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RNA in blood is altered prior to hemorrhagic transformation in ischemic stroke

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Abstract

Objective—Hemorrhagic transformation (HT) is a major complication of ischemic stroke that worsens outcomes and increases mortality. Disruption of the blood brain barrier is a central feature to HT pathogenesis, and leukocytes may contribute to this process. We sought to determine whether ischemic strokes that develop HT have differences in RNA expression in blood within 3 hours of stroke onset prior to treatment with thrombolytic therapy.

Methods—Stroke patient blood samples were obtained prior to treatment with thrombolysis, and leukocyte RNA assessed by microarray analysis. Strokes that developed HT (n=11) were compared to strokes without HT (n=33) and controls (n=14). Genes were identified (corrected p-value <0.05, fold change ≥|1.2|) and functional analysis performed. RNA prediction of HT in stroke was evaluated using cross-validation, and in a second stroke cohort (n=52).

Results—Ischemic strokes that developed HT had differential expression of 29 genes in circulating leukocytes prior to treatment with thrombolytic therapy. A panel of 6 genes could predict strokes that later developed HT with 80% sensitivity and 70.2% specificity. Key pathways involved in HT of human stroke are described, including amphiregulin, a growth factor that regulates matrix metalloproteinase-9; a shift in transforming growth factor-beta signaling involving SMAD4, INPP5D and IRAK3; and a disruption of coagulation factors V and VIII.

Interpretation—Identified genes correspond to differences in inflammation and coagulation that may predispose to HT in ischemic stroke. Given the adverse impact of HT on stroke outcomes,

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further evaluation of the identified genes and pathways is warranted to determine their potential as therapeutic targets to reduce HT and as markers of HT risk.

Keywords

Ischemic stroke; hemorrhagic transformation; genomics; immune response

Introduction

Hemorrhagic transformation (HT) is the most feared complication of thrombolytic therapy in ischemic stroke. When it occurs HT exacerbates ischemic brain injury by promoting neuronal and glial cell death, resulting in worse stroke outcomes and increased mortality [1–3]. Identifying ischemic strokes at high risk for HT is challenging, and therapies to reduce HT in those at risk remains a great need. Given the clinical significance of HT, improved understanding of its complex pathophysiology is required to better identify patients at risk and develop novel prevention therapies.

Breakdown of the blood brain barrier (BBB) is an important component in the development of HT in ischemic stroke. Neuroimaging studies have shown HT to be associated with increased BBB permeability [4–7]. The mechanisms of enhanced BBB disruption in HT remain unclear, though free radical damage and proteolytic degradation of the neurovascular matrix are important [8]. Indeed, levels of matrix metalloproteinase-9 (MMP-9) protein are increased in HT ischemic stroke [9–12]. Moreover, inhibition of MMP-9 in experimental stroke can decrease the risk of thrombolysis related HT [13, 14].

The immune response may also contribute to HT in ischemic stroke. Ischemia of brain parenchyma elicits the production of cytokines and adhesion molecules that promote peripheral leukocyte activation, adhesion, and migration. This process alters the structure of the BBB, reorganizing tight-junction proteins and the actin cytoskeleton [15–17]. Thus, circulating leukocytes may contribute to the enhanced BBB disruption that is associated with HT. Indeed, in experimental stroke depletion of circulating neutrophils reduces thrombolysis related hemorrhage [18, 19]. In mice with circulating leukocytes derived from MMP-9 null bone marrow, BBB disruption is decreased [20]. Exogenous activation of the immune system increases BBB disruption in ischemic stroke and increases MMP-9 dependent HT [13, 21]. Additionally, neutrophils accumulate in greater numbers in regions of HT and correlate with disruption of the basal lamina [22].

We have shown in both patients and experimental animal studies that the peripheral immune response in ischemic stroke can be characterized by RNA expression in circulating leukocytes [23–28]. In this study, we sought to determine whether differences in gene expression in circulating leukocytes are present in ischemic strokes within 3 hours of stroke onset and prior to treatment with thrombolytic. Such genes and associated pathways may be early markers of HT risk or therapeutic targets to reduce HT.

