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Genetic variation contributes to gene expression response in ischemic stroke: an eQTL study

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Abstract

Objective: Single nucleotide polymorphisms (SNPs) contribute to complex disorders such as ischemic stroke (IS). Since SNPs could affect IS by altering gene expression, we studied the association of common SNPs with changes in mRNA expression (i.e. expression quantitative trait loci; eQTL) in blood after IS.

Methods: RNA and DNA were isolated from 137 patients with acute IS and 138 vascular risk factor controls (VRFC). Gene expression was measured using Affymetrix HTA 2.0 microarrays and SNP variants were assessed with Axiom Biobank Genotyping microarrays. A linear model with a genotype (SNP) × diagnosis (IS and VRFC) interaction term was fit for each SNP-gene pair.

Results: The eQTL interaction analysis revealed significant genotype × diagnosis interaction for four SNP-gene pairs as cis-eQTL and 70 SNP-gene pairs as trans-eQTL. Cis-eQTL involved in the inflammatory response to IS included rs56348411 which correlated with neurogranin expression (NRGN), rs78046578 which correlated with CXCL10 expression, rs975903 which correlated with SMAD4 expression, and rs62299879 which correlated with CD38 expression. These four genes are important in regulating inflammatory response and BBB stabilization. SNP rs148791848 was a strong trans-eQTL for anosmin-1 (ANOS1) which is involved in neural cell adhesion and axonal migration and may be important after stroke. Interpretation: This study highlights the contribution of genetic variation to regulating gene expression following IS. Specific inflammatory response to stroke is at least partially influenced by genetic variation. This has implications for progressing toward personalized treatment strategies. Additional research is required to investigate these genes as therapeutic targets.

Introduction

Gene expression studies of blood have shown different gene profiles for ischemic stroke (IS) compared to controls, and different profiles for IS compared to intracerebral hemorrhage. There are different profiles for varying causes of IS that can predict causes of cryptogenic strokes where the cause is not otherwise known. Moreover, gene expression profiles in blood of IS patients prior to administration of tPA predict those who develop hemorrhagic transformation one day later. These data raise the question of whether some changes of gene expression might be genetically programmed, given that stroke has a heritability ranging from 0.16 to 0.40. Thus, this study assessed the effects of single nucleotide polymorphisms (SNPs) on gene expression (mRNA levels) following IS.

SNPs that affect RNA expression are called expression quantitative trait loci (eQTL). These are widespread in the genome and account for part of the genetic effects that contribute to complex genetic diseases. eQTLs are divided into those with local effects (cis-eQTLs), where the genetic variant is located within 1 megabase (Mb) of the affected gene, and those with distant effects (trans-eQTLs), where the genetic variant is further away or on a different chromosome. Analysis of eQTL in large cohorts (e.g., GTEx) has shown many diseases associated loci regulate nearby genes, though a substantial fraction of disease associated loci still remain unexplained and are...
likely trans-eQTL found mainly in noncoding regions of the genome.9

Blood is used here in part because it is readily accessible in humans. More importantly, studying blood following stroke provides an index of the coagulation status of each patient as well as inflammatory and immune response mechanisms following stroke that in part determine outcome.1

In this study, we have explored the influence of SNP genotype on expression of genes that are different between blood of IS and controls. These eQTLs could provide possible mechanisms by which SNPs influence IS outcomes and provide prognostic and treatment targets.

Materials and Methods

The research protocol was approved by institutional review boards of the University of California at Davis, University of California at San Francisco and the University of Alberta. All subjects provided written informed consent and RNA and DNA were isolated from blood samples collected from 137 ischemic stroke (IS) patients and 138 vascular risk factor matched controls including diabetes and/or hypertension and/or hypercholesterolemia (VRFC). Gene expression of all protein-coding transcripts was quantified by Affymetrix HTA 2.0 microarrays10 and variants assessed by Axiom Biobank Genotyping microarrays. To identify a linear regression model with a genotype-diagnosis interaction term for each SNP-gene pair was utilized and tested for significance. All the analyses were conducted using the Matrix eQTL package in the R statistical environment as described previously.11 Additional detailed information is provided in the Supplementary Materials and Methods File.

Results

Patient characteristics

Subject characteristics including age, sex, race, smoking status, alcohol consumption, and vascular risk factors (hypertension, diabetes, and hypercholesterolemia) for 137 IS and 138 VRFC subjects are presented in Table 1. The mean age (± standard deviation (SD)) of the male (n = 86) and the female (n = 51) stroke subjects were 59.5 ± 12.2 and 64.6 ± 14.2, respectively. Average ages of the male (n = 70) and female (n = 68) VRFC subjects were 59.1 ± 14.4 and 62.8 ± 11.9, respectively. There were no significant differences in subject demographics for age, sex, race, smoking status, alcohol consumption or vascular risk factors including diabetes and/or hypertension and/or hypercholesterolemia between IS and VRFC groups (Table 1).

Analysis of genotype (SNP) × diagnosis effect on gene expression

The SNP-gene pair interactions show the impact of genotype (SNP) on gene expression when the interaction significantly differs between IS and VRFC subjects. These SNP-gene pairs from the interaction analysis can indicate one of three different biological properties. First, they can represent eQTL in VRFC or IS but not both. Second, eQTLs can indicate an opposite directional effect between VRFC and IS. Third, eQTL may be in the same direction but of significantly different magnitude of impact between VRFC and IS. More formally, the interaction term assesses whether there is a significant difference in the slope of the genotype-expression regression line between VRFC individuals and IS patients (Figure 1).
We also investigated the significant cis-eQTL and trans-eQTL genes found in genes associated with stroke. The Harmonizome web portal “http://amp.pharm.mssm.edu/Harmonizome/gene_set/Stroke/CTD+Gene-Disease+Associations” includes 1187 genes significantly associated with stroke.12 We found that three (3/36 = 8.33%) and four (4/23 = 17.39%) of our genes from cis-eQTL and trans-eQTL results, respectively, were significantly associated with stroke. The significant associated genes from our eQTL results were PTTPC, UCCG, ZBTB16, CCL2, CD38, and ITGA1 (Tables 2, 3 and 4).

