Transient ischemic attacks characterized by RNA profiles in blood

X. Zhan, MD, PhD*
G.C. Jickling, MD*
Y. Tian, PhD
B. Stamova, PhD
H. Xu, MD, PhD
B.P. Ander, PhD
R.J. Turner, PhD
M. Mesias, BS
P. Verro, MD
C. Bushnell, MD, MHS
S.C. Johnston, MD, PhD
F.R. Sharp, MD

Address correspondence and reprint requests to Dr. Xinhua Zhan, MIND Institute Research Wet Labs—Room 2415, University of California at Davis, 2805 50th Street, Sacramento, CA 95817
xzhan@ucdavis.edu

ABSTRACT

Objective: Transient ischemic attacks (TIA) are common. Though systemic inflammation and thrombosis are associated with TIA, further study may provide insight into TIA pathophysiology and possibly lead to the development of treatments specifically targeted to TIA. We sought to determine whether gene expression profiles in blood could better characterize the proinflammatory and procoagulant states in TIA patients.

Methods: RNA expression in blood of TIA patients (n = 26) was compared to vascular risk factor control subjects without symptomatic cardiovascular disease (n = 26) using Affymetrix U133 Plus 2.0 microarrays. Differentially expressed genes in TIA were identified by analysis of covariance and evaluated with cross-validation and functional analyses.

Results: Patients with TIA had different patterns of gene expression compared to controls. There were 480 probe sets, corresponding to 449 genes, differentially expressed between TIA and controls (false discovery rate correction for multiple comparisons, $p \leq 0.05$, absolute fold change ≥ 1.2). These genes were associated with systemic inflammation, platelet activation, and prothrombin activation. Hierarchical cluster analysis of the identified genes suggested the presence of 2 patterns of RNA expression in patients with TIA. Prediction analysis identified a set of 34 genes that discriminated TIA from controls with 100% sensitivity and 100% specificity.

Conclusion: Patients with recent TIA have differences of gene expression in blood compared to controls. The 2 gene expression profiles associated with TIA suggests heterogeneous responses between subjects with TIA that may provide insight into cause, risk of stroke, and other TIA pathophysiology. *Neurology*® 2011;77:1718-1724

GLOSSARY

CRP = C-reactive protein; **FDR** = false discovery rate; **LDA** = linear discriminant analysis; **TIA** = transient ischemic attack; **vWF** = von Willebrand factor.

Transient ischemic attacks (TIAs) are common, affecting over 300,000 people per year in the United States alone. Although TIA symptoms resolve by definition, they are far from benign, with a risk of stroke, myocardial infarction, and death as high as 25% within 90 days.¹⁻³ Better understanding of TIA pathogenesis is needed to improve our ability to treat this condition and potentially prevent stroke.

We have previously shown blood gene expression changes following experimental TIAs⁴ are associated with microglial activation and macrophage infiltration in brain.⁵ In an experimental rat model of TIA, we demonstrated differences in blood immune cell activation using whole genome expression analysis.⁴ In humans, TIAs have elevated levels of cytokines and leukocytes,^{6,7} fibrinogen,⁸ D-dimer,⁹ and platelet collagen receptor glycoprotein VI.¹⁰ Furthermore, levels of high-sensitivity C-reactive protein (CRP) and Lp-PLA2 are associated with recurrent ischemic events in

Editorial, page 1716

Supplemental data at
www.neurology.org

Supplemental Data

*These authors contributed equally to this work.

From the Department of Neurology (X.Z., G.C.J., Y.T., B.S., H.X., B.P.A., R.J.T., M.M., P.V., F.R.S.), MIND Institute, University of California at Davis, Sacramento; Department of Neurology (C.B.), Wake Forest University Health Sciences, Winston-Salem, NC; and Department of Neurology (S.C.J.), University of California at San Francisco, San Francisco.

Study funding: This study was supported by NIH/NINDS grant NS056302, the AHA Bugher Foundation (F.R.S., B.P.A., H.X., and Y.T.), and NIH/NINDS K02 NS058760 (C.D.B.). Dr. Jickling is a fellow of the Canadian Institutes of Health Research (CIHR). Drs. Xu, Ander, and Tian are AHA Bugher Fellows. Dr. Turner is supported by a National Health and Medical Research Council (Australia) Postdoctoral Fellowship. Dr. Bushnell receives research support from NIH/NINDS K02 NS058760.

