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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,[1] and different profiles for IS compared to intracerebral hemorrhage.[2] There are different profiles for varying causes of IS[3] that can predict causes of cryptogenic strokes where the cause is not otherwise known.[4] Moreover gene expression profiles in blood of IS patients prior to administration of tPA predict those who develop hemorrhagic transformation one day later.[5] 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.[6] 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.[7] 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[8] 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 microarrays[10] 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) VCRF 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).

Table 1. Demographic and clinical characteristics for ischemic stroke (IS) patients and vascular risk factor controls (VRFC)

	Vascular risk factor controls (n = 138)	Ischemic stroke patients (n = 137)	*P* value
Age, y (SD)	60.9 (13.3)	61.4 (13.2)	0.780
Sex, female, n (%)	68 (49.3)	51 (37.2)	0.051
Race/ethnicity, n (%)			0.424
Caucasian	81 (58.7)	86 (62.8)	
African American	14 (10.1)	20 (14.6)	
Latino, Hispanic	16 (11.6)	9 (6.6)	
Asian	13 (9.4)	12 (8.8)	
Other	14 (10.1)	10 (7.3)	
Hypertension, n (%)	86 (62.3)	98 (71.5)	0.124
Diabetes, n (%)	24 (17.4)	36 (26.3)	0.081
Hypercholesterolemia, n (%)	64 (46.4)	66 (48.2)	0.809
Cause of stroke, n (%)			
Cardioembolic	---	24 (17.5)	
Large vessel disease	---	23 (16.8)	
Lacunar	---	42 (30.7)	
Cryptogenic	---	44 (32.1)	
Other	---	4 (2.9)	
Smoking status, n (%)			0.423
Current	24 (17.4)	32 (23.3)	
Former	40 (28.9)	40 (29.2)	
Never	74 (53.6)	65 (47.4)	
Alcohol consumption, n (%)			0.113
Heavy	4 (2.9)	12 (10.14)	
Mild	63 (45.65)	52 (37.96)	
Former Heavy	7 (5.07)	11 (8.03)	
Never	64 (46.38)	62 (45.25)	

P values represent the comparison between IS and VRFC using a two-tailed *t* test or Fisher's exact test/chi-square test.
Alcohol consumption as heavy and mild defined as ≥3 drinks/day and ≤2 drinks/day, respectively.

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 VFRC subjects. These SNP-gene pairs from the interaction analysis can indicate one of three different biological properties. First, they can represent eQTL in VFRC or IS but not both. Second, eQTLs can indicate an opposite directional effect between VFRC and IS. Third, eQTL may be in the same direction but of significantly different magnitude of impact between VFRC 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).

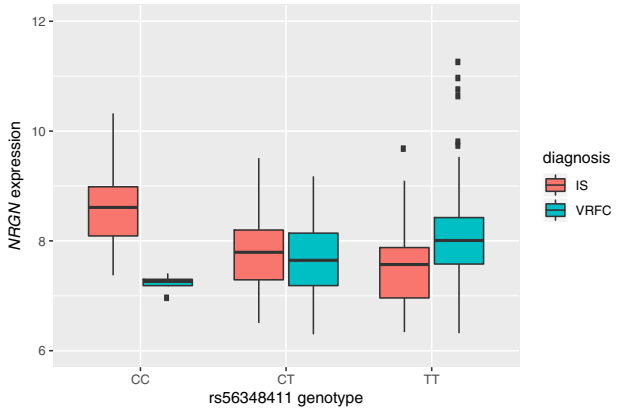

Figure 1. *cis*-eQTL rs56348411 for *NRGN*. Linear interaction between genotype (*x*-axis) of rs56348411 and diagnosis (IS and VRFC) on gene expression of *NRGN* (*y*-axis). Mean gene expression from the signal space transformation, in conjunction with regular robust multiple-array average normalization 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.

The *cis*-eQTL analysis indicated 38 SNP-gene pairs with a *P* value cut-off below 1.0×10^{-5} (Table 2). Four of these *cis*-eQTL had FDR < 0.25. The significant associated genes for these four *cis*-eQTL SNPs were: *NRGN* (rs56348411) (Figure 1), *CXCL10* (rs78046578), *SMAD4* (rs975903) and *CD38* (rs62299879) (Table 2). Note that genotype rs56348411 (C/T) is a variant associated with a strong eQTL (*P* value = 2.10×10^{-8}, FDR = 0.08) for *NRGN* expression in blood (Table 2).