Methods

1. Study Patients

Patients with acute ischemic stroke were enrolled from the CLEAR trial and from the University of California Davis (UCD). The institutional review board of each site approved the study protocol and written informed consent was obtained from each patient. Standardized evaluation was performed on all patients, including clinical examination, brain imaging and investigations to determine cause. The CLEAR trial was a multicenter, randomized, double-blind safety study of recombinant tissue-plasminogen activator (rt-PA) and eptifibatide that has been previously described (NCT00250991 at Clinical-Trials.gov) [29]. In brief, patients with acute ischemic stroke were randomized to receive either rt-PA alone or rt-PA plus eptifibatide within 3 hours of stroke onset. Eligible patients were 18–80 years of age, an National Institutes of Health Stroke Scale (NIHSS) >5, and met criteria for rt-PA therapy including no evidence of hemorrhage on imaging prior to tPA therapy, no history of intracerebral hemorrhage, blood pressure <185/110, glucose >50 and <400 mg/dl, international normalized ratio (INR) <1.4, and platelet count >100 000 /mm^3 [29]. Blood samples were drawn into PAXgene tubes (PreAnalytiX, Hilden, Germany) and used for gene expression analysis. There were 44 ischemic strokes analyzed in the derivation cohort, and 52 ischemic strokes analyzed in the validation cohort.

Hemorrhagic transformation was determined by repeat brain CT scan at 24 hours. HT was classified according to ECASS as HT-1, HT-2, PH-1, PH-2 [30]. PH-1, PH-2 and HT-2 were considered to have relevant HT, given the association with worse outcomes in ischemic stroke [1, 2]. Control blood samples were drawn from 14 subjects similar in age, gender and race to stroke subjects. These subjects were either healthy relatives of stroke patients, patients seen in outpatient clinics or healthy subjects with no known medical disease. They had no history of ischemic stroke or cardiovascular disease, no recent infection, and no hematological disease. The control group was included to ensure identified genes are unique to patients with acute ischemic stroke.

2. RNA Sample Processing

Venous blood was drawn into PAXgene tubes within 3 hours of stroke onset and stored at −80°C. PAXgene tubes stabilize RNA in blood which predominantly is from circulating leukocytes, megakaryocytes and red blood cells. Samples were processed at the same time and in the same laboratory to reduce technical variation. Total RNA was isolated according to the manufacturer's protocol (PAXgene blood RNA kit; Pre-AnalytiX). RNA concentration was determined by Nano-Drop (Thermo Fisher) and RNA quality by Agilent 2100 Bioanalyzer. Samples required A_{260}/A_{280} absorbance ratios of purified RNA ≥ 2.0 and 28S/18S rRNA ratios ≥ 1.8. NuGEN's Ovation Whole Blood Solution (NuGEN Technologies, San Carlos, CA) was used for reverse transcription, amplification, and sample labeling. Hybridization of each RNA sample was performed according to the manufacturer's protocol on Affymetrix Human U133 Plus 2.0 GeneChips (Affymetrix Santa Clara, CA). Arrays were washed and processed on a Fluidics Station 450 and scanned on a GeneChip Scanner 3000. Samples were randomly assigned to microarray batch stratified by diagnosis.

3. Microarray Data Analysis

Microarray CEL file data were normalized using robust multichip averaging (RMA), mean centering standardization, and log2 transformation (Partek Genomics Suite 6.4, Partek Inc., St. Louis, MO). Univariate analysis was performed to identify factors different between ischemic strokes that developed HT and ischemic stroked that did not develop HT. Ischemic strokes that developed HT were compared to matched ischemic strokes without HT and to controls using Analysis of Covariance (ANCOVA). All factors on univariate analysis with a p-value <0.2 (systolic blood pressure, platelet count) and those previously associated with risk of HT (age, NIHSS, glucose) were then included in the model. After Benjamini-Hochberg false discovery rate (FDR) correction for multiple comparisons, probesets with a corrected p-value <0.05 and fold change ≥ |1.2| were considered significant.

