HGG Advances

ARTICLE

Transcriptome-wide association study identifies novel candidate susceptibility genes for migraine

Travis J. Meyers,[1] Jie Yin,[1] Victor A. Herrera,[1] Alice R. Pressman,[2,3] Thomas J. Hoffmann,[2,4] Catherine Schaefer,[1] Andrew L. Avins,[1,2] and Hélène Choquet[1,5,*]

Summary

Genome-wide association studies (GWASs) have identified more than 130 genetic susceptibility loci for migraine; however, how most of these loci impact migraine development is unknown. To identify novel genes associated with migraine and interpret the transcriptional products of those genes, we conducted a transcriptome-wide association study (TWAS). We performed tissue-specific and multi-tissue TWAS analyses to assess associations between imputed gene expression from 53 tissues and migraine susceptibility using FUSION software. Meta-analyzed GWAS summary statistics from 26,052 migraine cases and 487,214 controls, all of European ancestry and from two cohorts (the Kaiser Permanente GERA and the UK Biobank), were used. We evaluated the associations for genes after conditioning on variant-level effects from GWAS, and we tested for colocalization of GWAS migraine-associated loci and expression quantitative trait loci (eQTLs). Across tissue-specific and multi-tissue analyses, we identified 53 genes for which genetically predicted gene expression was associated with migraine after correcting for multiple testing. Of these 53 genes, 10 (*ATF5*, *CNTNAP1*, *KTN1-AS1*, *NEIL1*, *NEK4*, *NNT*, *PNKP*, *RUFY2*, *TUBG2*, and *VAT1*) did not overlap known migraine-associated loci identified from GWAS. Tissue-specific analysis identified 45 gene-tissue pairs and cardiovascular tissues represented the highest proportion of the Bonferroni-significant gene-tissue pairs (n = 22 [49%]), followed by brain tissues (n = 6 [13%]), and gastrointestinal tissues (n = 4 [9%]). Colocalization analyses provided evidence of shared genetic variants underlying eQTL and GWAS signals in 18 of the gene-tissue pairs (40%). Our TWAS reports novel genes for migraine and highlights the important contribution of brain, cardiovascular, and gastrointestinal tissues in migraine susceptibility.

Introduction

Migraine (MIM: 157300) is a syndromic neurological disease[1] that affects approximately 14% of the global population and is the second most common source of years lived with disability.[2,3] While classified as a headache disorder primarily involving dysregulated sensory processing in the brain,[1] migraine has been associated with a wide range of symptoms, including gastroesophageal reflux, diarrhea, constipation, and nausea[4]; sound and light sensitivity and disturbed vision.[5–7] Migraine has also been associated with myocardial infarction and ischemic stroke, particularly in women, even after adjusting for cardiovascular disease risk factors.[8–14] This suggests that multiple mechanisms underly migraine pathophysiology and its symptoms; however, those mechanisms are still poorly understood.

Although family and twin-based studies have estimated the heritability of migraine ranging from 30% to 60%,[15–18] SNP array-based heritability is estimated to be approximately 10% to 15%.[19] Over the past decade, genome-wide association studies (GWASs) have identified more than 130 genetic susceptibility loci for migraine in adults[19–25]; however, these loci only explain a small fraction of migraine heritability, and the causal genes underlying those associations remain poorly understood.

We have previously conducted a multiethnic GWAS meta-analysis of migraine,[20] using the Kaiser Permanente Northern California (KPNC) Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, the UK Biobank, and data from the International Headache Genetics Consortium,[21] and identified 45 novel genetic loci associated at a genome-wide level of significance ($p < 5 \times 10^{-8}$) with migraine. However, for the majority of these loci, the impact on migraine etiology is unknown, and whether these loci regulate genes differentially across tissues has not yet been explored. Recently, transcriptome-wide association studies (TWAS), which integrate data from GWAS and tissue gene expression datasets, have emerged as an effective approach to identify gene-trait associations.[26–30]

The current study builds on our previous GWAS meta-analysis of migraine[20] that also reported strong positive genetic correlations between migraine with neck, shoulder, or back pain, and anxiety or depression, suggesting shared genetic factors and mechanisms underlying those conditions. Here, we conduct a TWAS of migraine to identify novel associated genes and interpret the transcriptional and disease risk mechanisms for putative migraine risk genes. We impute gene expression into GWAS data (26,052 migraine cases and 487,214 controls of European descent from the GERA and UK Biobank cohorts) from our previous study[20]

[1]Division of Research, Kaiser Permanente Northern California, Oakland, CA 94612, USA; [2]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA 94158, USA; [3]Sutter Health, San Francisco, CA 94107, USA; [4]Institute for Human Genetics, University of California, San Francisco, San Francisco, CA 94143, USA
[5]Lead contact
*Correspondence: helene.choquet@kp.org
https://doi.org/10.1016/j.xhgg.2023.100211.

using expression quantitative trait loci (eQTL) datasets[31] from multiple tissues (53 tissue reference panels). The different stages and datasets used for this study are summarized in an overview diagram (Figure 1). Furthermore, we analyze the frequency of TWAS genes across different tissue types, given evidence that migraine affects the not only the brain, but also the cardiovascular and gastrointestinal systems. We identify novel genes whose changes in expression may play a role in migraine susceptibility and identify tissues potentially relevant to migraine.

Material and methods

Migraine GWAS data

We used summary statistics from our recent GWAS study.[20] Briefly, we performed a meta-analysis, including 513,266 individuals of European descent (26,052 migraine cases and 487,214 controls) from two cohorts: GERA[32] and UK Biobank.[33,34] The meta-analysis was conducted using the R package "meta"[35] and fixed-effects summary estimates were calculated for an additive model. A total of 9,056,148 genetic variants passing quality control were used for the TWAS analysis.

For GERA, all study procedures were approved by the Institutional Review Board of the KPNC, and written informed consent was obtained from all participants. For UK Biobank, this research has been conducted using the UK Biobank Resource project number 14105.

eQTL data

Local (*cis*) eQTL datasets for 53 tissue types were downloaded from the FUSION website. These reference data were sourced from the Genotype Tissue Expression (GTEx) Project v7 (n = 48 tissue reference panels),[31] the CommonMind Consortium (CMC) (n = 2 tissue reference panels),[36] the Metabolic Syndrome in Men Study (METSIM) (n = 1 tissue reference panel),[37] the Netherlands Twin Registry (NTR) (n = 1 tissue reference panel),[38] and the Cardiovascular Risk in Young Finns Study (YFS) (n = 1 tissue reference panel).[39] Table S1 lists the study sources, number of participants, and number of imputable genes for each tissue reference panel.

Tissue-specific TWAS analyses

We performed a TWAS of migraine using FUSION,[30] which computes predictive models for eQTLs from reference data and tests the association between predicted gene expression with a trait from GWAS summary statistics. We conducted tissue-specific TWAS analyses using FUSION default settings and the three following data inputs: (1) the above-mentioned GWAS summary statistics for migraine; (2) FUSION gene expression predictive models for 53 reference tissues; and (3) 1000 Genomes (European ancestry) Phase 3 data from the 1000 Genomes Project[40] as a reference panel for linkage disequilibrium (LD). Model weights for tissue-specific gene expression regressed on SNPs were computed from best linear unbiased predictor, Bayesian sparse linear mixed model (BSLMM), least absolute shrinkage and selection operator, and elastic net regression, as well as from the model with the top associated SNP.

In total, we tested 260,598 gene-tissue-pair models (representing 26,434 unique genes across 53 tissue reference panels) for associations between imputed gene expression with migraine susceptibility. Associations with a Bonferroni significance p value of less than 1.92×10^{-7} (=0.05/260,598) were considered significant. Novel TWAS genes were defined as those located more than 1 Mb apart from any previously identified migraine GWAS loci (i.e., no prior GWAS SNPs within 1 Mb from the start or end of the gene).

Colocalization analyses

To assess whether GWAS SNPs colocalized with eQTLs, we conducted a Bayesian colocalization analysis using the COLOCv3.2.1 software, which is implemented in FUSION using marginal expression weights, for Bonferroni-significant TWAS associations.[41] Thus, we tested the hypothesis that a single variant in each TWAS-significant model was associated with both migraine (from the GWAS) and imputed gene expression. A Bayesian posterior probability greater than 0.9 was considered supporting evidence for colocalization.

Conditional and joint analyses

We performed conditional analyses to evaluate transcriptome-wide associations after adjusting for SNP-level effects from GWAS. We ran the COJO software program to adjust the GWAS summary statistics (the meta-analyzed results from the GERA and UK Biobank cohorts) by the most statistically significant risk variants within 1 Mb of each TWAS gene.[42] Using the marginal TWAS associations from the single-tissue analysis, we conducted a FUSION joint analysis for migraine-associated genes located on the same chromosome region within each reference panel.

Tissue enrichment analyses

To identify tissues potentially relevant to migraine, we assigned the 53 tissue reference panels to 12 anatomical categories as per Strunz et al. (2020)[43]: adipose (n = 3 reference panels), brain (n = 15), cardiovascular (n = 9), female reproductive (n = 3), gastrointestinal (n = 7), gland (n = 9), lung (n = 1), skeletal muscle (n = 1), skin (n = 2), spleen (n = 1), tibial nerve (n = 1), and transformed fibroblasts (n = 1). Table S1 lists the tissue reference panels and their corresponding anatomical categories used for this analysis. We calculated the frequency of Bonferroni-significant TWAS genes in each category. However, we expect more Bonferroni-significant TWAS genes from eQTL reference panels with more tissue donors and more imputable genes. Therefore, to compare TWAS genes across tissue types, we used the hypergeometric test to calculate the probability of observing at least as many TWAS-significant genes from all the gene-tissue pairs that we tested in each anatomical category. We also used the method of LD score regression applied to specifically expressed genes (LDSC-SEG) to test for migraine heritability enrichment near genes with the highest specific expression in a particular tissue.[44] The inputs for this analysis were the GWAS meta-analysis summary statistics from the above-mentioned migraine studies, and the "Multi_tissue_gene_expr" dataset (which included 205 cell types) from the LDSC-SEG authors' website. We used a Bonferroni-significance threshold for the number of cell types tested ($p < 0.05/205$, or approximately 2.4×10^{-4}).