The *trans*-eQTL analysis showed 70 SNP-gene pairs (39 SNPs) affecting 23 genes and meeting the cut-off *P* value < 1.0×10^{-11} with FDR < 0.01 (Table 3 and Table 4). In other words, using a 1% FDR threshold, we identified 23 genes with *trans*-eQTL exhibiting a genotype × diagnosis interaction effect. Among these genes, two X-linked genes *ANOS1* and *POF1B* were found. Expression of an X-linked gene *ANOS1* was significantly correlated with intergenic variants including rs148791848 and rs149957475. Expression of another X-linked premature ovarian failure gene *POF1B* was significantly correlated with intergenic variant rs950391 (Table 3).

For *trans*-eQTL a single SNP usually affected the expression of several genes, from two to five. For example, the AA variant of rs2369519 found on the X chromosome increased expression of: *ABCA6* on chromosome 17, *EML6* on chromosome 2, and *CLNK* on chromosome 4 in stroke compared to VRFC (Figure 2) (Table 3). The 70 *trans*-eQTL affected the expression of only 23 genes, meaning a given gene was regulated by multiple *trans*-eQTL.

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 *PTPRC*, *UGCG*, *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 thrombolysis 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

Table 2. *cis*-eQTL identified as ischemic stroke diagnosis dependent (genotype × diagnosis interaction)