**Discussion**

eQTL have revealed disease-associated variants and identified expression of genes that are influenced by a particular allele.13 In this study, we identified SNPs in both the cis and trans relation that correlated with changes in gene expression after ischemic stroke (IS). Though an increasing number of genetic studies are discovering many SNPs significantly associated with IS,14-16 how the genotypes modulate IS are usually unknown. The eQTL identified in this study are SNPs that drive changes of gene expression following IS and thus provide insight into their effect in stroke.

The strongest cis-eQTLs were involved in the inflammatory response to IS including rs78046578 that correlated with CXCL10 expression, rs975903 that correlated with SMAD4 expression, rs62299879 that correlated with CD38 expression, and rs56348411 that correlated with neurogranin (NRGN) expression. Chemokine (C-X-C motif) ligand 10 (CXCL10) mediates inflammatory responses and is a chemoattractant for activated T cells, natural killer (NK) cells, dendritic cells, and blood monocytes.17 CXCL10 directly binds IL6, both having key inflammatory roles in IS.18 CXCL10 level is increased in post-mortem ischemic stroke brain and is involved in blood–brain barrier (BBB) breakdown following IS.17

SMAD4 is associated with inflammation and hypercoagulation in ischemic stroke and development of thrombolyis related hemorrhagic transformation. A subset of stroke patients may be more prone to hemorrhagic transformation as a result of differences in SMAD4 signaling in circulating leukocytes.5 Mutations in SMAD4 cause the hereditary hemorrhagic telangiectasia syndrome and native SMAD4 regulates N-cadherin expression in endothelial cells to stabilize the BBB.19,20 The expression of SMAD4 is higher after IS, and as we observe in this study, particularly higher in those individuals with the GG allele of rs975903. SMAD4 could be important in endogenous thrombolysis following IS.

CD (cluster of differentiation) proteins, including CD38, play a role in cell signaling and cell adhesion. Our

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**Figure 1.** cis-eQTL rs56348411 for NRGN. Linear interaction between genotype (x-axis) of rs56348411 and diagnosis (IS and VRFC) on gene expression of NRGN (y-axis). Mean gene expression from the signal space transformation, in conjunction with regular robust multiple-array average normalization method (SST-RMA) (y-axis) with standard error bars are plotted by SNP genotype (x-axis: CC, CT, TT) and diagnosis status (red - IS; green - VRFC). The beta was 0.313, P value = 2.10E-08; and FDR = 0.088 (Table 2). IS - ischemic stroke. VRFC - vascular risk factor control.
### Table 2. cis-eQTL identified as ischemic stroke diagnosis dependent (genotype × diagnosis interaction)