Multi-tissue TWAS analysis

We carried forward the tissue-specific associations and performed an omnibus test in FUSION for associations across multiple tissues. That is, TWAS associations from all 53 tissue reference panels were jointly analyzed accounting for correlation between expression weights across tissues. We applied two filters to the omnibus test results to consider a multi-tissue gene expression test significant. First, using a Bonferroni correction, we divided the family-wise error rate of 0.05 by the effective number of genes tested (n = 14,575), and retained genes with omnibus test p values less

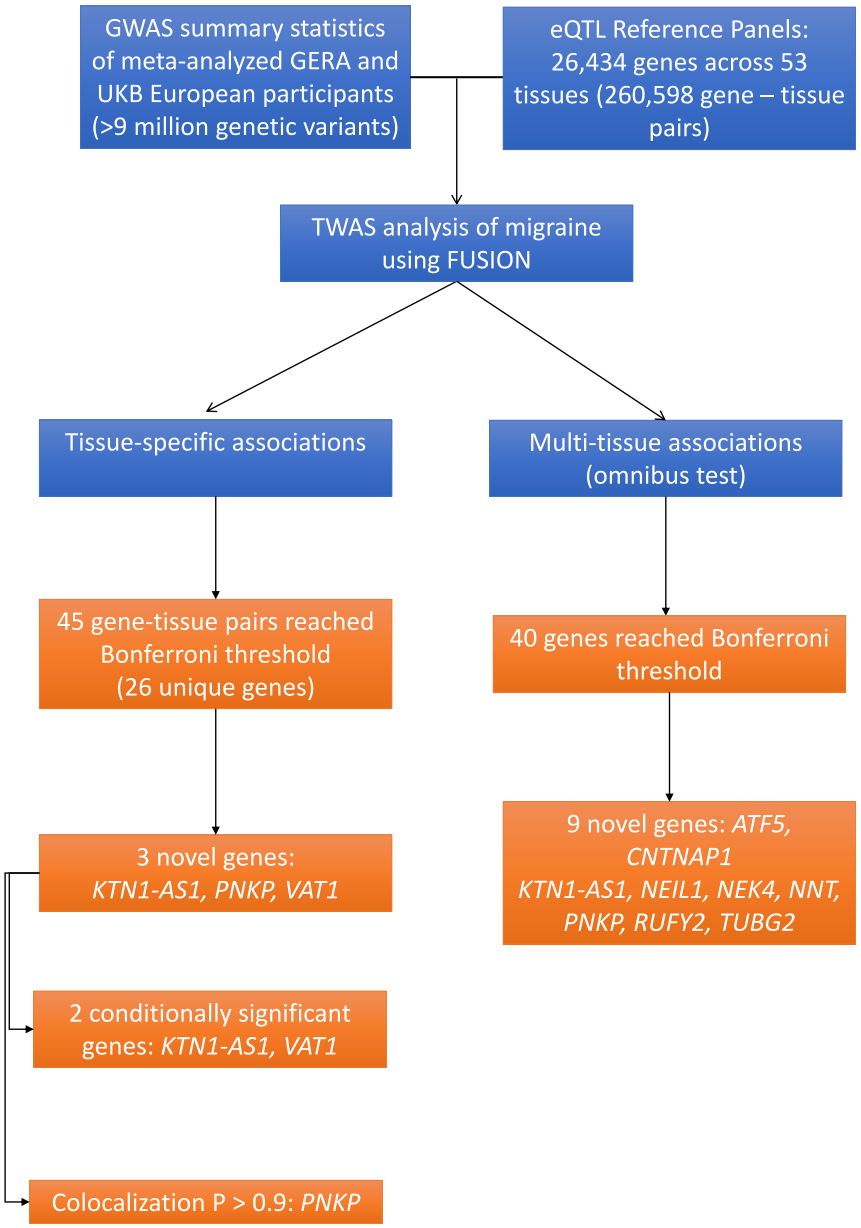

Figure 1. Diagram summarizing the datasets, analyses, and results for this study
Datasets and analyses are shown in blue. Summarized results are shown in orange.

than this value ($p < 3.43 \times 10^{-6}$); second we retained genes with a minimum tissue-specific p value suggestive of a significant association ($p < 1 \times 10^{-5}$) as described by Barbeira et al.[29]

Results

Tissue-specific TWAS analysis identified 45 gene-tissue pairs associated with migraine

We found that 45 gene-tissue pairs reached the Bonferroni significance threshold for their associations between imputed gene expression with migraine susceptibility (Table 1, Figure 2). One gene-tissue pair (*MRVI1* [MIM: 604673] in the CMC.BRAIN.RNASEQ_SPLICING panel) was associated with migraine according to three separate models (two elastic net models and the BSLMM). These 45 gene-tissue pairs were represented by 26 unique genes across 26 tissue reference panels. Importantly, 3 of the 26 unique genes did not overlap previously identified migraine GWAS loci (*KTN1-AS1* on chromosome 14 [no MIM number available], *PNKP* [MIM: 605610] on chromosome 19, and *VAT1* [MIM: 604631] on chromosome 17).

Furthermore, 17 genes were Bonferroni significant in only one tissue reference panel each, and nine were Bonferroni significant in more than one tissue reference panel. These included *UFL1* (MIM: 613372; eight panels: subcutaneous adipose, tibial artery, pancreas, tibial nerve, stomach, spleen, whole blood, and brain–cerebellum); *TJP2* (MIM: 607709; five panels: gastroesophageal junction, esophageal muscularis, spleen, and whole blood from two studies); *RP1-257A7.5* (no MIM number available; three panels: aortic, coronary, and tibial artery); and six genes significant in two panels each (*FXN* [MIM: 606829], *LRP1* [MIM: 107770], *PHACTR1* [OMIM: 608723], *STAT6* [MIM: 601512], *TMEM194A* [MIM: 616496], and *TSPAN2* [MIM: 613133]) (Table 1). Increased predicted expression was associated with increased migraine susceptibility for 32 of the Bonferroni-significant gene-tissue pairs (e.g., *LRP1*-tibial artery; $z = 10.9$), and with decreased migraine susceptibility for the other 13 Bonferroni-significant tests (e.g., *SUGCT* [MIM: 609187] aortic artery; $z = -6.85$).

In addition, we tested sex-specific TWAS associations using sex-specific GWAS summary statistics and tissue reference panels, that is, GWAS summary statistics from women for TWAS of ovary, uterus, and vagina eQTLs; and GWAS summary statistics from men for TWAS of prostate and testis eQTLs. None of the sex-specific tests reached the Bonferroni significance level that we applied to the main analysis ($p < 1.92 \times 10^{-7}$). Marginally significant associations ($p < 1 \times 10^{-5}$) are reported in Table S2.

Importance of brain, cardiovascular, and gastrointestinal tissues in migraine susceptibility

Across the 53 tissue reference panels, the greatest number of Bonferroni-significant gene-tissue pairs was observed

Table 1. TWAS analysis of migraine identified 45 Bonferroni-significant gene-tissue pairs