cis-eQTL										
SNP					mRNA					
rsID	Gene ID	Chr:Position	Variant Type	Ref allele/ Alt allele	Gene ID	Chr:Position	beta	*P* value	FDR	Appears in References
rs56348411	*TMEM218*	11:124974588	intron	C/T	*NRGN*	11:124609829-124617869	0.312936	2.10E-08	0.087925	7, 13
rs78046578	*NAAA*	4:76836362	intron	T/C	*CXCL10*	4:76942269-76944689	-0.52763	5.43E-08	0.113845	7
rs975903		18:49306115	intergenic	T/G	*SMAD4*	18:48556583-48611415	-0.16254	1.18E-07	0.165241	7, 13
rs62299879		4:16448976	intergenic	T/C	*CD38*	4:15779898-15851069	0.493781	1.99E-07	0.20815	7, 12, 13, 50
rs11809423	*HIVEP3*	1:41976529	missense	C/T	*ZNF684*	1:40997233-41013841	0.176159	5.87E-07	0.49235	
---		1:157485429		C/G	*FCRL4*	1:157543539-157567870	0.070146	2.26E-06	0.862336	
rs75608718	*CCDC61*	19:46515961	intron	T/C	*PPP1R37*	19:45596218-45650543	0.024433	2.12E-06	0.862336	13
rs75391517		3:196415355	intergenic	G/C	*UBXN7*	3:196074533-196159345	-0.08238	1.73E-06	0.862336	13
rs75368642	*SMARCD3*	7:150969808	intron	C/T	*ACTR3C*	7:149941005-150020814	0.099661	1.59E-06	0.862336	
rs17666226		18:49166695	intergenic	C/T	*SMAD4*	18:48556583-48611415	0.150092	1.86E-06	0.862336	7, 13
rs10958734	*HOOK3*	8:42801655	intron	C/T	*HOOK3*	8:42752033-42885682	0.098645	2.25E-06	0.862336	
rs3776738	*ARL15*	5:53224090	intron	G/A	*ITGA1*	5:52083730-52255037	0.145169	3.68E-06	0.943412	7, 12, 13, 50
rs79403922	*SDK1*	7:3959420	intron	A/C	*RADIL*	7:4834285-4923350	-0.04465	3.47E-06	0.943412	13
rs60839180	*KLK6*	19:51467289	intron	C/T	*KLK15*	19:51328545-51334779	0.082362	3.72E-06	0.943412	7
rs3730850	*LIG1*	19:48668709	intron	A/G	*SPHK2*	19:49122548-49133974	0.022658	3.22E-06	0.943412	7, 13
rs2180911		20:44949747	intergenic	T/C	*ZNF335*	20:44577292-44600833	-0.03493	4.05E-06	0.943412	
rs11243548		9:134716309	intergenic	G/A	*ABL1*	9:133589268-133763062	-0.10017	3.31E-06	0.943412	13
rs7250947	*PLIN4*	19:4510530	missense	G/A	*MYDGF*	19:4657557-4670415	-0.17133	3.91E-06	0.943412	
rs12110	*FXYD5*	19:35660508	missense	G/A	*IGFLR1*	19:36230151-36233520	0.146164	4.29E-06	0.945503	13
rs2892934	*CLCA4*	1:87037398	intron	C/T	*CLCA4*	1:87012759-87046437	0.101111	4.62E-06	0.968375	
rs7129315	*TMEM218*	11:124977280	intron	T/C	*NRGN*	11:124609829-124617869	-0.21486	5.04E-06	0.976903	7
rs2738360	*PPP2R3B*	X:302966	intron	G/A	*GTPBP6*	X:220013-230887	-0.11172	5.13E-06	0.976903	
rs12359932	*HPSE2*	10:100998381	upstream	T/C	*COX15*	10:101468505-101492423	-0.16858	5.38E-06	0.979881	7, 13
rs7757514	*CRYBG1*	6:106834984	intron	T/C	*RTN4IP1*	6:107018903-107078366	-0.10361	5.65E-06	0.986128	13
rs6662611		1:151936485	intergenic	A/G	*OAZ3*	1:151735445-151743808	0.044318	7.37E-06	1	
rs10943676		6:80606507	intergenic	G/T	*TTK*	6:80713604-80752244	0.049491	8.95E-06	1	7
rs2195310	*ZNF347*	19:53645291	missense	T/C	*NDUFA3*	19:54606036-54614898	-0.03663	9.68E-06	1	13
rs61733124	*PHLPP2*	16:71682830	missense	C/T	*MTSS1L*	16:70442867-70719954	0.03369	6.54E-06	1	13
rs6844790	*STOX2*	4:184946378	downstream	G/A	*CLDN24*	4:184242917-184243579	-0.06327	7.79E-06	1	
rs11068369	*FBXO21*	12:117586896	intron	T/G	*WSB2*	12:118470492-118499979	-0.1048	7.61E-06	1	7, 13
rs74517766		19:6870146	intergenic	C/T	*ZNF358*	19:7581004-7585912	0.058977	8.47E-06	1	7, 13
rs34517659	*WDR1*	4:10094042	intron	G/A	*DEFB131*	4:9446257-9452240	-0.2016	8.03E-06	1	
rs76287022	*SHC3*	9:91627100	3′ UTR	C/T	*SECISBP2*	9:91933412-91974561	0.128618	9.04E-06	1	7, 13
rs16986309	*PTPRH*	19:55710074	missense	G/A	*LILRA4*	19:54844456-54850421	0.081471	6.28E-06	1	7
rs12480811	*KCNQ2*	20:62059116	intron	C/T	*PTK6*	20:62159776-62168723	-0.05829	8.12E-06	1	
rs132642	*APOL3*	22:36545137	intron	A/T	*FOXRED2*	22:36883233-36903148	-0.04143	6.40E-06	1	7, 13
rs1326895	*LPAR1*	9:113678096	intron	C/T	*UGCG*	9:114659046-114697649	0.196689	9.22E-06	1	7, 12, 13
---		7:23300046		TTTA/-	*RAPGEF5*	7:22157908-22396763	0.028594	9.65E-06	1	13, 50

Table 3. *trans*-eQTL identified as ischemic stroke diagnosis dependent (genotype × diagnosis interaction)