Disclosure: Author disclosures are provided at the end of the article.

TIA.^{11,12} Thus, experimental and human studies of brief cerebral ischemia document evidence of inflammatory and prothrombotic states.

Gene expression profiles derived from blood can distinguish ischemic stroke from controls and identify procoagulant and proinflammatory features important to stroke pathophysiology.^{13–15} Whether such profiles exist in patients with acute TIA is unknown. Accordingly, in this study, we sought to determine whether gene expression profiles in blood could characterize the inflammatory and coagulant states in patients with acute TIA.

METHODS **Standard protocol approvals, registration, and patient consents.** Patients with TIA and controls were recruited from the University of California Davis Medical Center, University of California San Francisco Medical Center, and Wake Forest University Health Sciences. The institutional review board at each institution approved the study. Informed consent was obtained from all patients.

The diagnosis of TIA was made by board-certified neurologists. TIA was defined as an acute loss of focal cerebral or ocular function of vascular etiology lasting <24 hours. Neuroimaging was obtained on all TIAs, with 85% having MRI. Controls were persons with vascular risk factors but without symptomatic vascular disease, including no prior stroke, TIA, myocardial infarction, angina, or peripheral arterial disease. They were matched to patients with TIA based on age and gender.

Blood collection and RNA isolation. Blood was collected from 9.5 to 68.6 hours (mean 35.1 hours) from TIA symptom onset. A venous blood sample (15 mL) was collected into PAXgene Vacutainers (PreAnalytiX, Hilden, Germany) from each subject. PAXgene Vacutainers immediately stabilize blood RNA, thus reducing RNA degradation and changes of gene expression after phlebotomy. After 2 hours at room temperature, PAXgene Vacutainers were frozen at -70°C until processed. Total RNA

was isolated according to the manufacturer's protocol (PreAnalytiX) and processed together at the same time and site. The RNA from whole blood is from white blood cells (neutrophils, basophils, eosinophils, lymphocytes, and monocytes), immature platelets, and immature red blood cells.

Microarray hybridization. RNA was analyzed using an Agilent 2100 bioanalyzer for quality and Nano-drop for concentration. Biotin-labeled cDNA was synthesized from 50 ng of total RNA using the Ovation Whole Blood Solution (Nugen Technologies, San Carlos, CA) kit according to protocol. Each RNA sample was processed on Affymetrix Human Genome U133-Plus-2.0 microarrays (Affymetrix Santa Clara, CA).¹⁵

Statistical analysis. Microarray data were normalized using Robust Multichip Average and log₂ transformed. Individual probes were summarized into probe sets as described by Affymetrix, and used for probe set and gene level analysis. Analysis of covariance was conducted in Partek Genomics Suite 6.5 (Partek Inc., St. Louis, MO) to identify probe sets significantly different between patients with TIA and controls adjusting for age and microarray batch effect. Multiple comparison correction was performed using Benjamini-Hochberg false discovery rate (FDR) of 5%. Probe sets were considered significant with an absolute fold change ≥ 1.2 and a *p* value ≤ 0.05 after the FDR correction for multiple comparisons.

Differences in demographic and clinical data between groups were analyzed using Fisher exact test or *t* test. Prediction analysis was performed using linear discriminant analysis (LDA). Cross-validation was performed by randomly dividing subjects into 10 equal groups, developing a prediction model using 90% of the patients, and evaluating the model on the remaining 10%. This process was repeated 10 times to estimate the overall prediction ability of the developed model. Function and pathway analyses were performed using ingenuity pathways analysis.

RESULTS **Patients.** A total of 52 patients were analyzed: 26 with TIA and 26 vascular risk factor controls. The average age was 62.9 years. There were 26 men and 26 women. Patients were of mixed race and ethnicity, with Caucasian race accounting for 77% of the patients. A summary of the TIA and control subjects is shown in table 1. Since there was a significant difference in race between TIA and controls, we performed a secondary analysis restricted to Caucasians with TIA (*n* = 15) and controls (*n* = 25).