**cis-eQTL**

| rsID         | Gene ID | Chr:Position | Variant Type | Ref allele/ Alt allele | mRNA | Gene ID | Chr:Position | beta | P value | FDR  | Appears in References |
|--------------|---------|--------------|--------------|------------------------|------|---------|--------------|------|---------|------|------------------------|
| rs56348411   | TMEM218  | 11:124974588 | intron       | C/T                    | 0.319266 | 11:12460820-124617869 | 3.12936 | 2.10E-08 | 0.087925 | 7, 13                  |
| rs78046578   | NAAA    | 4:76826362   | intron       | T/C                    | -0.52763 | 4:76942269-76946899 | -0.52763 | 5.43E-08 | 0.113845 | 7                      |
| rs975903     | 18:49206115 | intergenic     | T/G            | -0.16254 | 1.18E-07 | 0.165241 | 7, 13                  |
| rs62298978   | HIVEP3  | 1:15748452   | intergenic     | T/C                    | 0.493781 | 1.99E-07 | 0.20815 | 7, 12, 13, 50        |
| rs1809423    | CHRNA3  | 1:15748452   | intergenic     | C/G                    | -0.0238 | 1.73E-06 | 0.862336 | 13                      |
| rs75608718   | CCDC61  | 19:4615961   | intergenic     | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs75391517   | SMARC3  | 7:150969808 | intron       | C/T                    | 0.070146 | 2.26E-06 | 0.862336 | 7, 12, 13, 50        |
| rs75368642   | --      | 1:15748452   | intergenic     | C/G                    | -0.0238 | 1.73E-06 | 0.862336 | 13                      |
| rs17666226   | --      | 18:49165965  | intergenic     | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs10958734   | HOOK3   | 8:42806155   | intergenic     | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs3776738    | ARL15   | 5:53224090   | intron       | G/A                    | 0.070146 | 2.26E-06 | 0.862336 | 7, 12, 13, 50        |
| rs79403922   | SDK1    | 7:399420     | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs60839180   | KUK6    | 19:51467289  | intron       | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs3730850    | UG1     | 19:48686709  | intron       | A/G                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs2180911    | --      | 20:44949747  | intergenic     | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs11243458   | --      | 9:13746130   | intron       | G/A                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs7250947    | PUN4    | 19:4510530   | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs21110      | FXD5    | 19:35660058  | intron       | G/A                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs892934     | CLCA4   | 1:8737398   | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs7129315    | TMEM218  | 11:124977208 | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs7238360    | PPPR3R8 | X:302966     | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs12359932   | HPSE2   | 10:100998381 | upstream     | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs7757514    | CRYBG1  | 6:106834984 | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs6662611    | --      | 1:151963485 | intron       | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs10943676   | --      | 6:80660507  | intron       | G/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs2195310    | ZNF347  | 19:53645291  | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs6173124    | PHLP2   | 16:71682830  | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs8644970    | STOX2   | 4:18494678  | downstream   | G/A                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs11068369   | FBXO21  | 12:117586896 | intron       | T/G                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs74517767   | --      | 19:6870146   | intergenic     | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs34517659   | WDR1    | 4:10094042  | intron       | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs62870112   | SHC3    | 9:91627100   | 3' UTR       | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs16986039   | PTPRH   | 19:55710074  | missense     | G/A                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs12480811   | KCNQ2   | 20:62059116  | intercon      | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs132642     | APOL3   | 22:3654137  | intron       | A/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs1326895    | LPAR1   | 9:113678096 | intron       | C/T                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rs72330046   | TTAT1   | 7:23230046   | intercon      | T/C                    | 0.20815 | 7, 12, 13, 50 | 13 |
| rsID           | Gene ID     | Chr:Position | Variant Type | Ref allele/Alt allele | SNP                          | mRNA | FDR  | Appears in References |
|---------------|-------------|--------------|--------------|-----------------------|------------------------------|------|------|-----------------------|
| rs148791848   |            | X:933686861  | intergenic   | T/C                   | rs148791848                  |      |      |                       |
| rs950391      |            | X:86453292   | intergenic   | G/A                   | rs950391                     |      |      |                       |
| rs2464504     | TEC         | 6:166247384  | intron       | C/T                   | rs2464504                    |      |      |                       |
| rs11761821    | PDE10A      | 6:16724734   | intron       | A/G                   | rs11761821                   |      |      |                       |
| rs11761821    | PDE10A      | 6:16724734   | intron       | A/G                   | rs11761821                   |      |      |                       |
| rs2944885     | LOC103374016| 3:102311450  | intron       | G/A                   | rs2944885                    |      |      |                       |
| rs950391      |            | X:86453292   | intergenic   | G/A                   | rs950391                     |      |      |                       |
| rs73507341    | NFX         | 19:13135197  | intron       | T/C                   | rs73507341                   |      |      |                       |
| rs950391      |            | X:86453292   | intergenic   | G/A                   | rs950391                     |      |      |                       |
| rs12833155    |            | X:42486482   | intergenic   | A/C                   | rs12833155                   |      |      |                       |
| rs11761821    | PDE10A      | 6:166247384  | intron       | A/G                   | rs11761821                   |      |      |                       |
| rs2369519     |            | X:86392534   | intergenic   | G/A                   | rs2369519                    |      |      |                       |
| rs11853524    | SNHG14      | 15:25509895  | intron       | G/T                   | rs11853524                   |      |      |                       |
| rs139929471   |            | X:88063578   | intergenic   | G/A                   | rs139929471                  |      |      |                       |
| rs79434685    | KREMEN1     | 4:21971817   | intron       | C/G                   | rs79434685                   |      |      |                       |
| rs7664829     | KCNIP4      | 10:635632    | mRnasense     | G/A                   | rs7664829                    |      |      |                       |
| rs9791781     | MICA        | 6:13178510   | mRnasense     | G/A                   | rs9791781                    |      |      |                       |
| rs2464504     | TEC         | 4:48232441   | intergenic   | C/T                   | rs2464504                    |      |      |                       |
| rs950391      |            | X:86453292   | intergenic   | G/A                   | rs950391                     |      |      |                       |
| rs139929471   |            | X:88063578   | intergenic   | G/A                   | rs139929471                  |      |      |                       |
| rs1051785     | MICA        | 6:13178388   | mRnasense     | G/A                   | rs1051785                    |      |      |                       |
| rs149957475   |            | X:93351607   | intergenic   | C/T                   | rs149957475                  |      |      |                       |
| rs9847733     | UBE2E2-AS1  | 2:32242050   | intron       | A/G                   | rs9847733                    |      |      |                       |
| rs1063632     | MICA        | 6:13178510   | mRnasense     | G/A                   | rs1063632                    |      |      |                       |
| rs12399124    | PRKX        | 3:35440896   | intergenic   | G/A                   | rs12399124                   |      |      |                       |
| rs139929471   |            | X:88063578   | intergenic   | G/A                   | rs139929471                  |      |      |                       |
| rs2369519     |            | X:86392534   | intergenic   | G/A                   | rs2369519                    |      |      |                       |
| rs17409498    |            | 20:56040855  | intergenic   | C/T                   | rs17409498                   |      |      |                       |
| rs2464504     | TEC         | 4:48232441   | intergenic   | C/T                   | rs2464504                    |      |      |                       |
| rs79434685    | KREMEN1     | 2:229556745  | intron       | C/G                   | rs79434685                   |      |      |                       |
| rs1051785     | MICA        | 6:13178388   | mRnasense     | G/A                   | rs1051785                    |      |      |                       |
| rs79434685    | KREMEN1     | 2:229556745  | intron       | C/G                   | rs79434685                   |      |      |                       |
| rs677787      | ENTPD3-AS1  | 3:40486470   | intron       | T/C                   | rs677787                     |      |      |                       |
| rs9797183     |            | 13:0309957   | intergenic   | T/C                   | rs9797183                    |      |      |                       |
| rs117781420   | DENND4C     | 9:19355687   | intron       | G/A                   | rs117781420                  |      |      |                       |
| rs14058068    |            | 19:9802257   | intergenic   | C/T                   | rs14058068                   |      |      |                       |
previous studies indicated CD46 and zinc-finger family, ZNF \((ZNF185 \text{ and } ZNF254)\) expression as a biomarker distinguishing the cause of ischemic stroke as cardioembolic or large-vessel disease.\(^{21}\) Leukemic blast cells over-express CD38 in pediatric ischemic stroke.\(^{22}\) Following focal ischemia, astrocytic release of extracellular mitochondrial particles is mediated by a calcium-dependent mechanism involving CD38.\(^{23}\) Suppression of CD38 signaling by short interfering RNA reduced extracellular mitochondria transfer and worsened neurological outcomes.\(^{23}\) CD38 is a NAD-consuming protein that synthesizes NADH and may be involved in vascular repair following stroke.\(^{24}\) In contrast, CD38-deficient mice have decreased chemokines, immune cell infiltration and infarct volumes following stroke.\(^{25}\) CD38 levels increase in monocytes, macrophages, and T and B lymphocytes following stroke in humans.\(^{26}\)

Neurogranin (NRGN) is expressed in telencephalic neurons, particularly dendritic spines, and is involved in synaptic signaling by regulating calmodulin (CaM) availability. NRGN levels in plasma reflect stroke volume.\(^{27}\) Neurogranin is involved in maintaining quiescent B cells\(^{28}\) and modulating T-cell apoptosis.\(^{29}\) Thus, neurogranin might play a role in B- and T-cell regulation and perhaps of other mononuclear cells in blood of patients with stroke. Our results show that there is a distinct difference in expression of NRGN that is higher in ischemic stroke patients that have the CC allele (rs56348411) and CC allele (rs7129315), both in the nearby gene TMEM218. Based on databases of known protein-protein interaction and biological pathways, there is no known existing relationship between these molecules. Identification of the \textit{cis}-eQTL involving the pair through our SNP \times diagnosis analysis may suggest a relational dependence related to a pathological state rather than functional relationship at baseline.