Gene	CHR	Tissue reference panel	Source of tissue reference panel	TWAS.Z	TWAS.P	TWAS.P.Conditional	COLOC.PP4
C12orf4	12	Artery_Tibial	GTEx	7.31	2.75×10^{-13}	8.95×10^{-01}	1
CCND2	12	Artery_Tibial	GTEx	7.33	2.23×10^{-13}	7.60×10^{-01}	1
FAM189A2	9	Small_Intestine_Terminal_Ileum	GTEx	−5.35	8.94×10^{-8}	9.60×10^{-01}	0.05
FXN	9	Muscle_Skeletal	GTEx	6.2	5.61×10^{-10}	1.80×10^{-01}	0.8
FXN	9	NTR.BLOOD.RNAARR	NTR	5.5	3.83×10^{-08}	9.69×10^{-02}	0.89
GJA1	6	Artery_Tibial	GTEx	5.87	4.46×10^{-09}	3.63×10^{-01}	1
HBCBP	12	Adrenal_Gland	GTEx	6.15	7.77×10^{-10}	2.06×10^{-01}	0.07
KIAA0776	6	NTR.BLOOD.RNAARR	NTR	9.45	3.55×10^{-21}	9.99×10^{-01}	0.02
KTN1-AS1[a]	14	Cells_Transformed_fibroblasts	GTEx	5.49	4.08×10^{-08}	6.65×10^{-04}[b]	0.15
LRP1	12	Artery_Tibial	GTEx	10.9	1.22×10^{27}	8.68×10^{-01}	1
LRP1	12	Skin_Sun_Exposed_Lower_leg	GTEx	8.4	4.51×10^{-17}	2.67×10^{-01}	0.97
MEF2D	1	Artery_Tibial	GTEx	5.97	2.44×10^{-09}	2.61×10^{-01}	0.9
MRVI1[c]	11	CMC.BRAIN.RNASEQ_SPLICING	CMC	6.81	1.01×10^{-11}	9.27×10^{-02}	0.97
MRVI1[d]	11	CMC.BRAIN.RNASEQ_SPLICING	CMC	−6.65	2.88×10^{-11}	2.74×10^{-01}	1
MRVI1[e]	11	CMC.BRAIN.RNASEQ_SPLICING	CMC	5.64	1.68×10^{-8}	9.31×10^{-01}	0.95
NXPH4	12	METSIM.ADIPOSE.RNASEQ	METSIM	6.15	7.86×10^{-10}	1.83×10^{-1}	0.63
PHACTR1	6	Artery_Aorta	GTEx	8.8	1.42×10^{-18}	2.76×10^{-01}	1
PHACTR1	6	Artery_Tibial	GTEx	8.34	7.15×10^{-17}	3.10×10^{-01}	1
PNKP[a]	19	CMC.BRAIN.RNASEQ	CMC	−5.23	1.70×10^{-07}	2.34×10^{-01}	0.99
RP11-264C15.2	9	Testis	GTEx	−5.51	3.67×10^{-08}	5.79×10^{-01}	0.42
RP1-257A7.5	6	Artery_Aorta	GTEx	8.67	4.48×10^{-18}	7.76×10^{-01}	1
RP1-257A7.5	6	Artery_Coronary	GTEx	6.94	3.92×10^{-12}	9.97×10^{-03}[b]	0.04
RP1-257A7.5	6	Artery_Tibial	GTEx	8.67	4.48×10^{-18}	7.76×10^{-01}	1
RP5-1115A15.1	1	Cells_EBV-transformed_lymphocytes	GTEx	−5.51	3.49×10^{-08}	3.90×10^{-01}	0.79
SLC45A1	1	CMC.BRAIN.RNASEQ	CMC	6.54	6.03×10^{-11}	7.99×10^{-01}	1
STAT6	12	CMC.BRAIN.RNASEQ	CMC	−6.28	3.35×10^{-10}	2.06×10^{-01}	0.03
STAT6	12	METSIM.ADIPOSE.RNASEQ	METSIM	6.08	1.22×10^{-09}	6.26×10^{-01}	0.58
SUGCT	7	Artery_Aorta	GTEx	−6.85	7.38×10^{-12}	8.24×10^{-01}	1
TAF5	10	Brain_Frontal_Cortex_BA9	GTEx	−5.65	1.61×10^{-08}	3.85×10^{-04}[b]	0.16
TJP2	9	Esophagus_Gastroesophageal_Junction	GTEx	6.22	4.87×10^{-10}	1.86×10^{-01}	0.79
TJP2	9	Esophagus_Muscularis	GTEx	6.16	7.18×10^{-10}	1.92×10^{-01}	0.76
TJP2	9	NTR.BLOOD.RNAARR	NTR	−5.72	1.05×10^{-08}	8.47×10^{-01}	0.06
TJP2	9	Spleen	GTEx	−5.5	3.80×10^{-08}	7.23×10^{-01}	0.99
TJP2	9	YFS.BLOOD.RNAARR	YFS	−5.28	1.30×10^{-07}	8.76×10^{-01}	0.08
TMEM194A	12	Heart_Atrial_Appendage	GTEx	−5.76	8.45×10^{-09}	9.34×10^{-02}	0.16
TMEM194A	12	Muscle_Skeletal	GTEx	−5.25	1.56×10^{-07}	2.10×10^{-01}	0.4
TSPAN2	1	Artery_Aorta	GTEx	7.39	1.46×10^{-13}	8.16×10^{-01}	0.99
TSPAN2	1	Artery_Tibial	GTEx	6.17	6.70×10^{-10}	3.24×10^{-01}	0.97
UFL1	6	Adipose_Subcutaneous	GTEx	9.85	7.13×10^{-23}	9.98×10^{-01}	0.42
UFL1	6	Artery_Tibial	GTEx	9.85	7.13×10^{-23}	9.98×10^{-01}	0.42

(*Continued on next page*)

Table 1. ***Continued***

Gene	CHR	Tissue reference panel	Source of tissue reference panel	TWAS.Z	TWAS.P	TWAS.P.Conditional	COLOC.PP4
UFL1	6	Brain_Cerebellum	GTEx	6.72	1.79×10^{-11}	6.42×10^{-01}	0.97
UFL1	6	Nerve_Tibial	GTEx	7.91	2.49×10^{-15}	4.38×10^{-01}	0
UFL1	6	Pancreas	GTEx	7.92	2.47×10^{-15}	4.11×10^{-01}	0.03
UFL1	6	Spleen	GTEx	7.81	5.80×10^{-15}	8.40×10^{-01}	0.89
UFL1	6	Stomach	GTEx	7.91	2.66×10^{-15}	5.96×10^{-01}	0.34
UFL1	6	Whole_Blood	GTEx	7.08	1.40×10^{-12}	8.36×10^{-01}	0.28
VAT1[a]	17	Artery_Coronary	GTEx	−5.32	1.06×10^{-07}	3.02×10^{-02}[b]	0.27

Chr, chromosome; COLOC.PP4, posterior probability of the hypothesis for a shared causal variant for both migraine and gene expression (colocalization); TWAS.P, TWAS p value; TWAS.P.Conditional, TWAS p value conditional on the top GWAS SNP within 1 Mb of the gene; TWAS.Z, TWAS z-score.
[a]These genes are novel, with no prior reported GWAS SNP within 1 Mb.
[b]Nominally significant p values ($p < 0.05$) for TWAS associations conditional on GWAS loci.
[c]From the elastic net model with eQTL = rs1863244.
[d]From the BSLMM with eQTL = rs4442541.
[e]From the elastic net model with eQTL = rs11042902.

for the GTEx tibial artery dataset (9 gene-tissue pairs) followed by the GTEx aorta dataset (4 gene-tissue pairs), the CMC RNA sequencing (RNA-seq) brain dataset (3 gene-tissue pairs), the NTR RNA array peripheral blood array dataset (3 gene-tissue pairs), and 4 panels with two significant TWAS genes (GTEx coronary artery, skeletal muscle, and spleen datasets, and the METSIM adipose RNA-seq dataset) (Table 1). To identify tissues potentially relevant to migraine, we assigned tissue reference panels to anatomical categories as listed above in the Methods (Table S1). In the tissue-specific TWAS results, cardiovascular tissues represented the highest proportion of the 45 Bonferroni-significant genes (22 genes [48.9%]), followed by brain tissues (6 genes [13.3%]), and gastrointestinal tissues (4 genes [8.9%]) (Figure 3). The p values from the hypergeometric tests accounting for the number of gene-tissue pairs tested per anatomical category were 2.36×10^{-6}, 0.77, and 0.93 for cardiovascular, brain, and gastrointestinal tissues, respectively (Table S3). Therefore, while we do not rule out the importance of brain- and gastrointestinal-specific gene expression for migraine, only cardiovascular tissues had an outsized number of TWAS associations, suggesting the importance of these tissues in migraine susceptibility. For LDSC-SEG, none of the reference cell types demonstrated enrichment for migraine GWAS heritability after multiple testing correction. Table S4 displays the complete results from LDSC-SEG, including marginally enriched cell types ($p < 0.05$).

Colocalization and conditional analyses provide additional support for migraine TWAS associations

To assess whether common genetic variants underly eQTL and GWAS associations with migraine, we conducted a colocalization analysis for Bonferroni-significant gene-tissue pairs. We found that 18 (40%) of the Bonferroni-significant gene-tissue pairs had a colocalized variant associated with both migraine risk (from GWAS) and predicted gene expression based on our TWAS results (Tables 1 and S5). Of the three TWAS associations that we identified in genes outside of known GWAS loci, colocalized GWAS and eQTL associations were evident at *PNKP* in the CMC RNA-seq brain panel (posterior probability = 0.988).

To identify TWAS signals for migraine independent of GWAS risk variants, we repeated the FUSION analysis with GWAS summary statistics conditioned on the top GWAS SNP in each of the 45 Bonferroni-significant gene-tissue pairs. We found that four gene-tissue pairs reached nominal significance ($p < 0.05$): *KTN1-AS1* (above-mentioned novel migraine gene) in GTEx-transformed fibroblast cells, *RP1-257A7.5* in GTEx coronary artery, *TAF5* (MIM: 601787) in GTEx brain frontal cortex (BA9), and *VAT1* in GTEx coronary artery (Table 1). Furthermore, we assessed joint TWAS associations in tissue reference panels with more than one Bonferroni-significant gene on the same chromosome region (Table S6). Of the five pairwise joint models including eight unique genes, all the associations were attenuated below the TWAS significance level, but retained marginal significance ($p < 0.05$). All eight of these genes (*PHACTR1*, *RP1-257A7.5*, *FXN*, *TJP2*, *C12orf4*, *CCND2*, *NXPH4*, and *STAT6*) are nearby previously identified GWAS loci for migraine.[19–21]

Multi-tissue TWAS identified nine migraine genes outside of known GWAS loci

The multi-tissue TWAS using the omnibus test in FUSION revealed 40 genes for which imputed expression was associated with migraine susceptibility (Bonferroni $p < 0.05/14{,}575$ effective gene tests approximately 3.43×10^{-6} and minimum tissue-specific $p < 1 \times 10^{-5}$) (Table 2). Of these 40 genes, 9 (22.5%) were located outside of the known GWAS migraine-associated loci: *ATF5* (MIM: 606398), *CNTNAP1* (MIM: 602346), *KTN1-AS1*, *NEIL1* (MIM: 608844), *NEK4* (MIM: 601959), *NNT* (MIM: 607878), *PNKP*, *RUFY2* (MIM: 610328), and *TUBG2* (MIM: 605785). In addition, 13 of the 40 multi-tissue associated genes