trans-eQTL

SNP					mRNA					
rsID	Gene ID	Chr:Position	Variant Type	Ref allele/Alt allele	Gene ID	Chr:Position	beta	*p* value	FDR	Appears in References
rs148791848		X:93386861	intergenic	T/C	*ANOS1*	X:8496915-8700227	0.349324	2.90E-28	1.54E-18	
rs950391		X:86454329	intergenic	G/A	*ABCA6*	17:67074847-67138015	0.478933	4.97E-17	1.32E-07	7, 13
rs2464504	*TEC*	4:48232441	intron	C/T	*ABCA6*	17:67074847-67138015	−0.39571	6.91E-16	1.22E-06	7, 13
rs11758921	*PDE10A*	6:166247384	intron	A/G	*ABCA6*	17:67074847-67138015	−0.39284	1.01E-15	1.34E-06	7, 13
rs11758921	*PDE10A*	6:166247384	intron	A/G	*SLC16A4*	1:110905470-110933704	−0.27329	7.70E-15	8.17E-06	7
rs72944885	*LOC105374016*	3:102311450	intron	G/A	*AP3B2*	15:83328033-83378666	−0.12245	1.15E-14	1.02E-05	7, 13
rs950391		X:86454329	intergenic	G/A	*CLNK*	4:10488019-10686489	0.26793	1.67E-14	1.26E-05	
rs73507341	*NFIX*	19:13135197	intron	T/C	*AP3B2*	15:83328033-83378666	0.131706	2.08E-14	1.38E-05	7, 13
rs950391		X:86454329	intergenic	G/A	*EML6*	2:54950636-55199157	0.325174	5.16E-14	3.04E-05	13
rs12833155		X:42486482	intergenic	A/C	*ZFAT*	8:135490031-135725292	0.148685	1.47E-13	7.79E-05	7, 13
rs11758921	*PDE10A*	6:166247384	intron	A/G	*CLNK*	4:10488019-10686489	−0.21307	4.09E-13	0.000184	
rs2369519		X:86392534	intergenic	G/A	*ABCA6*	17:67074847-67138015	−0.31608	4.16E-13	0.000184	7, 13
rs11853524	*SNHG14*	15:25508955	intron	G/T	*AP3B2*	15:83328033-83378666	−0.12449	6.88E-13	0.000281	7, 13
rs139929471		X:88063578	intergenic	G/A	*TTC21A*	3:39149152-39180394	0.15013	8.44E-13	0.00032	7, 13
rs79434685	*KREMEN1*	22:29556745	intron	C/G	*EML6*	2:54950636-55199157	−0.27044	9.51E-13	0.000336	13
rs7664829	*KCNIP4*	4:21791787	intron	A/G	*AP3B2*	15:83328033-83378666	0.130956	1.09E-12	0.000362	7, 13
rs1063632	*MICA*	6:31378510	missense	G/A	*PTPRC*	1:198607801-198726545	0.276078	1.26E-12	0.00037	7, 12
rs9779183		X:13009957	intergenic	T/C	*PTPRC*	1:198607801-198726545	−0.27337	1.21E-12	0.00037	7, 12
rs2464504	*TEC*	4:48232441	intron	C/T	*CLNK*	4:10488019-10686489	−0.21105	1.34E-12	0.000375	
rs950391		X:86454329	intergenic	G/A	*POF1B*	X:84532395-84634748	0.301167	1.69E-12	0.000427	
rs139929471		X:88063578	intergenic	G/A	*ZNF684*	1:40997233-41013841	0.23931	1.68E-12	0.000427	
rs1051785	*MICA*	6:31378388	missense	G/A	*PTPRC*	1:198607801-198726545	0.27801	2.38E-12	0.000536	7, 12
rs149957475		X:93351607	intergenic	C/T	*ANOS1*	X:8496915-8700227	−0.29645	2.27E-12	0.000536	
rs9847733	*UBE2E2-AS1*	3:23242050	intron	A/G	*AP3B2*	15:83328033-83378666	0.117811	2.43E-12	0.000536	7, 13
rs1063632	*MICA*	6:31378510	missense	G/A	*SMAD4*	18:48556583-48611415	0.241327	2.67E-12	0.000566	7, 13
rs12399124	*PRKX*	X:3544089	intron	G/A	*UGCG*	9:114659046-114697649	0.376897	2.87E-12	0.000586	7, 12, 13
rs139929471		X:88063578	intergenic	G/A	*LAMP3*	3:182840001-182881627	0.372997	3.06E-12	0.0006	7
rs2369519		X:86392534	intergenic	G/A	*EML6*	2:54950636-55199157	−0.22595	3.66E-12	0.000694	13
rs17409498		20:56044855	intergenic	C/T	*ABCA6*	17:67074847-67138015	0.295084	4.04E-12	0.000738	7, 13
rs2464504	*TEC*	4:48232441	intron	C/T	*EML6*	2:54950636-55199157	−0.25797	4.28E-12	0.000757	13
rs79434685	*KREMEN1*	22:29556745	intron	C/G	*SLC16A4*	1:110905470-110933704	−0.26083	5.40E-12	0.000895	7
rs1051785	*MICA*	6:31378388	missense	G/A	*SMAD4*	18:48556583-48611415	0.242621	5.35E-12	0.000895	7, 13
rs79434685	*KREMEN1*	22:29556745	intron	C/G	*ABCA6*	17:67074847-67138015	−0.35333	5.77E-12	0.000927	7, 13
rs6787784	*ENTPD3-AS1*	3:40486470	intron	T/C	*AP3B2*	15:83328033-83378666	0.118991	6.68E-12	0.001012	7, 13
rs9779183		X:13009957	intergenic	T/C	---	M:14857-15888	−0.44089	6.64E-12	0.001012	
rs117781420	*DENND4C*	9:19355687	intron	G/A	*CLNK*	4:10488019-10686489	0.201571	6.97E-12	0.001027	
rs140580619		X:98022257	intergenic	C/T	*TLR3*	4:186990306-187006255	−0.35134	7.28E-12	0.001043	7