4. Gene Function and Prediction Analysis

Functional pathways associated with the differentially expressed genes were identified using Ingenuity Pathway Analysis (IPA, Ingenuity Systems®, www.ingenuity.com) and review of relevant literature. Prediction analysis was performed using Partek Genomics Suite 6.4. The probesets differentially expressed between ischemic stroke with HT and ischemic stroke without HT were used to develop a prediction model. Genes best able to distinguish stroke with HT from stroke without HT were selected using forward selection linear discriminant analysis (LDA). The genes found to discriminate ischemic strokes with HT from ischemic strokes without HT were used to develop a LDA prediction model. LDA is an analytical method that determines a combination of features that can separate two or more classes. For this analysis, differentially expressed genes are the features and the classes are stroke with HT and stroke without HT. The ability of the LDA prediction model to identify strokes that develop HT was first evaluated using 10-fold leave-one-out cross-validation analysis and principal components analysis on the derivation cohort. The LDA prediction model was then evaluated on a second cohort of stroke subjects (validation cohort, n=52). This was performed by first measuring the expression of each predictor gene included in the LDA model for all stroke subjects by microarray. A subject was classified as stroke with HT when the probability to have HT predicted by the LDA model was >50%. The gene based prediction of strokes that develop HT was then compared to the known HT status of stroke patients, and used to determine sensitivity and specificity of the LDA prediction model to predict HT in stroke.

5. Statistics

Differences in patient characteristics between groups were compared using Kruskal Wallis, Chi-square test or Fisher's exact test, and Analysis of Variance (ANOVA) as appropriate (Stata 10.1, College Station, TX, USA). Differences in gene expression between groups were compared using ANCOVA (Partek Genomics Suite 6.4). Correction for multiple comparisons was performed using the Benjamini-Hochberg method. Data are presented as mean with standard deviation (SD) for continuous variables and median with interquartile range (IQR) for ordinal variables. Multiple logistic regression was performed to identify independent predictors of HT.

Results

There were 44 acute ischemic strokes in the derivation cohort, comprised of 33 strokes that did not develop HT and 11 strokes that did develop HT (Table 1). The mean age was 68.7 years (SD 9.9), and 63.9% were male. The median baseline NIHSS was 13 (IQR 7.8, 16.3). Among the strokes that developed HT, there were three subjects with an increase in NIHSS by ≥4 points within the first 36 hours of stroke onset deemed to be symptomatic. By the ECASS classification system there were 3 subjects with PH-2, 3 with PH-1, and 5 with HT-2 [30]. Cardioembolism was the cause of stroke in 47.7% of subjects, large vessel atherosclerosis in 20.5% and 31.8% were cryptogenic. There were no significant differences characteristics between ischemic strokes that did and did not develop HT (Table 1). Control subjects had a mean age of 67.4 years, and 71.4% were male. They did not have a history of prior stroke, coronary artery disease or peripheral vascular disease. There were 52 ischemic strokes in the validation cohort, 47 without HT and 5 that developed HT (Supplementary Table 1). The mean age was 65.6 years (SD 15.2), 53.8% were male, and the median baseline NIHSS was 10.5 (IQR 6, 14.8). Among the strokes that developed HT, 3 had an increase in NIHSS by ≥4 within the first 36 hours of stroke and were deemed to be symptomatic. By the ECASS classification system there were 2 subjects with PH-2, 1 with PH-1, and 2 with HT-2. Cardioembolism was the cause of stroke in 42.3% of subjects, large vessel atherosclerosis in 23.1% and 34.6% were cryptogenic.