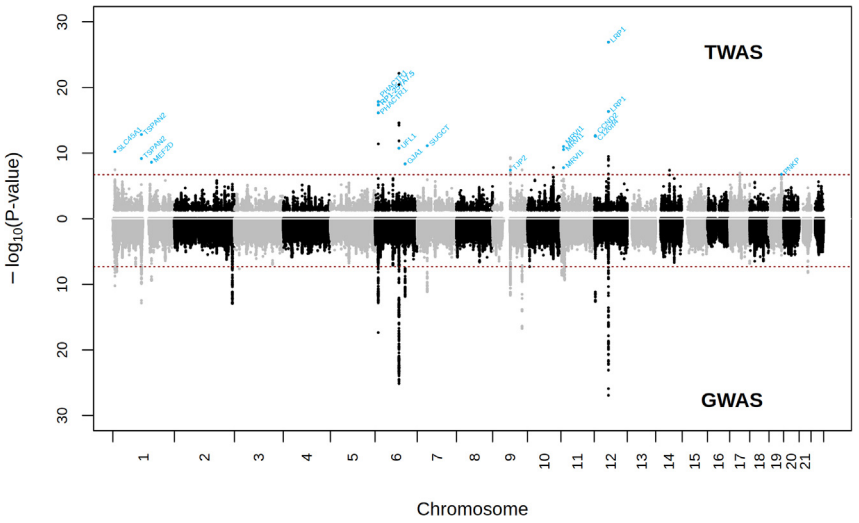

Figure 2. Miami plot of genome and transcriptome-wide associations with migraine
The top figure is the TWAS Manhattan plot for the tissue-specific analysis using information from all 53 tissues. Each point corresponds with an association test between predicted gene expression with migraine risk. The bottom figure is the Manhattan plot corresponding with the GWAS meta-analysis combining results from GERA and the UK Biobank, where each dot corresponds with an association test for an SNP with migraine risk. Blue labels indicate colocalized eQTL and GWAS signals in tissue-specific models. Red lines indicate the Bonferroni significance levels for GWAS ($p < 5 \times 10^{-8}$) and tissue-specific TWAS ($p < 1.92 \times 10^{-7}$), respectively.

were also associated with migraine in tissue-specific models, including 2 located outside of previously described migraine risk loci (*KTN1-AS1* and *PNKP*).

Discussion

Integrating GWAS summary statistics with pre-specified models for tissue-specific gene expression, we identified 53 genes associated with migraine susceptibility (13 from the tissue-specific analysis alone, 27 from the multi-tissue analysis alone, and 13 from both analyses). Of these 53, 10 were novel to the extent they did not overlap known migraine risk loci from GWAS (*ATF5*, *CNTNAP1*, *KTN1-AS1*, *NEIL1*, *NEK4*, *NNT*, *PNKP*, *RUFY2*, *TUBG2*, and *VAT1*). We also highlighted the importance of cardiovascular, brain, and gastrointestinal tissues in migraine etiology.

A recent, large GWAS meta-analysis of migraine, using PrediXcan to assess TWAS associations, identified 206 genes that were significant after Bonferroni correction.[19] Of the 53 TWAS genes that we identified in the current study (across single-tissue and multi-tissue analyses), 21 were outside of the genomic regions previously identified from PrediXcan (Table S7). These included nine of the ten genes that did not overlap known GWAS loci. As for the 10th gene, *NEK4*, which we detected from the multi-tissue test, Hautakangas et al.[19] reported PrediXcan associations for *GLT8D1*, less than 65 kb away on the same (negative) strand, from spleen and transverse colon tissue panels.

Interestingly, *KTN-AS1* and *VAT1* were associated with migraine after conditioning on the lead GWAS SNP in the respective gene regions, and *PNKP* indicated a colocalized genetic variant for both migraine risk and modified gene expression. *KTN1-AS1* (KTN1 antisense RNA 1) is a long non-coding RNA gene that has been associated with cell cycle regulation and tumorigenesis,[45] as well as acute pancreatitis.[46] *PNKP* (polynucleotide kinase 3′-phosphatase) encodes a protein that repairs DNA damage from ionizing radiation and oxidative stress.[47] Mutations in *PNKP* have been associated with ataxia with oculomotor apraxia,[48] microcephaly, and severe seizures and can result in severe neurological disease.[49,50] *VAT1* (vesicle amine transport 1) encodes a synaptic vesicle protein[51] and has been reported as a pathogenic factor in a variety of tumors in the brain or spinal cord, including glioblastoma and gliomas.[52–54] Future investigations could confirm the role of these genes in migraine pathogenesis and determine their precise role in migraine susceptibility.

Among the novel migraine-associated genes identified in this study, we also identified *CNTNAP1*, *TUBG2*, and *RUFY2*, all expressed predominantly in the brain. *CNTNAP1*, or contactin associated protein 1, is an essential component of the paranodal junctions and may be the signaling subunit of contactin, enabling recruitment and activation of intracellular signaling pathways in neurons. Mutations in *CNTNAP1* led to defects in neuronal development[55] and have been associated with severe congenital hypomyelinating neuropathy.[56] *TUBG2*, or tubulin gamma 2, has been involved in microtubule cytoskeleton organization and mitotic sister chromatid segregation and cell growth.[57] Interestingly, genomic deletions in *CNTNAP1* and *TUBG2* correlate with the under-expression of those genes in pediatric pilocytic astrocytoma, a rare childhood brain tumor.[58] *RUFY2*, or RUN and FYVE domain containing 2, has been involved in the regulation of endocytosis, human glioblastoma multiforme,[59] and beta-amyloid precursor protein secretion associated with late-onset Alzheimer disease.[60] Those findings are consistent with our tissue-specific TWAS results, which provide evidence of the importance of brain tissues in migraine susceptibility.

Another gene implicated in our migraine TWAS was *NNT*, which encodes an integral protein of the inner mitochondrial membrane, nicotinamide nucleotide transhydrogenase. Importantly, *NNT* is broadly expressed

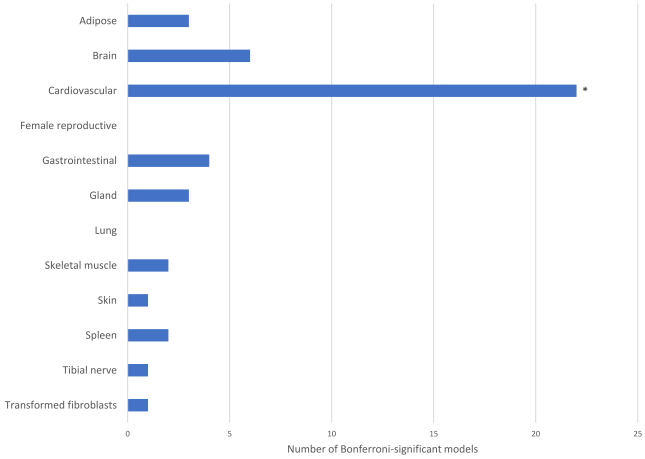

Figure 3. Bonferroni-significant TWAS genes by anatomical category

In the tissue-specific TWAS, 45 gene-tissue associations with migraine were Bonferroni-significant. To compare these TWAS associations by anatomical category, eQTL reference tissues were grouped where appropriate (see Table S1). No genes were Bonferroni significant for migraine in female reproductive or lung tissue panels. The asterisk indicates categories with a hypergeometric test p value of <0.05 accounting for the number of gene-tissue pairs tested per category.

in the heart and adrenal gland and has been implicated in free radical detoxification. Mutations in *NNT* have been shown to cause familial glucocorticoid deficiency.[61] Recently, misfolded NNT protein was found in the amygdalae of elderly, cognitively impaired subjects,[62] suggesting that *NNT* could play a key role in the neuropathologic feature of Alzheimer disease and many other neurodegenerative disorders. Another gene implicated in our migraine TWAS was *NEK4* (NIMA related kinase 4), encoding a serine/threonine protein kinase required for normal entry into replicative senescence, and involved in cell-cycle arrest in response to double-stranded DNA damage.[63] This gene also plays a role in maintaining cilium integrity, and defects have been associated with ciliopathies.[64] Finally, *NEK4* has been associated with bipolar disorder in GWAS[65] and TWAS.[66] Our study also identified *ATF5* as a novel migraine-associated gene. *ATF5* (activating transcription factor 5) promotes normal cell survival and proliferation[67–70] and modulates growth and differentiation of neural progenitor cells during murine brain development,[69,71–75] and expression has been associated with higher tumor grade and reduced patient survival for glioma.[67,69,76–79] Future studies will determine how these identified genes contribute to migraine susceptibility.

Using joint-conditional analysis, we attempted to differentiate causal versus non-causal genes for chromosomes with multiple TWAS associations, potentially attributable to gene co-regulation.[26] For example, we identified two marginally associated TWAS genes on chromosome 6p24.1 using the GTEx aorta and tibial artery references, *PHACTR1* and *RP1-257A7.5*, which span previously identified migraine GWAS risk loci. While *PHACTR1* and *RP1-257A7.5* are only 572 Kb apart, *PHACTR1* has been shown to regulate the actin cytoskeleton and endothelial cell survival,[80] and its RNA has outsized expression in the brain[81]; in contrast, *RP1-257A7.5* is a long non-coding RNA that has not been characterized yet. Therefore, *PHACTR1* has greater *a priori* evidence of involvement in migraine susceptibility compared with *RP1-257A7.5*.