(*Continued*)

Table 3 Continued.

trans-eQTL										
	SNP				mRNA					
rsID	Gene ID	Chr:Position	Variant Type	Ref allele/Alt allele	Gene ID	Chr:Position	beta	*p* value	FDR	Appears in References
rs73178117		X:3466525	intergenic	T/C	*GLDC*	9:6532464-6645692	0.210052	8.07E-12	0.001126	7, 13
rs1172922		9:93488534	intergenic	A/C	*ZBTB16*	11:113930315-114121398	−0.18066	8.84E-12	0.001202	7, 12, 13, 50
rs2464504	*TEC*	4:48232441	intron	C/T	*SLC16A4*	1:110905470-110933704	−0.24903	1.07E-11	0.001421	7
rs72906031		1:16845719		G/T	*AP3B2*	15:83328033-83378666	−0.11958	1.10E-11	0.001421	7, 13
rs57764234	*CHD8*	14:21897616	intron	C/T	*AP3B2*	15:83328033-83378666	−0.12591	1.16E-11	0.001434	7, 13
rs9873394	*ENTPD3*	3:40468206	intron	T/G	*AP3B2*	15:83328033-83378666	0.111598	1.14E-11	0.001434	7, 13
rs117781420	*DENND4C*	9:19355687	intron	G/A	*EML6*	2:54950636-55199157	0.247554	1.59E-11	0.001913	13
rs950391		X:86454329	intergenic	G/A	*WNT16*	7:120965421-120981158	0.246993	1.74E-11	0.002045	
rs7081076	*SORBS1*	10:97174537	missense	C/A	*TTC21A*	3:39149152-39180394	0.163531	1.89E-11	0.002184	7, 13
rs2369519		X:86392534	intergenic	G/A	*CLNK*	4:10488019-10686489	−0.177	2.18E-11	0.002458	
rs1063632	*MICA*	6:31378510	missense	G/A	*ZNF207*	17:30677128-30714780	0.358066	2.36E-11	0.002612	7, 13

previous studies indicated *CD46* and zinc-finger family, *ZNF* (*ZNF185* and *ZNF254*) expression as a biomarker distinguishing the cause of ischemic stroke as cardioembolic or large-vessel disease.[21] Leukemic blast cells overexpress 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 *cis*-eQTL involving the pair through our SNP × 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 *cis*-eQTL: rs11809423 (*ZNF684*), rs2180911 (*ZNF335*), and rs74517766 (*ZNF358*). Additionally, as *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 *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 *trans*-eQTL, which by current estimates

Table 4. *trans*-eQTL identified as ischemic stroke diagnosis dependent (genotype × diagnosis interaction)