There were 32 probesets corresponding to 29 genes that were significantly different between ischemic stroke that developed HT compared to strokes that did not develop HT (FDR<0.05, fold change ≥|1.2| (Supplementary Table 2, Supplementary Figure 1). When factors associated with risk of HT (age, NIHSS, glucose, systolic blood pressure, platelet count, time from onset to thrombolytic) were adjusted for in the ANCOVA model, the identified genes remained significantly associated with HT. Several genes and pathways of interest were identified relating to TGF-B to SMAD4 signaling, VEGI (TL1A, TNFSF-15) mediated inflammation, amphiregulin growth factor expression, MARCH7 regulation of membrane receptors, and MCFD2 regulation of coagulation factors. Box plots of selected genes are shown in Figure 1 for strokes that developed HT (red) and strokes that did not develop HT (blue).

A prediction model was developed to distinguish stroke patients that develop HT from those that did not. Using the 29 genes identified as differentially expressed at baseline, those that optimally distinguish stroke with HT from stroke without HT were identified using forward selection linear discriminant analysis. Six genes were identified and combined into a single predictive model, including SMAD4, INPP5D, VEGI, AREG, MARCH7, and MCFD2. This model was evaluated using 10-fold leave-one-out cross-validation analysis. On cross validation analysis, the model was able to distinguish 72.7% of stroke that developed HT (8/11), and 93.9% of stroke subjects that did not develop HT (31/33). This corresponds to an accuracy of 88.6%. The ability of the genes to distinguish stroke with HT from stroke without HT is shown in a principal components analysis plot in Figure 2. The model was further evaluated in the validation cohort of ischemic strokes (n=52). Strokes that did not develop HT were correctly predicted in 70.2% of subjects (33/47), and strokes that did develop HT were correctly predicted in 80% of subjects (4/5).

To evaluate the relationship between the identified RNA panel to clinical risk factors associated with HT, multiple logistic regression was performed (Table 2). Factors included in the multiple logistic regression model were age, baseline NIHSS, baseline glucose, admission systolic blood pressure, history of hypertension, history of diabetes, admission glucose, admission platelet count, and time from stroke onset to thrombolytic therapy [31–33]. RNA was included in the model as the posterior probability to be HT as predicted by LDA model. The 6 gene RNA panel remained an independent predictor of HT (Table 2).

Discussion

Ischemic strokes that developed HT had differential RNA expression of 29 genes in blood, including SMAD4, INPP5D, VEGI, AREG, MCFD2, and MARCH7. Importantly, the genes were expressed within 3 hours of stroke onset prior to administration of thrombolytic, and prior to the development of HT. Identified genes correspond to differences in inflammation and coagulation in ischemic stroke patients that developed HT. As such, they represent factors that may increase the propensity of HT formation in stroke. Given the adverse impact of HT on outcomes in ischemic stroke, further evaluation of identified genes and pathway is warranted to determine their potential as therapeutic targets to reduce HT and as very early markers of HT risk.

Among the genes found to be differentially expressed in strokes that developed HT was SMAD4. SMAD4 codes for a member of the Smad family of signal transduction proteins which are activated by TGF-beta signaling to regulate the transcription of target genes. Little is known about SMAD4 in ischemic stroke. However, the gene is a cause of hereditary hemorrhagic telangiectasia syndrome, and regulates N-cadherin expression in endothelial cells to stabilize the BBB [34, 35]. SMAD4 and TGF-B signaling have also been associated with extracellular matrix remodeling and the formation of vascular pathology including aortic dissections and aneurysms [36, 37]. In our study of HT, SMAD4 was increased in blood in addition to several of its target genes including IRAK3 (interleukin-1 receptor-associated kinase 3) and INPP5D. IRAK-3 is involved in toll-like receptor signaling and is primarily expressed in monocytes and macrophages [38]. INPP5D regulates proliferation and programming of myeloid cells [39]. Though the significance of these genes to HT in ischemic stroke remains unclear, a subset of stroke patients may be more prone to HT as a result of differences in SMAD4 signaling in circulating leukocytes.