We recognize potential limitations of our study. First, in the current study gene expression is predicted, not measured, so the reported TWAS associations cannot be considered causal and should be interpreted cautiously.[26] As a corollary, our TWAS reference panels model gene expression on *cis*-eQTLs, which only explain approximately 10% of the variance in genetically predicted expression.[26,82] Second, our method of testing all gene-tissue associations could produce spurious results in tissues not related to migraine.[26] Although migraine is primarily a brain disorder, the location of migraine initiation is unknown[83]; therefore, we could not prioritize any of the 13 brain reference tissues either *a priori* or from our LDSC-SEG analysis. In addition, given previous evidence implicating cardiovascular and gastrointestinal tissues in migraine susceptibility,[19,84,85] we tested models across all tissues to compare the frequency of TWAS genes by anatomical category. Moreover, we aggregated all the tissue-specific associations in FUSION's omnibus test, as recommended to decrease the likelihood of this potential bias.[26] Third, model weights from GTEx Version 8 were published on the FUSION website shortly after we completed our main analysis using GTEx Version 7 weights. Version 8 includes 49% more RNA-seq samples from 33% more tissue donors compared with Version 7, as well as splicing eQTLs.[86] Despite these limitations, our study is based on results from a large GWAS meta-analysis on more than 26,000 migraine cases, enabling causal-gene prioritization for migraine. Although a larger GWAS meta-analysis was recently published with 102,000 migraine cases,[19] our current study used GERA data from KPNC members with migraine ascertained from the electronic health record system and our previously validated migraine probability algorithm.[87] Therefore, our current study represents an independent and valuable discovery cohort for TWAS candidate genes. A general strength of TWAS is that is prioritizes effects of predicted gene expression, not just SNPs. Further, in this study, we conducted multi-tissue TWAS analysis, which enables increased statistical precision compared with single-tissue approaches.[29,88,89]

In conclusion, we identified 53 genes associated with migraine susceptibility, of which 10 did not overlap with known migraine risk loci. Our study also highlighted the important contributions of brain, cardiovascular, and gastrointestinal tissues in migraine susceptibility, consistent with previous work. Therefore, the expression of genes associated with migraine seems not to be restricted to brain tissues, as could be expected for this syndromic

Table 2. Multi-tissue omnibus test of migraine identified 40 genes meeting criteria for statistical significance

Gene	NUM.REF	MIN.TWAS.P	NUM.REF.PRUNED	OMNIBUS.P	CHR	P0	P1
AC007405.2	6	3.98×10^{-6}	6	1.16×10^{-6}	2	171568961	171571077
ANKDD1B	20	3.50×10^{-6}	18	2.60×10^{-12}	5	74907284	74967671
ARL3	18	4.75×10^{-6}	17	8.79×10^{-62}	10	104433488	104474164
ATF5[a]	20	2.79×10^{-6}	20	1.02×10^{-19}	19	50433021	50437192
C12orf4	3	2.75×10^{-13}	3	2.97×10^{-15}	12	4596894	4647674
CCND2	5	2.23×10^{-13}	5	5.78×10^{-11}	12	4382938	4414516
CNNM2	5	1.63×10^{-6}	5	2.70×10^{-7}	10	104678050	104849978
CNTNAP1[a]	25	2.44×10^{-7}	16	1.81×10^{-91}	17	40835936	40851832
DLST	5	7.46×10^{-7}	5	5.41×10^{-8}	14	75348593	75370450
FAM189A2	12	8.94×10^{-8}	12	2.34×10^{-7}	9	71939488	72007371
FXN	6	5.61×10^{-10}	6	1.31×10^{-23}	9	71650175	71715094
INA	4	4.52×10^{-6}	4	2.73×10^{-6}	10	105036920	105050108
INHBE	3	7.28×10^{-7}	3	2.76×10^{-6}	12	57846106	57853063
INPP5B	49	2.36×10^{-6}	31	2.74×10^{-31}	1	38326764	38412729
KTN1-AS1[a]	19	4.08×10^{-8}	15	2.26×10^{-42}	14	55965996	56046828
LRP1	4	1.22×10^{-27}	4	1.90×10^{-30}	12	57522276	57607134
MLXIPL	22	2.13×10^{-6}	18	6.29×10^{-91}	7	73007524	73038873
MRVI1	25	1.01×10^{-11}	24	2.10×10^{-10}	11	10594638	10715535
MSL3P1	27	5.30×10^{-6}	24	3.64×10^{-22}	2	234774083	234777090
MTMR3	48	2.35×10^{-6}	32	1.41×10^{-151}	22	30279144	30426855
NEIL1[a]	19	6.00×10^{-6}	18	1.63×10^{-13}	15	75639296	75647550
NEK4[a]	30	7.62×10^{-6}	14	7.46×10^{-8}	3	52744800	52804965
NNT[a]	31	6.62×10^{-6}	28	4.28×10^{-50}	5	43602794	43707507
NT5C2	16	4.68×10^{-7}	15	2.45×10^{-112}	10	104850368	104953056
NXPH4	3	7.86×10^{-10}	3	3.66×10^{-10}	12	57610578	57620232
PHACTR1	13	1.42×10^{-18}	13	3.17×10^{-14}	6	12717893	13288645
PIP4K2C	8	3.29×10^{-6}	8	7.15×10^{-11}	12	57984957	57997198
PNKP[a]	10	1.70×10^{-7}	9	3.06×10^{-6}	19	50364461	50371166
POC5	36	3.50×10^{-6}	24	2.98×10^{-142}	5	74969949	75013313
RBBP8	7	3.72×10^{-6}	7	1.91×10^{-7}	18	20378224	20606451
RERE	22	1.17×10^{-6}	14	3.35×10^{-10}	1	8412457	8877702
RP11-724N1.1	12	2.16×10^{-6}	9	2.64×10^{-15}	10	104674342	104675161
RP11-739L10.1	5	2.65×10^{-6}	5	5.97×10^{-10}	18	20279444	20513727
RUFY2[a]	26	8.24×10^{-6}	16	1.75×10^{-21}	10	70100864	70167051
SFXN2	25	4.22×10^{-7}	17	6.27×10^{-188}	10	104474295	104503249
STAT6	10	3.35×10^{-10}	9	6.46×10^{-14}	12	57489260	57525922
TJP2	22	4.87×10^{-10}	20	2.13×10^{-14}	9	71736224	71870124
TMEM194A	16	8.45×10^{-9}	16	6.51×10^{-10}	12	57449426	57481846
TUBG2[a]	42	1.85×10^{-6}	17	1.18×10^{-11}	17	40811323	40819024
UTP11L	15	5.20×10^{-6}	14	1.10×10^{-6}	1	38474930	38490496

CHR, chromosome; MIN.TWAS.P, minimum p value from tissue-specific models; NUM.REF, number of tissue reference panels tested for each gene; NUM.REF.PRUNED, number of tissue reference panels tested for each gene after pruning for highly LD-correlated genes; OMNIBUS.P, p value from the omnibus test; P0, start of gene (hg19); P1, end of gene (hg19).
[a]These gene are novel, with no prior reported GWAS SNP within 1 Mb.

neurological disease, and the processes underlying migraine pathology seem to be systemic as observed for other diseases.[43] Identifying which biological processes are genetically influenced and in which tissue is important for understanding migraine etiology and developing novel therapies.

Data and code availability

The combined European ancestry (GERA and UKB) meta-analysis GWAS summary statistics for migraine will be made available through the NHGRI-EBI GWAS Catalog (https://www.ebi.ac.uk/gwas/downloads/summary-statistics).

The GERA genotype data are available upon application to the KP Research Bank (https://researchbank.kaiserpermanente.org/).

FUSION models trained on the GTEx version 7 data are available here: http://gusevlab.org/projects/fusion/. Gene expression and eQTL data are freely available at https://gtexportal.org/home/datasets.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2023.100211.

Acknowledgments

We are grateful to the Kaiser Permanente Northern California members who have generously agreed to participate in the Kaiser Permanente Research Program on Genes, Environment, and Health. Support for participant enrollment, survey completion, and biospecimen collection for the RPGEH was provided by the Robert Wood Johnson Foundation, the Wayne and Gladys Valley Foundation, the Ellison Medical Foundation, and Kaiser Permanente Community Benefit Programs. Genotyping of the GERA cohort was funded by a grant from the National Institute on Aging, National Institute of Mental Health, and National Institutes of Health Common Fund (RC2 AG036607 to C.S.). H.C. was supported by the National Eye Institute (NEI) grants R01 EY027004 and R01 EY033010, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) R01 DK116738, the National Cancer Institute (NCI) R01CA241623, and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) R21 AR076009. The Genetics and Comorbidity of Migraine study was funded by a grant from the National Institute of Neurological Disorders and Stroke (NINDS) R01NS080863 (A.R.P.). The authors would also like to thank Dr. Mark Kvale for helpful discussion about the analyses.

Declaration of interests

The authors declare no competing interests.

Received: December 15, 2022
Accepted: June 5, 2023

Web resources

FUSION: http://gusevlab.org/projects/fusion/
GTEx Project v7: https://gtexportal.org/home/datasets
OMIM: https://www.omim.org/
R: https://www.R-project.org
LDSC-SEG: https://github.com/bulik/ldsc/wiki/Cell-type-specific-analyses