TIA genomic profiles. There were 480 probe sets corresponding to 449 genes that were differentially expressed between TIA and control subjects (FDR ≤ 0.05 ; fold change ≥ 1.2) (table e-1 on the *Neurology*[®] Web site at www.neurology.org). Of the 480 probe sets, 129 probe sets were downregulated (table e-1A) and 351 probe sets were upregulated (table e-1B). Each gene was represented by 1 to 6 probe sets. Hierarchical cluster analysis of the 480 probe sets is shown in figure 1. This visually demonstrates how TIAs separate from controls based on pattern of gene expression. A secondary analysis restricted to Caucasian patients with TIA and controls revealed similar results. This comparison of Caucasians demonstrated

Table 1 Demographic and clinical features of patients with transient ischemic attack and control subjects^a

	Controls (n = 26)	TIA (n = 26)	<i>p</i> Value
Age (years $\pm$ SE)	60.4 $\pm$ 0.76	65.2 $\pm$ 2.35	0.06
Gender female, n (%)	13 (50.0)	13 (50.0)	1.00
Race Caucasian, n (%)	25 (96.2)	15 (57.7)	0.0007
Vascular risk factor, n (%)			
Hypertension	17 (65.4)	19 (70.1)	0.55
Diabetes	6 (20.1)	9 (34.6)	0.36
Hyperlipidemia	19 (70.1)	15 (57.7)	0.25
Smoker	10 (38.5)	13 (50.0)	0.48
Medication	20 (76.9)	23 (88.5)	0.28

Abbreviation: TIA = transient ischemic attack.

^a Differences in demographic data between groups were analyzed using Fisher exact test or *t* test as appropriate. There was no significant difference in age, gender, vascular risk factors, and medications between the 2 groups analyzed using *t* test or χ^2 test. Race is significantly different between the two groups analyzed using χ^2 test. Therefore, we performed a secondary analysis restricted to Caucasian TIA (*n* = 25) and Caucasian controls (*n* = 15).

Figure 1 Hierarchical cluster analysis of identified genes in transient ischemic attack (TIA) vs matched vascular risk factor controls

Hierarchical cluster analysis of 480 gene probe sets differentially expressed in blood between patients with TIA and control subjects (false discovery rate ≤ 0.05 , absolute fold change > 1.2). Each column on the x-axis represents 1 patient, with 26 patients with TIA (blue) and 26 controls (orange). Each row on the y-axis represents individual probe sets (usually for individual genes). TIAs cluster separately from controls as indicated by the green arrow (top). Within subjects with TIA, at least 2 clusters are apparent as indicated by the red arrow. These 2 TIA clusters are labeled TIA1 and TIA2. One patient with TIA (ID: IT-062) clustered with controls. Two controls (ID: IT-155 and IT-177) clustered with TIAs. Diagnosis = blue (TIA) and orange (controls). Green = downregulation; ID = subject ID; Red = upregulation.

that $> 90\%$ of the differentially expressed genes were common to the non-race-stratified analysis (453 out of 480 probe sets; data not shown).

The presence of 2 TIA groups, labeled as TIA1 and TIA2, was identified on the cluster plot (figure 1). To determine whether a clinical feature was associated with the 2 TIA groups based on RNA patterns, we compared the following features between TIA1 and TIA2: age, gender, race, time after TIA, hypertension, diabetes, hyperlipidemia, smoking, history of stroke, cardiovascular disorder, atrial fibrillation, large vessel disease, ABCD2 score, DWI lesion, recurrent ischemic event within 90 days, and medications. None of these features were statistically different between the TIA1 and TIA2 groups. However, the power to detect a difference was limited due to the small sample size. Notably, the 3 patients with

DWI lesions were in the TIA1 group, as was the one patient who had a subsequent stroke by 90 days (table 2). The genes associated with these TIA subtypes are shown in table e-1, C and D.

Prediction analysis for TIA. A list of 34 probe sets that optimally distinguished TIA from control patients was identified using forward selection linear discriminate analysis on the 480 probe sets (table 3). A LDA prediction model was developed using these 34 probe sets and evaluated using 10-fold leave-one-out cross-validation. TIA could be distinguished from controls with 100% sensitivity (26 out of 26 TIAs correctly classified) and 100% specificity (26 out of 26 controls correctly classified) (figure 2).