VEGI (vascular endothelial growth inhibitor; VEGI, TL1A, TNFSF15) was another gene of interest found to have decreased expression in strokes that developed HT. VEGI codes for a cytokine in the tumor necrosis factor (TNF) ligand family. It is associated with tumorigenesis, artherosclerosis, and several immune diseases including rheumatoid arthritis, inflammatory bowel disease, and autoimmune encephalitis[40]. VEGI modulates the innate and adaptive immune response, and has anti-angiogenic effects through suppression of endothelial cell proliferation [41, 42]. Whether such effects contribute to HT in stroke require further study. For example, decreased VEGI may contribute to HT-stroke by altering apoptosis of inflammatory cells mediated by caspase-8 / Fas ligand, or by impeding repair of damaged endothelium [43, 44].

Amphiregulin (AREG) was increased in ischemic strokes with HT. Amphiregulin is a ligand for the epidermal growth factor receptor (EGFR). It is expressed by several blood cells including neutrophils, monocytes, T-lymphocytes and B-lymphocytes [45–47]. To our knowledge, this is the first study reporting amphiregulin in ischemic stroke, thus its role remains unclear. However, it has several functions that may potentially contribute to HT in stroke. Amphiregulin enhances migration of neutrophils across epithelial cell layers by altering E-cadherin dependent tight junctions [48]. Amphiregulin activates epithelial cells and promotes release of IL-8 [49]. In cancer amphiregulin promotes proliferation, angiogenesis, invasion and metastasis [50, 51]. Amphiregulin increases expression of MMP-9 and VEGF through MAP kinase dependent pathways [52–54]. As such, it may promote MMP-9 expression in ischemic stroke and thus promote HT.

Multiple coagulation factor deficiency 2 (MCFD2) had decreased expression in strokes that developed HT. MCFD2 protein is involved in the transport of coagulation factors V and VIII from the endoplasmic reticulum to the Golgi apparatus [55, 56]. Mutations of the MCFD2 gene cause a bleeding disorder as a result of a deficiency in coagulation factors V and VIII [57, 58]. In our study, patients with HT were found to have decreased expression of MCFD2. This may indicate an alteration in coagulation may be present in some ischemic strokes that develop HT, with impaired processing of coagulation factors V and VIII from reduced MCFD2 expression. Alteration in the coagulation system has been associated with HT in stroke. Increased prothrombin time (factors I, II, V, VII, X) or thrombocytopenia increase the risk of HT. Fibrinolysis inhibitors PAI-1 and TAFI (thrombin activated fibrinolysis inhibitor) may be decreased in HT [59, 60]. A polymorphism in coagulation factor XIII has also been associated with tPA related HT [61]. Additionally, therapy with anticoagulants predisposes to tPA related HT, including warfarin (coagulation factors II, VII, IX, and X) and heparin (binds antithrombin III). Thus, an alteration in the coagulation can promote HT in ischemic stroke, and differential expression of MCFD2 may be a contributing factor warranting further evaluation.

MARCH7 (membrane associated RING-CH, axotrophin) is an E3 ubiquitin ligase that had increased expression in strokes that developed HT. It regulates membrane receptor expression in several tissues including leukocytes. Over expression of MARCH genes can downregulate cell surface receptors such as MHC class I, MHC class II, CD86 and ICAM1 [62]. MARCH7 can modulate T cell activity through its effects on the LIF receptor. An increase in MARCH7 can shift from a LIF-T-regulatory cell response to an IL-6 Th17 pro-inflammatory response [63], which has been associated with inflammatory disease including multiple sclerosis[64]. Whether a similar shift toward a Th17 response is present in ischemic strokes that develop HT requires further evaluation.

