Dongen J, van Iterson M, Swertz MA, Jan Bonder M, Dmitrieva J, Elansary M, Fairfax BP, Georges M, Heijmans BT, Hewitt AW, Kähönen M, Kim Y, Knight JC, Kovacs P, Krohn K, Li S, Loeffler M, Marigorta UM, Mei H, Momozawa Y, Müller-Nurasyid M, Nauck M, Nivard MG, Penninx BWJH, Pritchard JK, Raitakari OT, Rotzschke O, Slagboom EP, Stehouwer CDA, Stumvoll M, Sullivan P, 't Hoen PAC, Thiery J, Tönjes A, van Dongen J, van Iterson M, Veldink JH, Völker U, Warmerdam R, Wijmenga C, Swertz M, Andiappan A, Montgomery GW, Ripatti S, Perola M, Kutalik Z, Dermitzakis E, Bergmann S, Frayling T, van Meurs J, Prokisch H, Ahsan H, Pierce BL, Lehtimäki T, Boomsma DI, Psaty BM, Gharib SA, Awadalla P, Milani L, Ouwehand WH, Downes K, Stegle O, Battle A, Visscher PM, Yang J, Scholz M, Powell J, Gibson G, Esko T, Franke L, BIOS Consortium, i2QTL Consortium (2021) Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nature Genetics* **53**, 1300–1310.

[23] Gamazon ER, Wheeler HE, Shah KP, Mozaffari SV, Aquino-Michaels K, Carroll RJ, Eyler AE, Denny JC, Nicolae DL, Cox NJ, Im HK (2015) A gene-based association method for mapping traits using reference transcriptome data. *Nature Genetics* **47**, 1091–1098.

[24] Li B, Ritchie MD (2021) From GWAS to Gene: Transcriptome-Wide Association Studies and Other Methods to Functionally Understand GWAS Discoveries. *Front Genet* **12**, 713230.

[25] Chen H-H, Petty LE, Sha J, Zhao Y, Kuzma A, Valladares O, Bush W, Naj AC, Gamazon ER, Below JE, Alzheimer's Disease Genetics Consortium IG of AP (2021) Genetically regulated expression in late-onset Alzheimer's disease implicates risk genes within known and novel loci. *Translational Psychiatry* **11**, 618.

[26] Sun Y, Zhu J, Zhou D, Canchi S, Wu C, Cox NJ, Rissman RA, Gamazon ER, Wu L (2021) A transcriptome-wide association study of Alzheimer's disease using prediction models of relevant tissues identifies novel candidate susceptibility genes. *Genome Medicine* **13**, 141.

[27] Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, Hasz R, Walters G, Garcia F, Young N, Foster B, Moser M, Karasik E, Gillard B, Ramsey K, Sullivan S, Bridge J, Magazine H, Syron J, Fleming J, Siminoff L, Traino H, Mosavel M, Barker L, Jewell S, Rohrer D, Maxim D, Filkins D, Harbach P, Cortadillo E, Berghuis B, Turner L, Hudson E, Feenstra K, Sobin L, Robb J, Branton P, Korzeniewski G, Shive C, Tabor D, Qi L, Groch K, Nampally S, Buia S, Zimmerman A, Smith A, Burges R, Robinson K, Valentino K, Bradbury D, Cosentino M, Diaz-Mayoral N, Kennedy M, Engel T, Williams P, Erickson K, Ardlie K, Winckler W, Getz G, DeLuca D, MacArthur D, Kellis M, Thomson A, Young T, Gelfand E, Donovan M, Meng Y, Grant G, Mash D, Marcus Y, Basile M, Liu J, Zhu J, Tu Z, Cox NJ, Nicolae DL, Gamazon ER, Im HK, Konkashbaev A, Pritchard J, Stevens M, Flutre T, Wen X, Dermitzakis ET, Lappalainen T, Guigo R, Monlong J, Sammeth M, Koller D, Battle A, Mostafavi S, McCarthy M, Rivas M, Maller J, Rusyn I, Nobel A, Wright F, Shabalin A, Feolo M, Sharopova N, Sturcke A, Paschal J, Anderson JM, Wilder EL, Derr LK, Green ED, Struewing JP, Temple G, Volpi S, Boyer JT, Thomson EJ, Guyer MS, Ng C, Abdallah A, Colantuoni D, Insel TR, Koester SE, Little AR, Bender PK, Lehner T, Yao Y, Compton CC, Vaught JB, Sawyer S, Lockhart NC, Demchok J, Moore HF (2013) The Genotype-Tissue Expression (GTEx) project. *Nature Genetics* **45**, 580.