trans-eQTL										
SNP					mRNA					
rsID	Gene ID	Chr:Position	Variant type	Ref allele/ Alt allele	Gene ID	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		X:13461054	intergenic	C/A	*ZNF684*	1:40997233-41013841	0.239354	2.96E-11	0.00308	
rs6665585	*LINC01748*	1:61090200	upstream	A/G	*ABCA6*	17:67074847-67138015	−0.28688	3.12E-11	0.003181	7, 13
rs627635		18:66904739	intergenic	T/C	*AP3B2*	15:83328033-83378666	−0.11228	3.55E-11	0.003493	7, 13
rs9779183		X:13009957	intergenic	T/C	*SMAD4*	18:48556583-48611415	−0.227	3.56E-11	0.003493	7, 13
rs1063632	*MICA*	6:31378510	missense	G/A	---	M:14857-15888	0.425948	3.74E-11	0.00361	
rs6665585	*LINC01748*	1:61090200	upstream	A/G	*CLNK*	4:10488019-10686489	−0.17066	4.42E-11	0.004111	
rs148991762		X:13461054	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*	X:11320892	intron	T/C	*PTPRC*	1:198607801-198726545	−0.26358	5.63E-11	0.005058	7, 12
rs11922093	*SYNPR*	3:63269389	intron	T/C	*CCL2*	17:32582296-32584222	−0.3581	5.76E-11	0.005092	7, 12
rs6113722	*LINC00261*	20:22557099	intron	G/A	*AP3B2*	15:83328033-83378666	−0.12357	6.28E-11	0.005454	7, 13
rs7081076	*SORBS1*	10:97174537	missense	C/A	*LAMP3*	3:182840001-182881627	0.406578	6.47E-11	0.005534	7
rs12399124	*PRKX*	X:3544089	intron	G/A	*SLC16A4*	1:110905470-110933704	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*	X:11320892	intron	T/C	---	M:14857-15888	−0.4336	7.05E-11	0.005754	
rs5955819	*SH3KBP1*	X:19599813	intron	C/T	*UGCG*	9:114659046-114697649	0.315262	7.58E-11	0.006076	7, 12, 13
rs77599711	*NLRP13*	19:56425689	intron	G/A	*SLC16A4*	1:110905470-110933704	0.208622	7.68E-11	0.006076	7
rs117781420	*DENND4C*	9:19355687	intron	G/A	*ABCA6*	17:67074847-67138015	0.322396	8.03E-11	0.006261	7, 13
rs2158937	*LOC100129935*	19:40132472	intron	C/T	*PUS7*	7:105080108-105162714	−0.19093	8.65E-11	0.006647	7, 13
rs58232949		3:40693259	intergenic	G/A	*AP3B2*	15:83328033-83378666	−0.09515	9.28E-11	0.007026	7, 13