References

1. Goadsby, P.J., Holland, P.R., Martins-Oliveira, M., Hoffmann, J., Schankin, C., and Akerman, S. (2017). Pathophysiology of migraine: a disorder of sensory processing. Physiol. Rev. *97*, 553–622. https://doi.org/10.1152/physrev.00034.2015.
2. GBD 2016 Headache Collaborators (2018). Global, regional, and national burden of migraine and tension-type headache, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. *17*, 954–976. https://doi.org/10.1016/S1474-4422(18)30322-3.
3. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators (2017). Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet *390*, 1211–1259. https://doi.org/10.1016/S0140-6736(17)32154-2.
4. Aamodt, A.H., Stovner, L.J., Hagen, K., and Zwart, J.A. (2008). Comorbidity of headache and gastrointestinal complaints. The Head-HUNT Study. Cephalalgia *28*, 144–151. https://doi.org/10.1111/j.1468-2982.2007.01486.x.
5. Olesen, J., Bolay, H., Charles, A., Evers, S., First, M., Hershey, A., Lantéri-Minet, M., Lipton, R., MacGregor, E.A., Schytz, H.W., et al. (2018). Migraine. Headache classification committee of the international headache society (IHS). In The international classification of headache disorders, 3rd edition, *Vol. 38*, J. Olesen, L. Bendtsen, D. Dodick, A. Ducros, S. Evers, M. First, P.J. Goadsby, A. Hershey, Z. Katsarava, and M. Levin, et al., eds. (Cephalalgia), pp. 1–211. https://doi.org/10.1177/0333102417738202.
6. Global Burden of Disease Study 2013 Collaborators (2015). Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet *386*, 743–800. https://doi.org/10.1016/S0140-6736(15)60692-4.
7. Steiner, T.J., Stovner, L.J., and Birbeck, G.L. (2013). Migraine: the seventh disabler. J. Headache Pain *14*, 1. https://doi.org/10.1186/1129-2377-14-1.
8. Adelborg, K., Szépligeti, S.K., Holland-Bill, L., Ehrenstein, V., Horváth-Puhó, E., Henderson, V.W., and Sørensen, H.T. (2018). Migraine and risk of cardiovascular diseases: Danish population based matched cohort study. BMJ *360*, k96. https://doi.org/10.1136/bmj.k96.
9. Elgendy, I.Y., Nadeau, S.E., Bairey Merz, C.N., Pepine, C.J.; American College of Cardiology Cardiovascular Disease in Women Committee; and American College of Cardiology Cardiovascular Disease in Women Committee (2019). Migraine headache: an under-appreciated risk factor for cardiovascular disease in women. J. Am. Heart Assoc. *8*, e014546. https://doi.org/10.1161/JAHA.119.014546.
10. Gudmundsson, L.S., Scher, A.I., Aspelund, T., Eliasson, J.H., Johannsson, M., Thorgeirsson, G., Launer, L., and Gudnason,

V. (2010). Migraine with aura and risk of cardiovascular and all cause mortality in men and women: prospective cohort study. BMJ *341*, c3966. https://doi.org/10.1136/bmj.c3966.

11. Kurth, T., Gaziano, J.M., Cook, N.R., Logroscino, G., Diener, H.C., and Buring, J.E. (2006). Migraine and risk of cardiovascular disease in women. JAMA *296*, 283–291. https://doi.org/10.1001/jama.296.3.283.
12. Kurth, T., Winter, A.C., Eliassen, A.H., Dushkes, R., Mukamal, K.J., Rimm, E.B., Willett, W.C., Manson, J.E., and Rexrode, K.M. (2016). Migraine and risk of cardiovascular disease in women: prospective cohort study. BMJ *353*, i2610. https://doi.org/10.1136/bmj.i2610.
13. Peng, K.P., Chen, Y.T., Fuh, J.L., Tang, C.H., and Wang, S.J. (2017). Migraine and incidence of ischemic stroke: a nationwide population-based study. Cephalalgia *37*, 327–335. https://doi.org/10.1177/0333102416642602.
14. Rambarat, C.A., Elgendy, I.Y., Johnson, B.D., Reis, S.E., Thompson, D.V., Sharaf, B.L., Bittner, V., Sopko, G., Bairey Merz, C.N., Pepine, C.J., and Ahmed, B. (2017). Migraine headache and long-term cardiovascular outcomes: an extended follow-up of the women's ischemia Syndrome evaluation. Am. J. Med. *130*, 738–743. https://doi.org/10.1016/j.amjmed.2016.12.028.
15. Honkasalo, M.L., Kaprio, J., Winter, T., Heikkilä, K., Sillanpää, M., and Koskenvuo, M. (1995). Migraine and concomitant symptoms among 8167 adult twin pairs. Headache *35*, 70–78. https://doi.org/10.1111/j.1526-4610.1995.hed3502070.x.
16. Mulder, E.J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D.A., Nyholt, D.R., Martin, N.G., MacGregor, A.J., Cherkas, L.F., et al. (2003). Genetic and environmental influences on migraine: a twin study across six countries. Twin Res. *6*, 422–431. https://doi.org/10.1375/136905203770326420.
17. Polderman, T.J.C., Benyamin, B., de Leeuw, C.A., Sullivan, P.F., van Bochoven, A., Visscher, P.M., and Posthuma, D. (2015). Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat. Genet. *47*, 702–709. https://doi.org/10.1038/ng.3285.
18. Sutherland, H.G., Albury, C.L., and Griffiths, L.R. (2019). Advances in genetics of migraine. J. Headache Pain *20*, 72. https://doi.org/10.1186/s10194-019-1017-9.
19. Hautakangas, H., Winsvold, B.S., Ruotsalainen, S.E., Bjornsdottir, G., Harder, A.V.E., Kogelman, L.J.A., Thomas, L.F., Noordam, R., Benner, C., Gormley, P., et al. (2022). Genome-wide analysis of 102,084 migraine cases identifies 123 risk loci and subtype-specific risk alleles. Nat. Genet. *54*, 152–160. https://doi.org/10.1038/s41588-021-00990-0.
20. Choquet, H., Yin, J., Jacobson, A.S., Horton, B.H., Hoffmann, T.J., Jorgenson, E., Avins, A.L., and Pressman, A.R. (2021). New and sex-specific migraine susceptibility loci identified from a multiethnic genome-wide meta-analysis. Commun. Biol. *4*, 864. https://doi.org/10.1038/s42003-021-02356-y.
21. Gormley, P., Anttila, V., Winsvold, B.S., Palta, P., Esko, T., Pers, T.H., Farh, K.H., Cuenca-Leon, E., Muona, M., Furlotte, N.A., et al. (2016). Meta-analysis of 375,000 individuals identifies 38 susceptibility loci for migraine. Nat. Genet. *48*, 856–866. https://doi.org/10.1038/ng.3598.
22. Anttila, V., Stefansson, H., Kallela, M., Todt, U., Terwindt, G.M., Calafato, M.S., Nyholt, D.R., Dimas, A.S., Freilinger, T., Müller-Myhsok, B., et al. (2010). Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1. Nat. Genet. *42*, 869–873. https://doi.org/10.1038/ng.652.
23. Anttila, V., Winsvold, B.S., Gormley, P., Kurth, T., Bettella, F., McMahon, G., Kallela, M., Malik, R., de Vries, B., Terwindt, G., et al. (2013). Genome-wide meta-analysis identifies new susceptibility loci for migraine. Nat. Genet. *45*, 912–917. https://doi.org/10.1038/ng.2676.
24. Chasman, D.I., Schürks, M., Anttila, V., de Vries, B., Schminke, U., Launer, L.J., Terwindt, G.M., van den Maagdenberg, A.M.J.M., Fendrich, K., Völzke, H., et al. (2011). Genome-wide association study reveals three susceptibility loci for common migraine in the general population. Nat. Genet. *43*, 695–698. https://doi.org/10.1038/ng.856.
25. Freilinger, T., Anttila, V., de Vries, B., Malik, R., Kallela, M., Terwindt, G.M., Pozo-Rosich, P., Winsvold, B., Nyholt, D.R., van Oosterhout, W.P.J., et al. (2012). Genome-wide association analysis identifies susceptibility loci for migraine without aura. Nat. Genet. *44*, 777–782. https://doi.org/10.1038/ng.2307.
26. Wainberg, M., Sinnott-Armstrong, N., Mancuso, N., Barbeira, A.N., Knowles, D.A., Golan, D., Ermel, R., Ruusalepp, A., Quertermous, T., Hao, K., et al. (2019). Opportunities and challenges for transcriptome-wide association studies. Nat. Genet. *51*, 592–599. https://doi.org/10.1038/s41588-019-0385-z.
27. Nica, A.C., Montgomery, S.B., Dimas, A.S., Stranger, B.E., Beazley, C., Barroso, I., and Dermitzakis, E.T. (2010). Candidate causal regulatory effects by integration of expression QTLs with complex trait genetic associations. PLoS Genet. *6*, e1000895. https://doi.org/10.1371/journal.pgen.1000895.
28. Mancuso, N., Gayther, S., Gusev, A., Zheng, W., Penney, K.L., Kote-Jarai, Z., Eeles, R., Freedman, M., Haiman, C., Pasaniuc, B.; and PRACTICAL consortium (2018). Large-scale transcriptome-wide association study identifies new prostate cancer risk regions. Nat. Commun. *9*, 4079. https://doi.org/10.1038/s41467-018-06302-1.
29. Barbeira, A.N., Pividori, M., Zheng, J., Wheeler, H.E., Nicolae, D.L., and Im, H.K. (2019). Integrating predicted transcriptome from multiple tissues improves association detection. PLoS Genet. *15*, e1007889. https://doi.org/10.1371/journal.pgen.1007889.
30. Gusev, A., Ko, A., Shi, H., Bhatia, G., Chung, W., Penninx, B.W.J.H., Jansen, R., de Geus, E.J.C., Boomsma, D.I., Wright, F.A., et al. (2016). Integrative approaches for large-scale transcriptome-wide association studies. Nat. Genet. *48*, 245–252. https://doi.org/10.1038/ng.3506.
31. (2018). GTEx project maps wide range of normal human genetic variation: a unique catalog and follow-up effort associate variation with gene expression across dozens of body tissues. Am. J. Med. Genet. A *176*, 263–264. https://doi.org/10.1002/ajmg.a.38426.
32. Banda, Y., Kvale, M.N., Hoffmann, T.J., Hesselson, S.E., Ranatunga, D., Tang, H., Sabatti, C., Croen, L.A., Dispensa, B.P., Henderson, M., et al. (2015). Characterizing race/ethnicity and genetic ancestry for 100,000 subjects in the genetic Epidemiology research on adult health and aging (GERA) cohort. Genetics *200*, 1285–1295. https://doi.org/10.1534/genetics.115.178616.
33. Bycroft, C., Freeman, C., Petkova, D., Band, G., Elliott, L.T., Sharp, K., Motyer, A., Vukcevic, D., Delaneau, O., O'Connell, J., et al. (2018). The UK Biobank resource with deep phenotyping and genomic data. Nature *562*, 203–209. https://doi.org/10.1038/s41586-018-0579-z.