Function analysis of TIA-associated genes. Function analysis of the 480 probe sets that distinguished TIA

Table 2 Demographic and clinical features of TIA1 and TIA2 subgroups^a

	TIA1 (n = 13)	TIA2 (n = 13)	p Value
Age, y, mean ± SE	61.8 ± 3.1	68.6 ± 3.4	0.15
Female, n (%)	6 (46.2)	7 (53.8)	0.71
Caucasian, n (%)	7 (53.8)	8 (61.5)	0.71
Vascular risk factor, n (%)			
Hypertension	10 (76.9)	9 (69.2)	0.67
Diabetes	5 (38.5)	4 (30.8)	0.69
Hyperlipidemia	7 (53.8)	8 (61.5)	0.71
Smoker	7 (53.8)	6 (46.2)	0.71
Hours since TIA, mean ± SE	33.9 ± 7.5	36.4 ± 4.8	0.78
History of stroke, n (%)	4 (30.8)	4 (30.8)	1.00
CVD, n (%)	3 (23.1)	4 (30.8)	0.67
AF, n (%)	0 (0)	1 (7.7)	0.33
LVD, n (%)	0 (0)	1 (7.7)	0.33
ABCD ² score, mean ± SE	5.23 ± 0.4	4.77 ± 0.4	0.42
DWI lesion on MRI	3	0	0.07
Recurrent ischemic event within 90 days	1	0	0.31
Medication, n (%)			
Antithrombotic	9 (69.2)	10 (76.9)	0.67
Antihypertension	8 (61.5)	9 (69.2)	0.69
Lipid-lowering agent	8 (61.5)	11 (84.6)	0.20
Diabetes	3 (23.1)	1 (7.7)	0.30

Abbreviations: AF = atrial fibrillation; CVD = cardiovascular disease; DWI = diffusion-weighted imaging; LVD = large vessel disease; TIA = transient ischemic attack.

^a There was no significant difference between the 2 groups analyzed using t test or χ^2 test.

from controls demonstrated that they were significantly associated with immune functions. The genes associated with TIA demonstrated similar immune patterns as previously described in autoimmune diseases such as rheumatoid arthritis and Crohn disease. Function pathways for TIAs were also associated with immune patterns previously described in atherosclerosis and coronary artery disease (table e-2A). The pathways and associated differentially expressed genes are shown in table e-3A.

Function analysis of TIA subtype-associated genes. Patients with TIA clustered into 2 groups based upon their RNA expression profiles (figure 1). To better characterize the significance of this finding, we performed a second function analysis of the TIA1 and TIA2 subtypes. The subgroup labeled as TIA1 had statistically more genes associated with atherosclerosis, stroke, coronary artery disease, hypertension, and diabetes mellitus as compared to the TIA2 subgroup (table e-2B). The pathways and associated genes for the TIA RNA subgroups are shown in table e-3, B and C.

DISCUSSION Specific genes were expressed in blood following TIA associated with inflammation

and coagulation as compared to controls. Whether these genes represent potential targets for TIA-specific therapies or biomarkers of TIA requires further study. Two patterns of RNA expression were observed in patients with TIA. The clinical significance of these RNA subtypes remains unclear, though these data strongly suggest that different patterns of immune activation may exist in TIA.

Patients with TIA may have unique patterns of inflammation that are associated with the development of subsequent ischemic vascular events. Previous studies have demonstrated a relationship between TIA and increased leukocyte activation and systemic inflammatory response,^{6,7} presence of infection,^{16–18} and other inflammatory conditions.^{19–22} A number of genes differentially expressed in patients with TIA were similar to those previously associated with inflammatory bowel disease, rheumatoid arthritis, and cardiovascular disease. Whether this pattern of inflammation existed prior to TIA onset or was a result of acute TIA remains unclear. If the inflammation associated with TIA is an acute process, one would expect it to change over time. When we compared gene expression in TIAs with blood drawn within 24 hours (n = 11) to TIAs with blood drawn between 24 and 72 hours (n = 15), >90% of the genes expressed by 24 hours were similar to those expressed between 24 and 72 hours (data not shown). Though further studies of gene expression over time are required, this suggests that there may be a chronic inflammatory state in TIA that contributes to the development of symptomatic cerebrovascular disease.