[28] Battle A, Mostafavi S, Zhu X, Potash JB, Weissman MM, McCormick C, Haudenschild CD, Beckman KB, Shi J, Mei R, Urban AE, Montgomery SB, Levinson DF, Koller D (2014) Characterizing the genetic basis of transcriptome diversity through RNA-sequencing of 922 individuals. *Genome Research* **24**, 14–24.

[29] Allen M, Carrasquillo MM, Funk C, Heavner BD, Zou F, Younkin CS, Burgess JD, Chai H-S, Crook J, Eddy JA, Li H, Logsdon B, Peters MA, Dang KK, Wang X, Serie D, Wang C, Nguyen T, Lincoln S, Malphrus K, Bisceglio G, Li M, Golde TE, Mangravite LM, Asmann Y, Price ND, Petersen RC, Graff-Radford NR, Dickson DW, Younkin SG, Ertekin-Taner N (2016) Human whole genome genotype and transcriptome data for Alzheimer's and other neurodegenerative diseases. *Sci Data* **3**, 160089.

[30] Keur N, Ricaño-Ponce I, Kumar V, Matzaraki V (2022) A systematic review of analytical methods used in genetic association analysis of the X-chromosome. *Briefings in Bioinformatics* **23**, bbac287.

[31] Stegle O, Parts L, Piipari M, Winn J, Durbin R (2012) Using probabilistic estimation of expression residuals (PEER) to obtain increased power and interpretability of gene expression analyses. *Nature Protocols* **7**, 500–507.

[32] Mahoney E, Janve V, Hohman TJ, Dumitrescu L (2021) Evaluation of Sex-Aware PrediXcan Models for Predicting Gene Expression. In *Biocomputing 2022* WORLD SCIENTIFIC, Kohala Coast, Hawaii, USA, pp. 361–372.

[33] Wheeler HE, Shah KP, Brenner J, Garcia T, Aquino-Michaels K, GTEx Consortium, Cox NJ, Nicolae DL, Im HK (2016) Survey of the Heritability and Sparse Architecture of Gene Expression Traits across Human Tissues. *PLoS Genet* **12**, e1006423.

[34] Barbeira AN, Dickinson SP, Bonazzola R, Zheng J, Wheeler HE, Torres JM, Torstenson ES, Shah KP, Garcia T, Edwards TL, Stahl EA, Huckins LM, Aguet F, Ardlie KG, Cummings BB, Gelfand ET, Getz G, Hadley K, Handsaker RE, Huang KH, Kashin S, Karczewski KJ, Lek M, Li XX,

MacArthur DG, Nedzel JL, Nguyen DT, Noble MS, Segrè AV, Trowbridge CA, Tukiainen T, Abell NS, Balliu B, Barshir R, Basha O, Battle A, Bogu GK, Brown A, Brown CD, Castel SE, Chen LS, Chiang C, Conrad DF, Damani FN, Davis JR, Delaneau O, Dermitzakis ET, Engelhardt BE, Eskin E, Ferreira PG, Frésard L, Gamazon ER, Garrido-Martín D, Gewirtz ADH, Gliner G, Gloudemans MJ, Guigo R, Hall IM, Han B, He Y, Hormozdiari F, Howald C, Jo B, Kang EY, Kim Y, Kim-Hellmuth S, Lappalainen T, Li G, Li XX, Liu B, Mangul S, McCarthy MI, McDowell IC, Mohammadi P, Monlong J, Montgomery SB, Muñoz-Aguirre M, Ndungu AW, Nobel AB, Oliva M, Ongen H, Palowitch JJ, Panousis N, Papasaikas P, Park YS, Parsana P, Payne AJ, Peterson CB, Quan J, Reverter F, Sabatti C, Saha A, Sammeth M, Scott AJ, Shabalin AA, Sodaei R, Stephens M, Stranger BE, Strober BJ, Sul JH, Tsang EK, Urbut S, Van De Bunt M, Wang G, Wen X, Wright FA, Xi HS, Yeger-Lotem E, Zappala Z, Zaugg JB, Zhou YH, Akey JM, Bates D, Chan J, Claussnitzer M, Demanelis K, Diegel M, Doherty JA, Feinberg AP, Fernando MS, Halow J, Hansen KD, Haugen E, Hickey PF, Hou L, Jasmine F, Jian R, Jiang L, Johnson A, Kaul R, Kellis M, Kibriya MG, Lee K, Li JB, Li Q, Lin J, Lin S, Linder S, Linke C, Liu Y, Maurano MT, Molinie B, Nelson J, Neri FJ, Park YS, Pierce BL, Rinaldi NJ, Rizzardi LF, Sandstrom R, Skol A, Smith KS, Snyder MP, Stamatoyannopoulos J, Tang H, Wang L, Wang M, Van Wittenberghe N, Wu F, Zhang R, Nierras CR, Branton PA, Carithers LJ, Guan P, Moore HM, Rao A, Vaught JB, Gould SE, Lockart NC, Martin C, Struewing JP, Volpi S, Addington AM, Koester SE, Little AR, Brigham LE, Hasz R, Hunter M, Johns C, Johnson M, Kopen G, Leinweber WF, Lonsdale JT, McDonald A, Mestichelli B, Myer K, Roe B, Salvatore M, Shad S, Thomas JA, Walters G, Washington M, Wheeler J, Bridge J, Foster BA, Gillard BM, Karasik E, Kumar R, Miklos M, Moser MT, Jewell SD, Montroy RG, Rohrer DC, Valley DR, Davis DA, Mash DC, Undale AH, Smith AM, Tabor DE, Roche NV, McLean JA, Vatanian N, Robinson KL, Sobin L, Barcus ME, Valentino KM, Qi L, Hunter S, Hariharan P, Singh S, Um KS, Matose T, Tomaszewski MM, Barker LK, Mosavel M, Siminoff LA, Traino HM, Flicek P, Juettemann T, Ruffier M, Sheppard D, Taylor K, Trevanion SJ, Zerbino DR, Craft B, Goldman M, Haeussler M, Kent WJ, Lee CM, Paten B, Rosenbloom KR, Vivian J, Zhu J, Nicolae DL, Cox NJ, Im HK (2018) Exploring the phenotypic consequences of tissue specific gene expression variation inferred from GWAS summary statistics. *Nature Communications* **9**, 1–20.