34. Sudlow, C., Gallacher, J., Allen, N., Beral, V., Burton, P., Danesh, J., Downey, P., Elliott, P., Green, J., Landray, M., et al. (2015). UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med. *12*, e1001779. https://doi.org/10.1371/journal.pmed.1001779.
35. Balduzzi, S., Rücker, G., and Schwarzer, G. (2019). How to perform a meta-analysis with R: a practical tutorial. Evid. Based. Ment. Health *22*, 153–160. https://doi.org/10.1136/ebmental-2019-300117.
36. Hoffman, G.E., Bendl, J., Voloudakis, G., Montgomery, K.S., Sloofman, L., Wang, Y.C., Shah, H.R., Hauberg, M.E., Johnson, J.S., Girdhar, K., et al. (2019). CommonMind Consortium provides transcriptomic and epigenomic data for schizophrenia and bipolar disorder. Sci. Data *6*, 180. https://doi.org/10.1038/s41597-019-0183-6.
37. Laakso, M., Kuusisto, J., Stančáková, A., Kuulasmaa, T., Pajukanta, P., Lusis, A.J., Collins, F.S., Mohlke, K.L., and Boehnke, M. (2017). The Metabolic Syndrome in Men study: a resource for studies of metabolic and cardiovascular diseases. J. Lipid Res. *58*, 481–493. https://doi.org/10.1194/jlr.O072629.
38. Ligthart, L., van Beijsterveldt, C.E.M., Kevenaar, S.T., de Zeeuw, E., van Bergen, E., Bruins, S., Pool, R., Helmer, Q., van Dongen, J., Hottenga, J.J., et al. (2019). The Netherlands twin register: longitudinal research based on twin and twin-family designs. Twin Res. Hum. Genet. *22*, 623–636. https://doi.org/10.1017/thg.2019.93.
39. Akerblom, H.K., Uhari, M., Pesonen, E., Dahl, M., Kaprio, E.A., Nuutinen, E.M., Pietikäinen, M., Salo, M.K., Aromaa, A., Kannas, L., et al. (1991). Cardiovascular risk in young Finns. Ann. Med. *23*, 35–39. https://doi.org/10.3109/07853899109147928.
40. 1000 Genomes Project Consortium, Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., and Abecasis, G.R. (2015). A global reference for human genetic variation. Nature *526*, 68–74. https://doi.org/10.1038/nature15393.
41. Giambartolomei, C., Vukcevic, D., Schadt, E.E., Franke, L., Hingorani, A.D., Wallace, C., and Plagnol, V. (2014). Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. PLoS Genet. *10*, e1004383. https://doi.org/10.1371/journal.pgen.1004383.
42. Yang, J., Ferreira, T., Morris, A.P., Medland, S.E., Genetic Investigation of ANthropometric Traits GIANT Consortium; and DIAbetes Genetics Replication And Meta-analysis DIAGRAM Consortium, Madden, P.A.F., Heath, A.C., Martin, N.G., Montgomery, G.W., et al. (2012). Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. Nat. Genet. *44*, 369–375. S1-S3. https://doi.org/10.1038/ng.2213.
43. Strunz, T., Lauwen, S., Kiel, C., International AMD Genomics Consortium IAMDGC, Hollander, A.D., and Weber, B.H.F. (2020). A transcriptome-wide association study based on 27 tissues identifies 106 genes potentially relevant for disease pathology in age-related macular degeneration. Sci. Rep. *10*, 1584. https://doi.org/10.1038/s41598-020-58510-9.
44. Finucane, H.K., Reshef, Y.A., Anttila, V., Slowikowski, K., Gusev, A., Byrnes, A., Gazal, S., Loh, P.R., Lareau, C., Shoresh, N., et al. (2018). Heritability enrichment of specifically expressed genes identifies disease-relevant tissues and cell types. Nat. Genet. *50*, 621–629. https://doi.org/10.1038/s41588-018-0081-4.
45. Kim, T., Jeon, Y.J., Cui, R., Lee, J.H., Peng, Y., Kim, S.H., Tili, E., Alder, H., and Croce, C.M. (2015). Role of MYC-regulated long noncoding RNAs in cell cycle regulation and tumorigenesis. J. Natl. Cancer Inst. *107*, dju505. https://doi.org/10.1093/jnci/dju505.
46. Liu, H., Huang, W., Feng, Y., Li, Y., Li, J., and Mai, G. (2022). Identification of ceRNA regulatory network in acute pancreatitis and acute recurrent pancreatitis. Eur. J. Gastroenterol. Hepatol. *34*, 1031–1041. https://doi.org/10.1097/MEG.0000000000002421.
47. Jilani, A., Ramotar, D., Slack, C., Ong, C., Yang, X.M., Scherer, S.W., and Lasko, D.D. (1999). Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3′-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. J. Biol. Chem. *274*, 24176–24186. https://doi.org/10.1074/jbc.274.34.24176.
48. Bras, J., Alonso, I., Barbot, C., Costa, M.M., Darwent, L., Orme, T., Sequeiros, J., Hardy, J., Coutinho, P., and Guerreiro, R. (2015). Mutations in PNKP cause recessive ataxia with oculomotor apraxia type 4. Am. J. Hum. Genet. *96*, 474–479. https://doi.org/10.1016/j.ajhg.2015.01.005.
49. Reynolds, J.J., Walker, A.K., Gilmore, E.C., Walsh, C.A., and Caldecott, K.W. (2012). Impact of PNKP mutations associated with microcephaly, seizures and developmental delay on enzyme activity and DNA strand break repair. Nucleic Acids Res. *40*, 6608–6619. https://doi.org/10.1093/nar/gks318.
50. Shen, J., Gilmore, E.C., Marshall, C.A., Haddadin, M., Reynolds, J.J., Eyaid, W., Bodell, A., Barry, B., Gleason, D., Allen, K., et al. (2010). Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. Nat. Genet. *42*, 245–249. https://doi.org/10.1038/ng.526.
51. Linial, M., Miller, K., and Scheller, R.H. (1989). VAT-1: an abundant membrane protein from Torpedo cholinergic synaptic vesicles. Neuron *2*, 1265–1273. https://doi.org/10.1016/0896-6273(89)90311-5.
52. Mertsch, S., Becker, M., Lichota, A., Paulus, W., and Senner, V. (2009). Vesicle amine transport protein-1 (VAT-1) is upregulated in glioblastomas and promotes migration. Neuropathol. Appl. Neurobiol. *35*, 342–352. https://doi.org/10.1111/j.1365-2990.2009.00993.x.
53. Shan, X., Wang, K., Tong, X., Wang, Z., Wu, F., Liu, X., Yang, P., and Wang, J. (2019). High expression of VAT1 is a prognostic biomarker and predicts malignancy in glioblastoma. Oncol. Rep. *42*, 1422–1430. https://doi.org/10.3892/or.2019.7276.
54. Yang, P., Wang, K., Zhang, C., Wang, Z., Liu, Q., Wang, J., Jiang, T., and Shan, X. (2021). Novel roles of VAT1 expression in the immunosuppressive action of diffuse gliomas. Cancer Immunol. Immunother. *70*, 2589–2600. https://doi.org/10.1007/s00262-021-02865-z.
55. Li, W., Yang, L., Tang, C., Liu, K., Lu, Y., Wang, H., Yan, K., Qiu, Z., and Zhou, W. (2020). Mutations of CNTNAP1 led to defects in neuronal development. JCI Insight *5*, e135697. https://doi.org/10.1172/jci.insight.135697.
56. Low, K.J., Stals, K., Caswell, R., Wakeling, M., Clayton-Smith, J., Donaldson, A., Foulds, N., Norman, A., Splitt, M., Urankar, K., et al. (2018). Phenotype of CNTNAP1: a study of patients demonstrating a specific severe congenital hypomyelinating neuropathy with survival beyond infancy. Eur. J. Hum. Genet. *26*, 796–807. https://doi.org/10.1038/s41431-018-0110-x.