Several zinc-finger family (ZNF) transcripts were identified as \textit{cis}-eQTL: rs11809423 (ZNF684), rs2180911 (ZNF335), and rs74517766 (ZNF358). Additionally, as \textit{trans}-eQTL we also found genotypes rs1063632 and rs1051785 significantly affected the expression of ZNF207, while rs148991762 and rs139929471 significantly affected the expression of ZNF684. Changes in ZNFs are associated with neurodegenerative disorders. These ZNF proteins can also be used as predictive markers for different diseases such as cancer. ZNFs can also act as chromatin modifiers and cofactors affecting gene regulation at a broader level.\(^{30}\)

A prevailing thought for years placed more importance on the impact of \textit{cis}-eQTL in which the SNP was close to the expressed gene. However, growing evidence suggests expression of a typical gene is associated with large numbers of \textit{trans}-eQTL, which by current estimates

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**Table 3. Continued.**

| SNP | Gene ID | Chr:Position | Variant Type | Ref allele/Alt allele | beta | p value | FDR |
|-----|---------|--------------|--------------|----------------------|------|---------|-----|
| rs7317917 | X:3466534 | intergenic | T/C | 0.210052 | 8.07E-12 | 0.001126 | 7, 13 |
| rs1172922 | 9:93488534 | intergenic | A/C | 0.18066 | 8.84E-12 | 0.001202 | 7, 12, 13, 50 |
| rs2464504 | TEC | 4:48232441 | intron | C/T | 0.24903 | 1.07E-11 | 0.001421 | 7 |
| rs72906031 | 1:16845719 | G/T | 0.11958 | 1.10E-11 | 0.001421 | 7, 13 |
| rs57764234 | CHD8 | 14:21897616 | intron | C/T | 0.12591 | 1.16E-11 | 0.001434 | 7, 13 |
| rs9873394 | ENTPD3 | 3:40468206 | intron | T/G | 0.111598 | 1.14E-11 | 0.001434 | 7, 13 |
| rs117781420 | DENND4C | 9:19355687 | intron | G/A | 0.247554 | 1.59E-11 | 0.001913 | 13 |
| rs950391 | WNT16 | X:86454329 | intergenic | G/A | 0.24595 | 2.18E-11 | 0.002458 | 7, 13 |
| rs7081076 | SORBS1 | 10:97174537 | missense | C/A | 0.358066 | 2.50E-11 | 0.002612 | 7, 13 |
| rs2369519 | CLNK | X:86392534 | intergenic | G/A | 0.177 | 3.50E-11 | 0.002962 | 7, 13 |
| rs1063632 | MICA | 6:31378510 | missense | G/A | 0.24595 | 2.60E-11 | 0.002962 | 7, 13 |

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### Table 4.

trans-eQTL identified as ischemic stroke diagnosis dependent (genotype $\times$ diagnosis interaction)

| rsID          | Gene ID | chr:position | Variant type | Ref allele/Alt allele | mRNAs                                                                 | chr:position | beta          | P value     | FDR          | Appears in References |
|---------------|---------|--------------|--------------|-----------------------|-------------------------------------------------------------------------|--------------|---------------|-------------|--------------|----------------------|
| rs9812616     | UBE2E2-AS1 | 3:23237608   | intron       | C/T                   | AP3B2                                                                   | 15:83328033-83378666 | -0.11036     | 2.69E-11    | 0.002908     | 7, 13                     |
| rs1051785     | MICA    | 6:31378388   | missense     | G/A                   | ZNF207                                                                  | 17:30677128-30714780 | 0.363794     | 2.85E-11    | 0.003022     | 7, 13                     |
| rs148991762   | MICA    | 7:13660854   | intergenic   | C/A                   | ZNF684                                                                   | 1:40997233-41013841 | 0.239354     | 2.96E-11    | 0.00308      | 7, 13                     |
| rs6665585     | LINC01748 | 1:61090200   | upstream     | A/G                   | ABCA6                                                                   | 17:6.7074847-67138015 | -0.28688     | 3.12E-11    | 0.003181     | 7, 13                     |
| rs627635      | MICA    | 18:66904739  | intergenic   | T/C                   | AP3B2                                                                   | 15:83328033-83378666 | -0.11228     | 3.55E-11    | 0.003493     | 7, 13                     |
| rs9779183     | MICA    | 1:3009957    | intergenic   | T/C                   | SMAD4                                                                   | 18:48556583-48611415 | -0.227      | 3.56E-11    | 0.003493     | 7, 13                     |
| rs1063632     | MICA    | 6:31378510   | missense     | G/A                   | CLNK                                                                     | 4:10488019-10686489 | -0.17066     | 4.42E-11    | 0.004111     | 7                         |
| rs148991762   | MICA    | 1:3461054    | intergenic   | C/A                   | C5                                                                       | 9:123714614-123837452 | 0.174565     | 4.41E-11    | 0.004111     | 7                         |
| rs1063632     | MICA    | 6:31378510   | missense     | G/A                   | MORN2                                                                   | 2:39103103-39109850 | 0.13416      | 4.60E-11    | 0.004205     | 13                        |
| rs149536248   | ARHGAP6 | 11:1320892   | intron       | C/T                   | PTPRC                                                                   | 1:198607801-198726545 | -0.26358     | 5.63E-11    | 0.005058     | 7, 12                     |
| rs1192093     | SYNR    | 3:6269389    | intron       | T/C                   | CCL2                                                                    | 17:3258296-32584222 | -0.3581      | 5.76E-11    | 0.005092     | 7, 12                     |
| rs6123722     | LINC00261 | 20:22557099  | intron       | G/A                   | AP3B2                                                                   | 15:83328033-83378666 | -0.12357     | 6.28E-11    | 0.005454     | 7, 13                     |
| rs7081076     | SORBS1  | 10:97147537  | missense     | C/A                   | LAMP3                                                                   | 3:18284001-182881627 | 0.406578     | 6.47E-11    | 0.005554     | 7                         |
| rs12399124    | PRX     | X:3544089    | missense     | A/G                   | SLCl1644                                                                 | 1:110905470-11093704 | 0.213551     | 6.66E-11    | 0.005604     | 7                         |
| rs1051785     | MICA    | 6:31378388   | missense     | G/A                   | MORN2                                                                   | 2:39103103-39109850 | 0.13566      | 6.82E-11    | 0.005653     | 13                        |
| rs149536248   | ARHGAP6 | 11:1320892   | intron       | C/T                   | ---                                                                     | M:14857-15888     | -0.4336      | 7.05E-11    | 0.005754     | 7                         |
| rs5955819     | SH3KBP1 | 19:19599713  | intron       | C/T                   | UGGC                                                                    | 9:11469546-114697649 | 0.315262     | 7.58E-11    | 0.006076     | 7, 12, 13                |
| rs77599711    | NLRP13  | 19:56425689  | intron       | G/A                   | SLCl1644                                                                 | 1:110905470-11093704 | 0.208622     | 7.68E-11    | 0.006076     | 7                         |
| rs117781420   | DENND4C | 9:19335687   | intron       | G/A                   | ABCA6                                                                   | 17:6.7074847-67138015 | 0.322396     | 8.03E-11    | 0.006261     | 7, 13                     |
| rs2158937     | LOC100129935 | 19:40132472  | intron       | C/T                   | PUS7                                                                    | 7:105081080-105162714 | -0.19093     | 8.65E-11    | 0.006647     | 7, 13                     |
| rs58232949    | MICAL   | 3:40693259   | intergenic   | G/A                   | AP3B2                                                                   | 15:83328033-83378666 | -0.0915      | 9.28E-11    | 0.007026     | 7, 13                     |
may account for up to 70% of heritability.\textsuperscript{31} Studies using Hi-C and eQTL corroborate our results that show regions containing the regulatory SNP do not necessarily interact with or influence expression of the nearest gene.\textsuperscript{31–32} There is still a large gap in understanding of the contribution of trans-eQTLs to complex disorders as most of these disease-causing SNPs are still unknown and understudied.