[35] López-Doménech G, Serrat R, Mirra S, D'Aniello S, Somorjai I, Abad A, Vitureira N, García-Arumí E, Alonso MT, Rodriguez-Prados M, Burgaya F, Andreu AL, García-Sancho J, Trullas R, Garcia-Fernàndez J, Soriano E (2012) The Eutherian Armcx genes regulate mitochondrial trafficking in neurons and interact with Miro and Trak2. *Nat Commun* **3**, 814.

[36] Wingo AP, Liu Y, Gerasimov ES, Vattathil SM, Liu J, Cutler DJ, Epstein MP, Blokland GAM, Thambisetty M, Troncoso JC, Duong DM, Bennett DA, Levey AI, Seyfried NT, Wingo TS (2023) Sex differences in brain protein expression and disease. *Nature Medicine*.

[37] Sollis E, Mosaku A, Abid A, Buniello A, Cerezo M, Gil L, Groza T, Güneş O, Hall P, Hayhurst J, Ibrahim A, Ji Y, John S, Lewis E, MacArthur JAL, McMahon A, Osumi-Sutherland D, Panoutsopoulou K, Pendlington Z, Ramachandran S, Stefancsik R, Stewart J, Whetzel P, Wilson R, Hindorff L, Cunningham F, Lambert SA, Inouye M, Parkinson H, Harris LW (2023) The NHGRI-EBI GWAS Catalog: knowledgebase and deposition resource. *Nucleic Acids Research* **51**, D977–D985.

[38] Okbay A, Wu Y, Wang N, Jayashankar H, Bennett M, Nehzati SM, Sidorenko J, Kweon H, Goldman G, Gjorgjieva T, Jiang Y, Hicks B, Tian C, Hinds DA, Ahlskog R, Magnusson PKE, Oskarsson S, Hayward C, Campbell A, Porteous DJ, Freese J, Herd P, Agee M, Alipanahi B, Auton A, Bell RK, Bryc K, Elson SL, Fontanillas P, Furlotte NA, Hinds DA, Huber KE, Kleinman A, Litterman NK, McCreight JC, McIntyre MH, Mountain JL, Northover CAM, Pitts SJ, Sathirapongsasuti JF, Sazonova OV, Shelton JF, Shringarpure S, Tung JY, Vacic V, Wilson CH, Fontana MA, Pers TH, Rietveld CA, Chen G-B, Emilsson V, Meddens SFW, Pickrell JK, Thom K, Timshel P, de Vlaming R, Abdellaoui A, Ahluwalia TS, Bacelis J, Baumbach C, Bjornsdottir G,