Patients with TIA were also found to have increased expression of genes associated with coagulation. Specifically, genes involved in primary hemostasis, including platelet activation and aggregation as well as secondary hemostasis including intrinsic and extrinsic pathways, were identified. Of interest, tissue factor pathway inhibitor, an inhibitor of thrombin and factor Xa, was upregulated in TIA, suggesting differential regulation of the coagulation cascade and clot formation may be present in TIA. Genes encoding collagens and peptidases that break down von Willebrand factor (vWF)²³ were also overexpressed in patients with TIA compared to controls. Though peptidases that break down vWF have been associated with stroke^{24,25} and thrombotic thrombocytopenic purpura,²⁶ this is the first report in TIA. These data suggest that procoagulant features are present in TIA that might provide treatment targets.

Hierarchical clustering demonstrated 2 TIA groups based upon genes differentially expressed between TIAs and controls. Despite this, we were unable to identify a clinical factor that was significantly

Table 3 The 34 genes derived using linear discriminant analysis that distinguished TIA from control with 100% specificity and 100% sensitivity

AFFY ID	Gene symbol	Gene title	Fold-change (TIA vs control)
206513_at	AIM2	Absent in melanoma 2	1.54
243365_s_at	AUTS2	Autism susceptibility candidate 2	1.34
1560558_at	C9orf80	Chromosome 9 open reading frame 80	1.82
239871_at	CLTC	Clathrin, heavy chain (Hc), mRNA (cDNA clone IMAGE:4812912)	-1.56
239309_at	DLX6	Distal-less homeobox 6	2.24
1553998_at	DMRTC1 /// DMRTC1B	DMRT-like family C1 /// DMRT-like family C1B	-1.57
1554333_at	DNAJA4	DnaJ (Hsp40) homolog, subfamily A, member 4	-1.63
1557155_a_at	FLJ30375	CDNA clone IMAGE:5301781	1.66
230047_at	FLJ32810	Hypothetical protein FLJ32810	1.57
217487_x_at	FOLH1	Folate hydrolase (prostate-specific membrane antigen) 1	2.04
1553613_s_at	FOXC1	Forkhead box C1	1.70
1553024_at	G30	Protein LG30-like	1.64
1559520_at	GYPA	Glycophorin A	1.91
220085_at	HELLS	Helicase, lymphoid-specific	-1.49
206858_s_at	HOXC4 /// HOXC6	Homeobox C4 /// homeobox C6	1.80
37566_at	KIAA1045	KIAA1045	-1.43
1558107_at	LOC283788	Hypothetical protein LOC283788	-1.50
231434_at	LOC728460	Similar to FLJ32921 protein	1.73
219789_at	NPR3	Natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide rec	1.41
214111_at	OPCML	Opioid binding protein/cell adhesion molecule-like	2.06
228825_at	PTGR1	Prostaglandin reductase 1	1.83
235638_at	RASSF6	Ras association (RalGDS/AF-6) domain family member 6	1.55
242385_at	RORB	RAR-related orphan receptor B	1.96
230730_at	SGCD	Sarcoglycan, δ (35 kDa dystrophin-associated glycoprotein)	1.46
206634_at	SIX3	SIX homeobox 3	1.86
232020_at	SMURF2	SMAD specific E3 ubiquitin protein ligase 2	-1.38
1559545_at	SNRPN	Small nuclear ribonucleoprotein polypeptide N	1.73
204913_s_at	SOX11	SRY (sex determining region Y)-box 11	2.22
202935_s_at	SOX9	SRY (sex determining region Y)-box 9	2.17
235342_at	SPOCK3	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3	1.60
1570035_at	TBC1D10A	CDNA clone IMAGE:4299416	-1.45
228121_at	TGFB2	Transforming growth factor, β 2	1.76
222835_at	THSD4	Thrombospondin, type I, domain containing 4	2.13
226899_at	UNC5B	Unc-5 homolog B (<i>C elegans</i>)	1.90

Abbreviation: TIA = transient ischemic attack.

different between these 2 groups. However, the TIA1 group contained all 3 of the patients with restricted diffusion identified on MRI. Such patients are known to be at high risk for subsequent stroke. In addition, one patient in the TIA1 group had a stroke within 3 months of the TIA. These patients suggest that the TIA1 group may represent patients at increased risk for recurrent ischemic events, though much larger studies will be required to ultimately address this hypothesis.