Figure 2. trans-eQTL rs2369519 for \texttt{ABCA6}, \texttt{EML6}, and \texttt{CLNK}. Linear interaction between genotype (x-axis) of rs2369519 (on X chromosome) and diagnosis (IS and VRFC) on expression of three genes on the y-axis: \texttt{CLNK}, \texttt{EML6}, and \texttt{ABCA6}. Mean gene expression from the signal space transformation, in conjunction with regular robust multiple-array average normalization method (SST-RMA) (y-axis) with standard error bars are plotted by SNP genotype (x-axis: GG, GA, AA) and diagnosis status (red – IS; green - VRFC). For \texttt{ABCA6} the beta was $-0.32$, \textit{P} value $= 4.15\text{E}-13$, and FDR $0.000184$; for \texttt{EML6} the beta was $-0.23$, \textit{P} value $= 3.66\text{E}-12$ and FDR $= 0.000694$; and for \texttt{CLNK} the beta was $-0.177$, \textit{P} value $= 2.18\text{E}-11$, and FDR $= 0.002184$ (Table 3). IS, ischemic stroke; VRFC, vascular risk factor control.
The data presented here suggest a role for trans-eQTL after stroke. We identified many SNP-gene pairs that linked expression of the gene to the specific genotype. Notably, there were often many trans-eQTL/multiple SNPs that influenced expression of a single gene and similarly single trans-eQTL/SNPs sometimes influenced expression of a number of genes. The most significant trans-eQTL was ANOS1 (anosmin 1) (Table 3). ANOS1 mutations are associated with Kallmann syndrome (anosmia and hypogonadotropic hypogonadism). During development ANOS1 works as a chemotropic cue contributing to axonal outgrowth and collateralization, and modulating the migration and proliferation of different cell types including neurons and oligodendrocytes. Thus, ANOS1 may play a role in recovery following stroke.

We have previously investigated differences in X-chromosome gene expression between men and women with ischemic stroke. Several cis- and trans-eQTL in our study show that variants in the X-chromosome contribute to changes in expression of nearby and distant genes. Among cis-eQTLs, rs2738360 (G/A) was correlated with the expression of (GTP binding protein 6 putative) GTPBP6 that was differentially expressed between 5h ischemic stroke and controls in our previous study. Regarding trans-eQTL, we found SNP rs950391 (G/A) affected the expression of premature ovarian failure (POF1B) that was expressed differentially between 24h ischemic stroke and controls in our previous study.

Two other genes identified as differentially expressed between ischemic stroke and control patients in our previous studies are now shown to be eQTL. The trans-eQTL genes including CCL2 (chemokine (C-C motif)) and UGCCG (UDP-glucose ceramide glucosyltransferase) were differentially expressed between ischemic stroke and control patients (FDR < 0.05, fold change>1.5). Some trans-eQTL SNPs affect expression of multiple genes in trans, of which some are altered in individuals after stroke. For example, the X-linked SNP rs950391 (G/A), was associated with altered gene expression of ABCA6, CLN6, EML6, POF1B, and WNT16. These X-linked SNP-gene pairs may account for aspects of sexual dimorphism in stroke in particular related to aspects of X-linked inactivation and dosing effects of related genes or alleles.

The majority of stroke eQTL SNPs are located in non-coding regions of the genome (Tables 2, 3 and 4). Non-coding variants play a major role in the genetics of complex traits. Genome-wide association studies (GWAS) have identified associations with stroke and stroke subtypes, but have yet to assess stroke diagnosis-dependent eQTL. An analysis of genome-wide association data from 19,602 white persons showed two intergenic SNPs on chromosome 12p13 is associated with an increase of risk of stroke. A multi-ancestry genome-wide association study of 520,000 subjects identified 32 loci associated with stroke and stroke subtype. Given differences in study cohorts, screening platforms, and analysis workflows, it is unsurprising that we did not find much overlap in variants. However, of the 32 SNPs reported by Malik et al., (2018) four were included in our variant set. Three of the four overlapping variants (rs3184504, rs12037987, and rs635634) had associations (p < 0.05) with nine gene transcripts, highlighting the importance of the identified SNPs and suggesting that they may influence the transcriptional response to ischemic stroke (Supplementary Table S1).