Brandsma JH, Concas MP, Derringer J, Galesloot TE, Girotto G, Gupta R, Hall LM, Harris SE, Hofer E, Horikoshi M, Huffman JE, Kaasik K, Kalafati IP, Karlsson R, Lahti J, van der Lee SJ, de Leeuw C, Lind PA, Lindgren K-O, Liu T, Mangino M, Marten J, Mihailov E, Miller MB, van der Most PJ, Oldmeadow C, Payton A, Pervjakova N, Peyrot WJ, Qian Y, Raitakari O, Rueedi R, Salvi E, Schmidt B, Schraut KE, Shi J, Smith AV, Poot RA, Pourcain BS, Teumer A, Thorleifsson G, Verweij N, Vuckovic D, Wellmann J, Westra H-J, Yang J, Zhao W, Zhu Z, Alizadeh BZ, Amin N, Bakshi A, Baumeister SE, Biino G, Bønnelykke K, Boyle PA, Campbell H, Cappuccio FP, Davies G, De Neve J-E, Deloukas P, Demuth I, Ding J, Eibich P, Eisele L, Eklund N, Evans DM, Faul JD, Feitosa MF, Forstner AJ, Gandin I, Gunnarsson B, Halldórsson BV, Harris TB, Heath AC, Hocking LJ, Holliday EG, Homuth G, Horan MA, Hottenga J-J, de Jager PL, Joshi PK, Jugessur A, Kaakinen MA, Kähönen M, Kanoni S, Keltigangas-Järvinen L, Kiemeney LALM, Kolcic I, Koskinen S, Kraja AT, Kroh M, Kutalik Z, Latvala A, Launer LJ, Lebreton MP, Levinson DF, Lichtenstein P, Lichtner P, Liewald DCM, Loukola A, Madden PA, Mägi R, Mäki-Opas T, Marioni RE, Marques-Vidal P, Meddens GA, McMahon G, Meisinger C, Meitinger T, Milaneschi Y, Milani L, Montgomery GW, Myhre R, Nelson CP, Nyholt DR, Ollier WER, Palotie A, Paternoster L, Pedersen NL, Petrovic KE, Räikkönen K, Ring SM, Robino A, Rostapshova O, Rudan I, Rustichini A, Salomaa V, Sanders AR, Sarin A-P, Schmidt H, Scott RJ, Smith BH, Smith JA, Staessen JA, Steinhagen-Thiessen E, Strauch K, Terracciano A, Tobin MD, Ulivi S, Vaccargiu S, Quaye L, van Rooij FJA, Venturini C, Vinkhuyzen AAE, Völker U, Völzke H, Vonk JM, Vozzi D, Waage J, Ware EB, Willemsen G, Attia JR, Bennett DA, Berger K, Bertram L, Bisgaard H, Boomsma DI, Borecki IB, Bültmann U, Chabris CF, Cucca F, Cusi D, Deary IJ, Dedoussis GV, van Duijn CM, Eriksson JG, Franke B, Franke L, Gasparini P, Gejman PV, Gieger C, Grabe H-J, Gratten J, Groenen PJF, Gudnason V, van der Harst P, Hoffmann W, Hyppönen E, Iacono WG, Jacobsson B, Järvelin M-R, Jöckel K-H, Kaprio J, Kardia SLR, Lehtimäki T, Lehrer SF, Martin NG, McGue M, Metspalu A, Pendleton N, Penninx BWJH, Perola M, Pirastu N, Pirastu M, Polasek O, Posthuma D, Power C, Province MA, Samani NJ, Schlessinger D, Schmidt R, Sørensen TIA, Spector TD, Stefansson K, Thorsteinsdottir U, Thurik AR, Timpson NJ, Tiemeier H, Uitterlinden AG, Vitart V, Vollenweider P, Weir DR, Wilson JF, Wright AF, Conley DC, Krueger RF, Smith GD, Hofman A, Laibson DI, Medland SE, Yang J, Esko T, Watson C, Jala J, Conley D, Koellinger PD, Johannesson M, Laibson D, Meyer MN, Lee JJ, Kong A, Yengo L, Cesarini D, Turley P, Visscher PM, Beauchamp JP, 23andMe Research Team, Social Science Genetic Association Consortium, LifeLines Cohort Study (2022) Polygenic prediction of educational attainment within and between families from genome-wide association analyses in 3 million individuals. *Nature Genetics* **54**, 437–449.

[39] Lu M, Zhang Y, Yang F, Mai J, Gao Q, Xu X, Kang H, Hou L, Shang Y, Qain Q, Liu J, Jiang M, Zhang H, Bu C, Wang J, Zhang Z, Zhang Z, Zeng J, Li J, Xiao J (2023) TWAS Atlas: a curated knowledgebase of transcriptome-wide association studies. *Nucleic Acids Research* **51**, D1179–D1187.

[40] Luningham J, Chen J, Tang S, De Jager P, Bennett D, Buchman A, Yang J (2020) Bayesian Genome-wide TWAS Method to Leverage both cis- and trans-eQTL Information through Summary Statistics. *The American Journal of Human Genetics* **107**, 1–13.

[41] Liu W, Li M, Zhang W, Zhou G, Wu X, Wang J, Lu Q, Zhao H (2020) Leveraging functional annotation to identify genes associated with complex diseases. *PLOS Computational Biology* **16**, e1008315.

[42] Mak TSH, Porsch RM, Choi SW, Zhou X, Sham PC (2017) Polygenic scores via penalized regression on summary statistics. *Genetic Epidemiology* **41**, 469–480.