HT is a complex process and several factors in addition to circulating leukocytes are important to HT of ischemic brain tissue. Indeed, a number of clinical factors have been associated with an increase risk of HT including age, NIHSS, serum glucose, platelet count, and blood pressure [31, 32]. However, as predictors of HT, clinical factors to date have shown limited accuracy to identify HT in stroke patients presenting within the time window for thrombolytic therapy [33]. In this preliminary study RNA differentially expressed in the blood of strokes that develop HT is shown to have potential to predict ischemic strokes that

develop HT. This predictive ability was independent of clinical factors associated with HT risk (Table 2), though additional larger studies are required. Further study is also of interest to evaluate the relationship between circulating leukocytes and other factors associated with HT such as levels of proteolytic enzymes, permeability of the BBB, and reperfusion status of the cerebral vasculature.

A strength of the study is the homogenous stroke population evaluated, with subjects meeting criteria for rt-PA therapy, and with blood samples collected within 3 hours of stroke onset prior to treatment with thrombolytic therapy. However, given these strict criteria sample size was small, which may have limited our ability to identify differences between groups. Additionally, evaluation of multiple genes as performed in microarray analysis increases the risk of false discovery. Though correction for multiple comparisons was performed, confirmation in by an independent study is required. It is also important to note that subjects from the CLEAR trial were randomized to rt-PA alone or rt-PA plus eptifibatide. Though no increase in HT was observed in the eptifibatide treated group, rt-PA combined with eptifibatide may not fully reflect the use of rt-PA in clinical practice. Finally, HT is often classified as symptomatic or asymptomatic based on acute neurological deterioration related to the intracerebral hemorrhage. In the current study 3 of the 11 HT subjects had increase in NIHSS by ≥4 points within the first 36 hours of stroke onset. Though differences in RNA expression were not evident between symptomatic and asymptomatic subjects, sample size was small. Further studies in larger samples evaluating RNA expression in blood as a function of size of hemorrhage, degree of BBB disruption, and status of symptomatic versus asymptomatic HT are required.

In summary, several genes associated with inflammation and coagulation were identified in ischemic strokes that developed thrombolysis related HT. These genes may increase the propensity to develop HT in ischemic strokes treated with thrombolysis. Further study is required to determine whether these may be targets to modulate HT risk in ischemic stroke or markers of ischemic strokes at increased risk of HT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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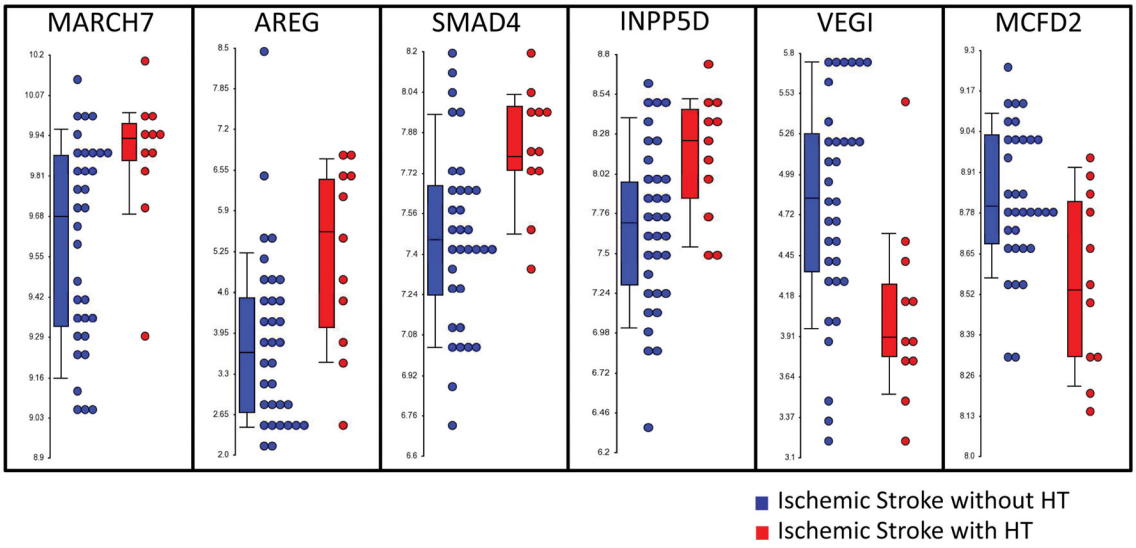

Figure 1.