Another GWAS discovered one significant variant and several variants with suggestive association with outcome and recovery three months after incidence of stroke. Furthermore, another study conducted by the NINDS-SIGN consortium discovered novel loci associated with ischemic stroke and its subtypes of European descent. Recent meta-analysis of GWAS in 71,128 individuals looking at carotid artery intima media thickness (cIMT), and 48,434 individuals for carotid plaque traits, identified 16 loci significantly associated with either cIMT or carotid plaque, of which nine were novel. Both cIMT and carotid plaque traits are relevant for large vessel ischemic stroke. A Dutch population-specific SNP imputation study identified an ABCA6 (ATP-binding cassette, subfamily A (ABC1), member 6) variant associated with cholesterol levels. We found several other variants associated with ABCA6 in our study, namely rs950391, rs2464504, rs11758921, rs2369519, rs17409498, rs79434685, rs6665585, and rs117781420, suggesting variants associated with specific traits of interest may be population-specific. ABCA6 is a membrane transporter likely involved in macrophage/leukocyte lipid/cholesterol homeostasis.

Since genes with trait-relevant function only contribute a small fraction of total disease risk, it seems reasonable that we found many eQTLs that were not reported in previous GWAS studies. Findings such as ours can provide deeper insight into the contribution of genetic variants to pathophysiological response to stroke and facilitate better genetic understanding and prediction of stroke outcomes related to cis and trans effects on gene expression. Association of rare and ultra-rare variants to disease is becoming more apparent as the breadth of knowledge expands. The exact mechanisms by which small changes in genetic variation aggregate to exert specific influence over specific gene expression effects remain unknown.

A number of our stroke eQTL have also been reported in other eQTL analyses highlighting their influence by genetic characteristics. In blood, NRGN, CXCL10,
SMAD4, CD38, ITGA1, KLK15, COX15, TTK, WSB2, ZNF358, FOXRED2, LILRA4, SECISBP2, SPHK2, UGCG, SLC16A4, ZFAT, ABCA6, AP3B2, TTC21A, PTTPC, TLR3, GLDC, ZBTB16, ZNF207, C5, LAMP3, CCL2, and PU57 have been reported as blood eQTL (Tables 2, 3 and 4).\(^7\)

Moreover, NRGN, PPP1R37, UBXN7, ITGA1, RADIL, SPHK2, ABL1, IGFRL1, COX15, RPN4IP1, NDUFA3, MTSS1L, WSB2, ZNF358, SECISBP2, FOXRED2, UGCG, RAPGEF5, ABCA6, AP3B2, EML6, ZFAT, TTC21A, SMAD4, GLDC, ZNF207, MORN2, PU57, and ZBTB16 genes have been reported as brain eQTL (Tables 2, 3 and 4).\(^{13}\)

Using the GRASP database, we found that expression of ITGA1, RAPGEF5, CD38, ZBTB16, C5 and ZNF gene family genes are associated with stroke.\(^{50}\) In addition, some stroke/cardiovascular disease risk factor SNPs including rs3776738, rs11809423, rs7250947, and rs2195310, identified in Tables 2, 3 and 4 overlapped with eQTL SNPs reported in the literature.\(^{50}\)

It is important to consider that our study examined the expression response in whole blood of IS patients. The components that make up whole blood, including immune cell subtypes, vesicles, and more, have important roles and responses to injury and also specific gene expression profiles that could be masked in whole blood analysis. Differentially expressed transcripts found in whole blood show enrichment of genes associated with monocyte- or neutrophil-specific inflammatory and immune response to IS.\(^{51}\) Two of the relevant genes we identify in eQTL here, NRGN and CXCL10 (cis-eQTL genes), have the highest expression levels in monocytes compared to other cell types based on the Human Blood Atlas.\(^{52}\) Future work will determine whether individual components of whole blood are preferred targets over strategies that more broadly affect the overall aggregate response, yet understanding candidate sources of key expressed transcripts is essential.

In summary, this genome-wide study examines and reveals the effect of genotype \(*\) diagnosis on gene expression of blood after IS. These eQTLs could play a role in post-ischemic stroke injury or recovery. The suggestion that the specific inflammatory response to stroke in each individual is at least partially influenced by genetic variation has implications for progressing towards personalized treatment strategies. Treatments guided by specific genetic architecture could help pinpoint the pathways and proteins most likely to be prominent and specifically activated or inactivated and thus could be modulated to improve outcome with fewer off target effects.

Additional studies of an independent cohort with large sample sizes are needed to validate the current findings. Future studies will also need to stratify the stroke eQTL by diagnosis subtype, since many of the genetic risk factors for stroke differ according to stroke subtype. Since the QTLs vary considerably between tissues and cell types and sex, eQTL analysis of different blood cell types of both sexes could provide insight into how risk loci influence disease susceptibility and response. While we included factors known to highly impact gene expression in our statistical model, any factors not included (e.g., diabetes, hypertension, alcohol consumption, or others that were not measured) may also influence gene expression in our subjects to some degree. The future work examining the above relationships will help determine treatment strategies to improve stroke outcome.

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### Conflict of Interest

Dr. Frank Sharp, Dr. Boryana Stamova and Dr. Xinhua Zhan are co-founders of Sanguinity, Inc. There are no conflicts of interest to report for the other authors.

### Compliance with Ethics Guidelines

#### Ethical Approval

All procedures involving human subjects were approved by the UC Davis and UC San Francisco Institutional Review Boards and the University of Alberta Health Research Ethics Board (Biomedical Panel) and adhere to all federal and state regulations related to the protection of human research subjects, including The Common Rule, the principles of The Belmont Report, and Institutional policies and procedures.

#### Informed Consent

Informed consent was obtained from all patients and participants or their proxy.