57. Ohashi, T., Yamamoto, T., Yamanashi, Y., and Ohsugi, M. (2016). Human TUBG2 gene is expressed as two splice variant mRNA and involved in cell growth. FEBS Lett. *590*, 1053–1063. https://doi.org/10.1002/1873-3468.12163.
58. Potter, N., Karakoula, A., Phipps, K.P., Harkness, W., Hayward, R., Thompson, D.N.P., Jacques, T.S., Harding, B., Thomas, D.G.T., Palmer, R.W., et al. (2008). Genomic deletions correlate with underexpression of novel candidate genes at six loci in pediatric pilocytic astrocytoma. Neoplasia *10*, 757–772. https://doi.org/10.1593/neo.07914.
59. Zheng, J., Su, Z., Kong, Y., Lin, Q., Liu, H., Wang, Y., and Wang, J. (2020). LncRNAs predicted to interfere with the gene regulation activity of miR-637 and miR-196a-5p in GBM. Front. Oncol. *10*, 303. https://doi.org/10.3389/fonc.2020.00303.
60. Majercak, J., Ray, W.J., Espeseth, A., Simon, A., Shi, X.P., Wolffe, C., Getty, K., Marine, S., Stec, E., Ferrer, M., et al. (2006). LRRTM3 promotes processing of amyloid-precursor protein by BACE1 and is a positional candidate gene for late-onset Alzheimer's disease. Proc. Natl. Acad. Sci. USA *103*, 17967–17972. https://doi.org/10.1073/pnas.0605461103.
61. Meimaridou, E., Kowalczyk, J., Guasti, L., Hughes, C.R., Wagner, F., Frommolt, P., Nürnberg, P., Mann, N.P., Banerjee, R., Saka, H.N., et al. (2012). Mutations in NNT encoding nicotinamide nucleotide transhydrogenase cause familial glucocorticoid deficiency. Nat. Genet. *44*, 740–742. https://doi.org/10.1038/ng.2299.
62. Gal, J., Chen, J., Katsumata, Y., Fardo, D.W., Wang, W.X., Artiushin, S., Price, D., Anderson, S., Patel, E., Zhu, H., and Nelson, P.T. (2018). Detergent insoluble proteins and inclusion body-like structures immunoreactive for PRKDC/DNA-PK/DNA-PKcs, FTL, NNT, and AIFM1 in the amygdala of cognitively impaired elderly persons. J. Neuropathol. Exp. Neurol. *77*, 21–39. https://doi.org/10.1093/jnen/nlx097.
63. Nguyen, C.L., Possemato, R., Bauerlein, E.L., Xie, A., Scully, R., and Hahn, W.C. (2012). Nek4 regulates entry into replicative senescence and the response to DNA damage in human fibroblasts. Mol. Cell Biol. *32*, 3963–3977. https://doi.org/10.1128/MCB.00436-12.
64. Coene, K.L.M., Mans, D.A., Boldt, K., Gloeckner, C.J., van Reeuwijk, J., Bolat, E., Roosing, S., Letteboer, S.J.F., Peters, T.A., Cremers, F.P.M., et al. (2011). The ciliopathy-associated protein homologs RPGRIP1 and RPGRIP1L are linked to cilium integrity through interaction with Nek4 serine/threonine kinase. Hum. Mol. Genet. *20*, 3592–3605. https://doi.org/10.1093/hmg/ddr280.
65. Scott, L.J., Muglia, P., Kong, X.Q., Guan, W., Flickinger, M., Upmanyu, R., Tozzi, F., Li, J.Z., Burmeister, M., Absher, D., et al. (2009). Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry. Proc. Natl. Acad. Sci. USA *106*, 7501–7506. https://doi.org/10.1073/pnas.0813386106.
66. Yao, S., Wu, H., Liu, T.T., Wang, J.H., Ding, J.M., Guo, J., Rong, Y., Ke, X., Hao, R.H., Dong, S.S., et al. (2021). Epigenetic element-based transcriptome-wide association study identifies novel genes for bipolar disorder. Schizophr. Bull. *47*, 1642–1652. https://doi.org/10.1093/schbul/sbab023.
67. Dluzen, D., Li, G., Tacelosky, D., Moreau, M., and Liu, D.X. (2011). BCL-2 is a downstream target of ATF5 that mediates the prosurvival function of ATF5 in a cell type-dependent manner. J. Biol. Chem. *286*, 7705–7713. https://doi.org/10.1074/jbc.M110.207639.
68. Li, G., Xu, Y., Guan, D., Liu, Z., and Liu, D.X. (2011). HSP70 protein promotes survival of C6 and U87 glioma cells by inhibition of ATF5 degradation. J. Biol. Chem. *286*, 20251–20259. https://doi.org/10.1074/jbc.M110.211771.
69. Sears, T.K., and Angelastro, J.M. (2017). The transcription factor ATF5: role in cellular differentiation, stress responses, and cancer. Oncotarget *8*, 84595–84609. https://doi.org/10.18632/oncotarget.21102.
70. Wang, H., Lin, G., and Zhang, Z. (2007). ATF5 promotes cell survival through transcriptional activation of Hsp27 in H9c2 cells. Cell Biol. Int. *31*, 1309–1315. https://doi.org/10.1016/j.cellbi.2007.05.002.
71. Angelastro, J.M., Ignatova, T.N., Kukekov, V.G., Steindler, D.A., Stengren, G.B., Mendelsohn, C., and Greene, L.A. (2003). Regulated expression of ATF5 is required for the progression of neural progenitor cells to neurons. J. Neurosci. *23*, 4590–4600.
72. Angelastro, J.M., Klimaschewski, L., Tang, S., Vitolo, O.V., Weissman, T.A., Donlin, L.T., Shelanski, M.L., and Greene, L.A. (2000). Identification of diverse nerve growth factor-regulated genes by serial analysis of gene expression (SAGE) profiling. Proc. Natl. Acad. Sci. USA *97*, 10424–10429. https://doi.org/10.1073/pnas.97.19.10424.
73. Angelastro, J.M., Mason, J.L., Ignatova, T.N., Kukekov, V.G., Stengren, G.B., Goldman, J.E., and Greene, L.A. (2005). Down-regulation of activating transcription factor 5 is required for differentiation of neural progenitor cells into astrocytes. J. Neurosci. *25*, 3889–3899. https://doi.org/10.1523/JNEUROSCI.3447-04.2005.
74. Lee, H.Y., Angelastro, J.M., Kenney, A.M., Mason, C.A., and Greene, L.A. (2012). Reciprocal actions of ATF5 and Shh in proliferation of cerebellar granule neuron progenitor cells. Dev. Neurobiol. *72*, 789–804. https://doi.org/10.1002/dneu.20979.
75. Mason, J.L., Angelastro, J.M., Ignatova, T.N., Kukekov, V.G., Lin, G., Greene, L.A., and Goldman, J.E. (2005). ATF5 regulates the proliferation and differentiation of oligodendrocytes. Mol. Cell. Neurosci. *29*, 372–380. https://doi.org/10.1016/j.mcn.2005.03.004.
76. Dong, S., Nutt, C.L., Betensky, R.A., Stemmer-Rachamimov, A.O., Denko, N.C., Ligon, K.L., Rowitch, D.H., and Louis, D.N. (2005). Histology-based expression profiling yields novel prognostic markers in human glioblastoma. J. Neuropathol. Exp. Neurol. *64*, 948–955. https://doi.org/10.1097/01.jnen.0000186940.14779.90.
77. Hua, X.M., Wang, J., Qian, D.M., Song, J.Y., Chen, H., Zhu, X.L., Zhou, R., Zhao, Y.D., Zhou, X.Z., Li, L., et al. (2015). DNA methylation level of promoter region of activating transcription factor 5 in glioma. J. Zhejiang Univ. - Sci. B *16*, 757–762. https://doi.org/10.1631/jzus.B1500067.
78. Huang, R., Qian, D., Hu, M., Zhang, X., Song, J., Li, L., Chen, H., and Wang, B. (2015). Association between human cytomegalovirus infection and histone acetylation level in various histological types of glioma. Oncol. Lett. *10*, 2812–2820. https://doi.org/10.3892/ol.2015.3638.
79. Sheng, Z., Li, L., Zhu, L.J., Smith, T.W., Demers, A., Ross, A.H., Moser, R.P., and Green, M.R. (2010). A genome-wide RNA interference screen reveals an essential CREB3L2-ATF5-MCL1 survival pathway in malignant glioma with therapeutic implications. Nat. Med. *16*, 671–677. https://doi.org/10.1038/nm.2158.

80. Jarray, R., Allain, B., Borriello, L., Biard, D., Loukaci, A., Larghero, J., Hadj-Slimane, R., Garbay, C., Lepelletier, Y., and Raynaud, F. (2011). Depletion of the novel protein PHACTR-1 from human endothelial cells abolishes tube formation and induces cell death receptor apoptosis. Biochimie *93*, 1668–1675. https://doi.org/10.1016/j.biochi.2011.07.010.
81. Fagerberg, L., Hallström, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K., et al. (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol. Cell. Proteomics *13*, 397–406. https://doi.org/10.1074/mcp.M113.035600.
82. Grundberg, E., Small, K.S., Hedman, Å.K., Nica, A.C., Buil, A., Keildson, S., Bell, J.T., Yang, T.P., Meduri, E., Barrett, A., et al. (2012). Mapping cis- and trans-regulatory effects across multiple tissues in twins. Nat. Genet. *44*, 1084–1089. https://doi.org/10.1038/ng.2394.
83. Charles, A., and Brennan, K.C. (2010). The neurobiology of migraine. Handb. Clin. Neurol. *97*, 99–108. https://doi.org/10.1016/S0072-9752(10)97007-3.
84. Arzani, M., Jahromi, S.R., Ghorbani, Z., Vahabizad, F., Martelletti, P., Ghaemi, A., Sacco, S., Togha, M.; and School of Advanced Studies of the European Headache Federation EHF-SAS (2020). Gut-brain Axis and migraine headache: a comprehensive review. J. Headache Pain *21*, 15. https://doi.org/10.1186/s10194-020-1078-9.
85. El-Metwally, A., Toivola, P., AlAhmary, K., Bahkali, S., AlKhathaami, A., Al Ammar, S.A., Altamimi, I.M., Alosaimi, S.M., Jawed, M., and Almustanyir, S. (2020). The Epidemiology of migraine headache in arab countries: a systematic review. Sci. World J. *2020*, 4790254. https://doi.org/10.1155/2020/4790254.
86. GTEx Consortium (2020). The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science *369*, 1318–1330. https://doi.org/10.1126/science.aaz1776.
87. Pressman, A., Jacobson, A., Eguilos, R., Gelfand, A., Huynh, C., Hamilton, L., Avins, A., Bakshi, N., and Merikangas, K. (2016). Prevalence of migraine in a diverse community–electronic methods for migraine ascertainment in a large integrated health plan. Cephalalgia *36*, 325–334. https://doi.org/10.1177/0333102415590242.
88. Grinberg, N.F., and Wallace, C. (2021). Multi-tissue transcriptome-wide association studies. Genet. Epidemiol. *45*, 324–337. https://doi.org/10.1002/gepi.22374.
89. Hu, Y., Li, M., Lu, Q., Weng, H., Wang, J., Zekavat, S.M., Yu, Z., Li, B., Gu, J., Muchnik, S., et al. (2019). A statistical framework for cross-tissue transcriptome-wide association analysis. Nat. Genet. *51*, 568–576. https://doi.org/10.1038/s41588-019-0345-7.