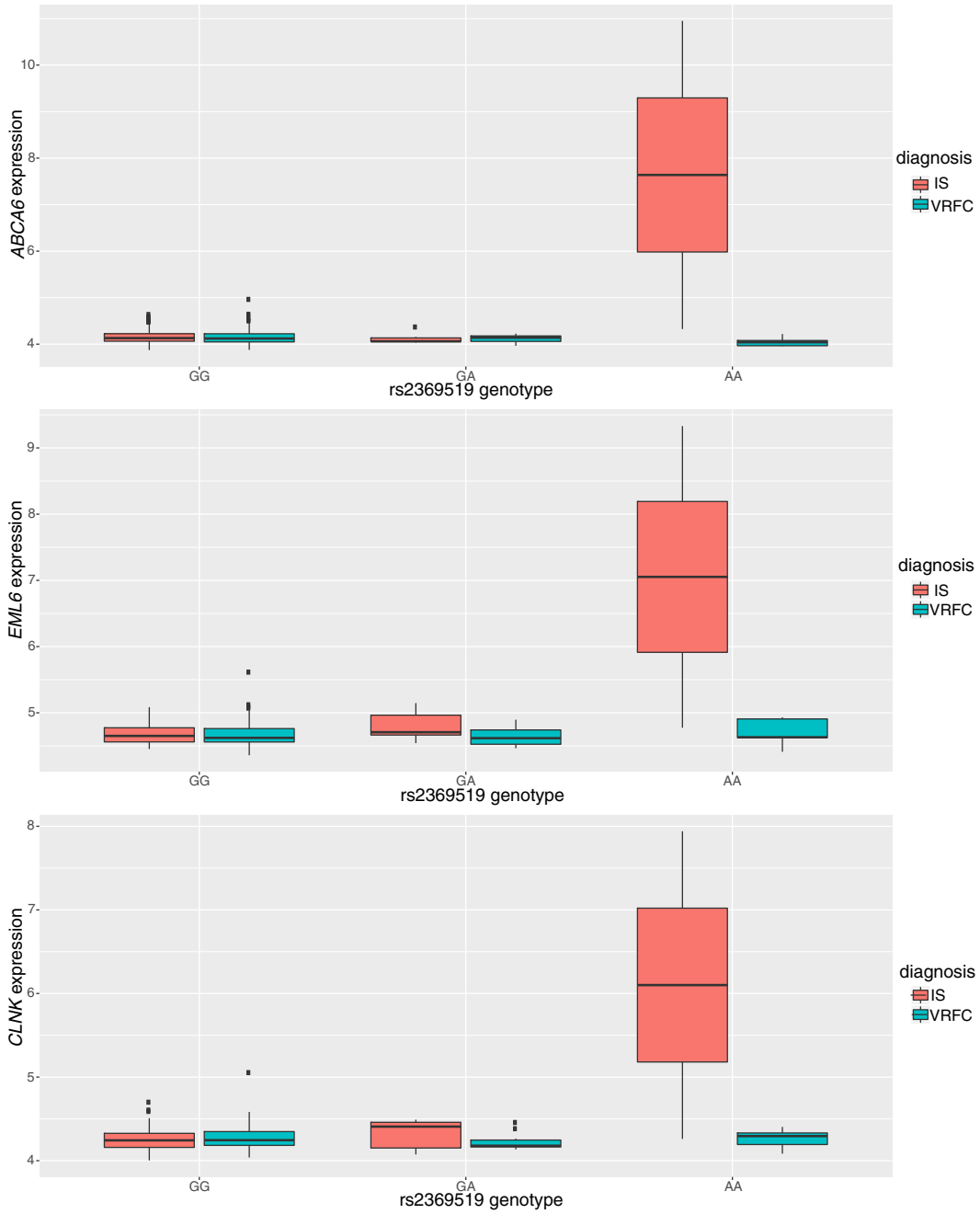

Figure 2. *trans*-eQTL rs2369519 for *ABCA6*, *EML6,* and *CLNK*. Linear interaction between genotype (*x*-axis) of rs2369519 (on X chromosome) and diagnosis (IS and VRFC) on expression of three genes on the *y*-axis: *CLNK, EML6,* and *ABCA6*. Mean gene expression from the signal space transformation, 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 *ABCA6* the beta was −0.32, *P* value = 4.15E-13, and FDR 0.000184; for *EML6* the beta was −0.23, *P* value = 3.66E-12 and FDR = 0.000694; and for *CLNK* the beta was −0.177, *P* value = 2.18E-11, and FDR = 0.002184 (Table 3). IS, ischemic stroke; VRFC, vascular risk factor control.

may account for up to 70% of heritability.[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.[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.

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).[33] 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.[34] 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.[35] 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.[35] 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.[35]

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 *UGCG* (UDP-glucose ceramide glucosyltransferase) were differentially expressed between ischemic stroke and control patients (FDR < 0.05, fold change>|1.5|).[3] Some *trans*-eQTL SNPs affect expression of multiple genes in *trans*, of which some are altered in individuals after stroke.[36] For example, the X-linked SNP rs950391 (G/A), was associated with altered gene expression of *ABCA6*, *CLNK, 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.[37] Genome-wide association studies (GWAS) have identified associations with stroke and stroke subtypes, but have yet to assess stroke diagnosis-dependent eQTL.[15,38–43] 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.[44] A multi-ancestry genome-wide association study of 520,000 subjects identified 32 loci associated with stroke and stroke subtype.[40] 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.[45] Furthermore, another study conducted by the NINDS-SiGN consortium discovered novel loci associated with ischemic stroke and its subtypes of European descent.[46] 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.[47] 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.[48] 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.[48] ABCA6 is a membrane transporter likely involved in macrophage/leukocyte lipid/cholesterol homeostasis.[49]

Since genes with trait-relevant function only contribute a small fraction of total disease risk,[31] 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*, *PTPRC*, *TLR3*, *GLDC*, *ZBTB16*, *ZNF207*, *C5*, *LAMP3*, *CCL2*, and *PUS7* have been reported as blood eQTL (Tables 2, 3 and 4).[7] Moreover, *NRGN*, *PPP1R37*, *UBXN7*, *ITGA1*, *RADIL*, *SPHK2*, *ABL1*, *IGFLR1*, *COX15*, *RTN4IP1*, *NDUFA3*, *MTSS1L*, *WSB2*, *ZNF358*, *SECISBP2*, *FOXRED2*, *UGCG*, *RAPGEF5*, *ABCA6*, *AP3B2*, *EML6*, *ZFAT*, *TTC21A*, *SMAD4*, *GLDC*, *ZNF207*, *MORN2*, *PUS7*, 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, rs10958734, rs7250947, rs6662611, 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.

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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.