Box plots of the six genes used to distinguish ischemic strokes that developed hemorrhagic transformation (red) from those that did not (blue). Expression levels are measured within three hours of stroke onset, prior to treatment with thrombolytic therapy. (MARCH7, Membrane-associated ring finger (C3HC4) 7; AREG, amphiregulin; SMAD4, SMAD family member 4; INPP5D, inositol polyphosphate-5-phosphatase; VEGI, Vascular endothelial growth inhibitor, TNFSF15, TL1A; MCFD2, multiple coagulation factor deficiency 2).

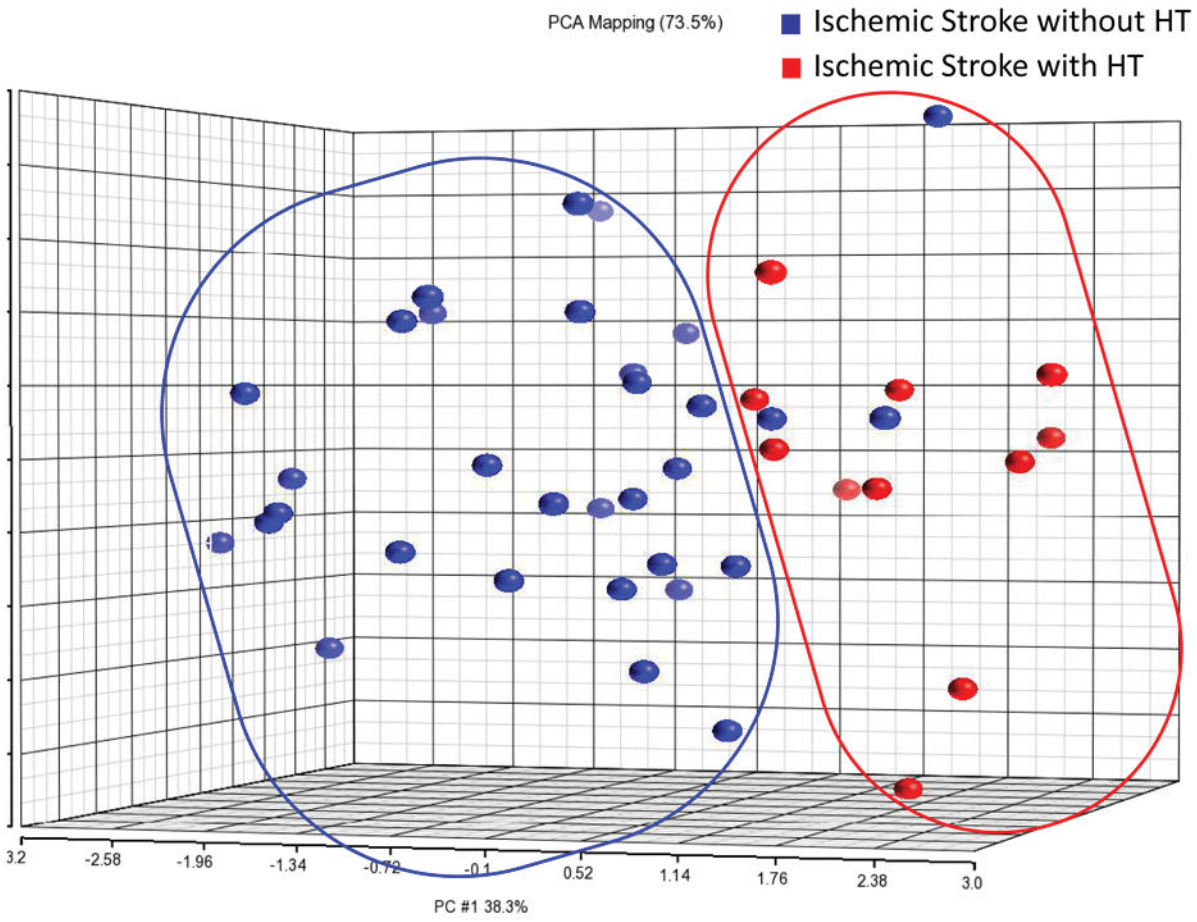

Figure 2.