### References

1. Stamova B, Xu H, Jickling G, et al. Gene expression profiling of blood for the prediction of ischemic stroke. Stroke 2010;41:2171–2177. https://doi.org/10.1161/STROKEAHA.110.588335.

2. Stamova B, Ander BP, Jickling G, et al. The intracerebral hemorrhage blood transcriptome in humans differs from...
the ischemic stroke and vascular risk factor control blood transcriptomes. J Cereb Blood Flow Metab 2019;39:1818–1835. https://doi.org/10.1177/027179X18769513
3. Jickling GC, Xu H, Stamova B, et al. Signatures of cardioembolic and large vessel ischemic stroke. Ann Neurol 2010;68:681–692. https://doi.org/10.1002/ana.22187
4. Jickling GC, Stamova B, Ander BP, et al. Prediction of cardioembolic, arterial, and lacunar causes of cryptogenic stroke by gene expression and infarct location. Stroke 2012;43:2036–2041. https://doi.org/10.1161/STROKEAHA.111.648725
5. Jickling GC, Ander BP, Stamova B, et al. RNA in blood is altered prior to hemorrhagic transformation in ischemic stroke. Ann Neurol 2013;74:232–240. https://doi.org/10.1002/ana.23883
6. Bevan S, Traylor M, Abid-Samii P, et al. Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genomewide associations. Stroke 2012;43:3161–3167. https://doi.org/10.1161/STROKEAHA.112.665760
7. Westra H-J, Peters MJ, Esko T, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet 2013;45:1238–1243. https://doi.org/10.1038/ng.2756
8. Strober Bj, Elorbany R, Rhodes K, et al. Dynamic genetic regulation of gene expression during cellular differentiation. Science 2019;364:1287–1290. https://doi.org/10.1126/science.aaw0040
9. Coolson W, Liang L, Abecasis G, et al. Mapping complex disease traits with global gene expression. Nat Rev Genet 2009;10:184. https://doi.org/10.1038/nrg2537
10. Microarray normalization using Signal Space Transformation with probe Guanine Cytosine Count Correction. 2015 [white paper].
11. Shabalina AA. Matrix eQTL: ultra fast eQTL analysis via large matrix operations. Bioinformatics 2012;28:1353–1358. https://doi.org/10.1093/bioinformatics/bts163
12. Rouillard AD, Gundersen GW, Fernandez NF, et al. The harmonizome: a collection of processed datasets gathered to serve and mine knowledge about genes and proteins. Database 2016;2016. https://doi.org/10.1093/database/baw100
13. Ng B, White CC, Klein H-U, et al. An XQTL map integrates the genetic architecture of the human brain’s transcriptome and epigenome. Nat Neurosci 2017;20:1418–1826. https://doi.org/10.1038/nn.4632
14. Miao L, Yin R-X, Yang S, et al. Association between single nucleotide polymorphism rs9534275 and the risk of coronary artery disease and ischemic stroke. Lipids Health Dis 2017;16:193. https://doi.org/10.1186/s12944-0170584-5.
15. Malik R, Rannikmüø K, Traylor M, et al. Genome-wide meta-analysis identifies 3 novel loci associated with stroke. Ann Neurol 2018;84:934–939. https://doi.org/10.1002/ana.25369
16. Matarín M, Brown WM, Scholz S, et al. A genome-wide genotyping study in patients with ischaemic stroke: initial analysis and data release. Lancet Neurol 2007;6:414–420. https://doi.org/10.1016/s1474-4422(07)70081-9
17. Chen C, Chu S-F, Liu D-D, et al. Chemokines play complex roles in cerebral ischemia. Neurochem Int 2018;112:146–158. https://doi.org/10.1016/j.neuint.2017.06.008
18. Quan Z, Quan Y, Wei B, et al. Protein-protein interaction network and mechanism analysis in ischemic stroke. Mol Med Rep 2015;11:29–36. https://doi.org/10.3892/mmr.2014.2696
19. Gallione CJ, Repetto GM, Legius E, et al. A combined syndrome of juvenile polyposis and hereditary haemorrhagic telangiectasia associated with mutations in MADH4 (SMAD4). Lancet 2004;363:852–859. https://doi.org/10.1016/S0140-6736(04)15732-2
20. Li F, Lan Yu, Wang Y, et al. Endothelial smad4 maintains cerebrovascular integrity by activating N-cadherin through cooperation with Notch. Dev Cell 2011;20:291–302. https://doi.org/10.1016/j.devcel.2011.01.011
21. Jickling GC, Sharp FR. Biomarker panels in ischemic stroke. Stroke 2015;46:915–920. https://doi.org/10.1161/STROKEAHA.114.005604.
22. Arning A, Hiersche M, Witten A, et al. A genome-wide association study identifies a gene network of ADAMTS genes in the predisposition to pediatric stroke. Blood 2012;120:5231–5236. https://doi.org/10.1182/blood-2012-07-442038
23. Hayakawa K, Esposito E, Wang X, et al. Transfer of mitochondria from astrocytes to neurons after stroke. Nature 2016;535:551–555. https://doi.org/10.1038/nature16000.
24. Wang P, Li W-L, Liu J-M, et al. NAMPT and NAMPT-controlled NAD metabolism in vascular repair. J Cardiovasc Pharmacol 2016;67:474–481. https://doi.org/10.1097/FJC.0000000000000332
25. Choe C-u, Lardong K, Gelderblom M, et al. CD38 exacerbates focal cytokine production, postischemic inflammation and brain injury after focal cerebral ischemia. PLoS One 2011;6:e19046. https://doi.org/10.1371/journal.pone.0019046
26. Kassner S, Kollmar R, Bonaterra Ga, et al. The early immune response to acute ischemic stroke: Differential gene expression in subpopulations of mononuclear cells. Neuroscience 2009;160:393–401. https://doi.org/10.1016/j.neuroscience.2009.02.050
27. De Vos A, Bjerke M, Brouns R, et al. Neurogranin and tau in cerebrospinal fluid and plasma of patients with acute ischemic stroke. BMC Neurol 2017;17:170.
28. Glynne R, Ghandour G, Rayner J, Mack DH, Goodnow CC. B-lymphocyte quiescence, tolerance and activation as viewed by global gene expression profiling on microarrays. Immunol Rev 2000;176:216–246. https://doi.org/10.1034/j.1600-065x.2000.00614.x
29. Devireddy LR, Green MR. Transcriptional program of apoptosis induction following interleukin 2 deprivation: identification of RC3, a calcium/calmodulin binding protein, as a novel proapoptotic factor. Mol Cell Biol 2003;23:4532–4541. https://doi.org/10.1128/mcb.23.13.4532-4541.2003

30. Cassandri M, Smirnov A, Novelli F, et al. Zinc-finger proteins in health and disease. Cell Death Discov 2017;3:17071. https://doi.org/10.1038/cddiscovery.2017.71

31. Liu X, Li Y, Pritchard JK. Trans effects on gene expression

32. Mumbach MR, Satpathy AT, Boyle EA, et al. Enhancer connectome in primary human cells identifies target genes of disease-associated DNA elements. Nat Genet 2017;49:1602–1612. https://doi.org/10.1038/ng.3963

33. Kim JH, Seo GH, Kim G-H, et al. Targeted gene panel sequencing for molecular diagnosis of Kallmann syndrome and normosmic idiopathic hypogonadotropic Hypogonadism. Exp Clin Endocrinol Diabetes 2019;127:538–544. https://doi.org/10.1055/a-0681-6608

34. Murcia-Belmonte V, Esteban PF, Martínez-Hernández J, et al. Anosmin-1 over-expression regulates oligodendrocyte precursor cell proliferation, migration and myelin sheath thickness. Brain Struct Funct 2016;221:1365–1385. https://doi.org/10.1007/s00429-014-0977-4

35. Stamova B, Tian Y, Jickling G, et al. The X-chromosome has a different pattern of gene expression in women compared to men with ischemic stroke. Stroke 2012;43:326–334. https://doi.org/10.1161/STROKEAHA.111.629337

36. Yao C, Joehanes R, Johnson AD, et al. Dynamic role of trans regulation of gene expression in relation to complex traits. Am J Hum Genet 2017;100:571–580. https://doi.org/10.1016/j.ajhg.2017.02.003

37. Li YI, van de Geijn B, Raj A, et al. RNA splicing is a primary link between genetic variation and disease. Science 2016;352:600–604. https://doi.org/10.1126/science.aad9417

38. Traylor M, Farrall M, Holliday EG, et al. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE Collaboration): a meta-analysis of genome-wide association studies. Lancet Neurol 2012;11:951–962. https://doi.org/10.1016/S1474-4422(12)70234-X

39. Traylor M, Malik R, Nalls MA, et al. Genetic variation at 16q24.2 is associated with small vessel stroke. Ann Neurol 2017;81(3):383–394. https://doi.org/10.1002/ana.24840

40. Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nat Genet 2018;50:524–537. https://doi.org/10.1038/s41588-018-0058-3

41. Chauhan G, Arnold CR, Chu AY, et al. Identification of additional risk loci for stroke and small vessel disease: a meta-analysis of genome-wide association studies. Lancet Neurol 2016;15:695–707. https://doi.org/10.1016/S1474-4422(16)00102-2

42. Rannikmae K, Davies G, Thomson PA, et al. Common variation in COL4A1/COL4A2 is associated with sporadic cerebral small vessel disease. Neurology 2015;84:918–926. https://doi.org/10.1212/WNL.0000000000001309

43. Chung J, Marini S, Pera J, et al. Genome-wide association study of cerebral small vessel disease reveals established and novel loci. Brain 2019;142:3176–3189. https://doi.org/10.1093/brain/awz233

44. Ikram MA, Seshadri S, Bis JC, et al. Genomewide association studies of stroke. N Engl J Med 2009;360:1718–1728. https://doi.org/10.1056/NEJMoA0900094.

45. Söderholm M, Pedersen A, Lorentzen E, et al. Genomewide association meta-analysis of functional outcome after ischemic stroke. Neurology 2019;92:e1271–e1283. https://doi.org/10.1212/WNL.0000000000007138

46. Pult SL, Mc Ardle PF, Wong Q, et al. The NINDS Stroke Genetics Network: a genome-wide association study of ischemic stroke and its subtypes. Lancet Neurol 2016;15:174–184. https://doi.org/10.1016/S1474-4422(15)00338-5

47. Franceschini N, Giambartolomei C, de Vries PS, et al. GWAS and colocalization analyses implicate carotid intima-media thickness and carotid plaque loci in cardiovascular outcomes. Nat Commun 2018;9:5141. https://doi.org/10.1038/s41467-018-07340-5

48. van Leeuwen EM, Karssen LC, Deelen J, et al. Genome of the Netherlands population-specific imputations identify an ABCA6 variant associated with cholesterol levels. Nat Commun 2015;6:6065. https://doi.org/10.1038/nc ommun.7065

49. Kaminski WE, Wenzel JJ, Pichler A, et al. ABCA6, a Novel A Subclass ABC Transporter. Biochem Biophys Res Commun 2001;285:1295–1301. https://doi.org/10.1006/bbrc.2001.5326

50. Leslie R, O’Donnell CJ, Johnson AD. GRASP: analysis of genotype–phenotype results from 1390 genome-wide association studies and corresponding open access database. Bioinformatics 2014;30:i185–i194. DOI: https://doi.org/10.1093/bioinformatics/btu273

51. Tang Y, Xu H, Du XL, et al. Gene expression in blood changes rapidly in neutrophils and monocytes after ischemic stroke in humans: a microarray study. J Cereb Blood Flow Metab 2007;26:1089–1102. https://doi.org/10.1038/sj.jcbfm.9600264

52. Thul PJ, Lindskog C. The human protein atlas: a spatial map of the human proteome. Protein Sci 2018;27:233–244.
Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Table S1. cis-eQTL identified in our cohort as ischemic stroke diagnosis dependent (genotype × diagnosis interaction) and shared with features identified in Malik et al. (2018).

Supplementary Materials and Methods. Detailed descriptions of subject recruitment, nucleic acid extractions from blood, genotyping and gene expression measurement, and eQTL analysis.