Principal components analysis of the 6 genes used to distinguish ischemic strokes that developed HT from ischemic strokes that did not develop HT. Genes included in the analysis were MARCH7, SMAD4, INPP5D, AREG, MCFD2, and VEGI.

Table 1

Characteristics of ischemic stroke patients with and without hemorrhagic transformation (HT).

	Ischemic Stroke No HT (n=33)	Ischemic Stroke with HT (n=11)	p-value
Age years (SD)	68.7 (9.8)	68.8 (10.7)	0.98
Male n(%)	21 (63.6%)	7 (63.6%)	0.72
Race Caucasian n(%)	27 (81.8%)	9 (81.8%)	0.65
Hypertension history n(%)	24 (72.7%)	8 (72.7%)	0.70
Systolic BP on admission mmHg (SD)	155.3 (27.6)	171.1 (46.5)	0.18
Diastolic BP on admission mmHg (SD)	82 (15.5)	84 (20.8)	0.73
Diabetes history n(%)	9 (27.3%)	3 (27.3%)	0.74
Glucose on admission mg/dL (SD)	124.3 (35.6)	125.2 (33.8)	0.95
Hyperlipidemia n(%)	10 (30.3%)	3 (27.3%)	0.85
Atrial fibrillation n(%)	6 (18.2%)	2 (18.2%)	0.65
NIHSS baseline (IQR)	13 (8.5,15)	14 (8,17)	0.83
Cardioembolic n(%)	15 (45.5%)	6 (54.5%)	0.93
Large vessel n(%)	7 (21.2%)	2 (18.2%)	0.83
Cryptogenic n(%)	11 (33.3%)	3 (27.3%)	1.00
tPA only n(%)	6 (18%)	2 (18%)	0.65
tPA + eptifibatide n(%)	27 (82%)	9 (82%)	0.65
Time stroke onset to blood draw hrs (SD)	1.26 (0.43)	1.21 (0.46)	0.74
Time stroke onset to thrombolytic hrs (SD)	2.53 (0.44)	2.54 (0.41)	0.97
INR baseline (SD)	1.03 (0.12)	1.05 (0.08)	0.73
Platelet baseline (10^3/mcL)	270.9 (77.2)	233.6 (62.6)	0.16
WBC baseline (10^3/mcL)	8.3 (1.8)	8.1 (2.6)	0.75
RBC baseline (10^6/mcL)	4.55 (0.48)	4.22 (0.78)	0.95

BP, blood pressure; hrs, hours; INR, international normalized ratio; IQR, interquartile range; mcL, microliter; NIHSS, NIH Stroke Scale; RBC, red blood cell; SD, standard deviation; tPA, tissue plasminogen activator; WBC, white blood cell.

Table 2

Multiple logistic regression analysis of clinical factors associated with hemorrhagic transformation and the identified RNA panel.

Factor	OR	95% CI	p-value
Age	1.03	0.90–1.18	0.66
NIHSS on admission	1.03	0.84–1.26	0.80
Systolic BP on admission	1.02	0.97–1.09	0.39
Hypertension history	1.86	0.08–45.20	0.70
Diabetes history	0.38	0.01–34.08	0.67
Glucose on admission	0.96	0.90–1.02	0.16
Platelet count baseline	0.98	0.95–1.01	0.13
Time from onset to thrombolytic therapy	14.3	0.08–2412	0.31
RNA-Panel (probability)	981.17	4.57–210597	0.01