The genes that were different between TIA1 and TIA2 groups also provide insight into the differences between these 2 TIA groups. The pattern of gene expression in TIA2 was more similar to controls as compared to the TIA1 group. This may suggest that the TIA2 group includes nonvascular TIA mimics, which would be consistent with previously reported challenges in making the diagnosis of TIA.²⁷ The TIA2 group may have less inflammation compared to TIA1. The genes that were overexpressed in TIA1

Figure 2 Predicted probability of transient ischemic attack (TIA) or control diagnosis based on a linear discriminant analysis (LDA) model

The LDA model was used to derive the 34 genes that optimally distinguished TIA from controls. Probabilities are based on 10-fold leave-one-out cross-validation analysis. The probability of predicted diagnosis is shown on the y-axis, and subjects are shown on the x-axis. Patients with TIA are shown on the right, and control subjects on the left. The predicted probability of TIA is shown in red, and the predicted probability of control is shown in blue. Patients with TIA could be distinguished from controls with 100% sensitivity and 100% specificity.

compared to the TIA2 group were mostly related to inflammation and extracellular matrix remodeling. Although these TIA subgroups are of interest, further evaluation in larger samples is required before definitive conclusions can be drawn.

There were several limitations to the present study. Sample size relative to the number of genes analyzed was small. Although correction for multiple testing was performed, replication in an independent larger cohort is required. PCR validation is needed before genes are claimed to be TIA-specific markers. Our control group had more Caucasians compared to the TIA group. Although a secondary analysis restricted to Caucasians revealed very similar results, the influence of racial differences contributing to identified genes requires further study. The identification of relevant activated biological pathways in individual patients with TIA might be potentially useful to personalize approaches to treatment.

Patients with acute TIAs have different patterns of gene expression compared to asymptomatic controls with vascular risk factors. Genes expressed in TIA were associated with systemic inflammation, platelet activation, and prothrombin activation. Further study of the 2 patterns of RNA expression in patients with TIA is warranted to ascertain any relevance of these genes to cause of TIA or risk for subsequent stroke.

AUTHOR CONTRIBUTIONS

Dr. Zhan: designed the studies, acquired the data, analyzed and interpreted the data, performed statistical analysis, drafted the manuscript, and

revised the manuscript. Dr. C. Jickling: acquired the data, analyzed and interpreted the data, performed statistical analysis, and revised the manuscript. Dr. Tian: acquired the data and revised the manuscript. Dr. Stamova: revised the manuscript. Dr. Xu: acquired the data and revised the manuscript. Dr. Ander: acquired the data and revised the manuscript. Dr. Turner: acquired the data and revised the manuscript. M. Mesias: acquired the data. Dr. Verro: acquired the data and revised the manuscript. Dr. Bushnell: acquired the data and revised the manuscript. Dr. Johnston: designed the studies and acquired the data. Dr. Sharp: conceived and designed the studies, acquired the data, analyzed and interpreted the data, obtained funding, helped draft the manuscript, and revised the manuscript.

DISCLOSURE

Dr. Jickling is a fellow of the Canadian Institutes of Health Research (CIHR). Drs. Xu, Ander, and Tian are AHA Bugher Fellows. Dr. Turner is supported by a National Health and Medical Research Council (Australia) Postdoctoral Fellowship. Dr. Zhan is co-holder of a patent re: The use of blood RNA in TIA. Dr. Jickling, Dr. Tian, and Dr. Stamova report no disclosures. Dr. Xu receives research support from the American Heart Association Bugher Foundation. Dr. Ander serves as a consultant for Ischemia Care and receives research support from the American Heart Association. Dr. Turner and M. Mesias report no disclosures. Dr. Verro has received speaker honoraria from Boehringer Ingelheim and receives research support from the American Heart Association Bugher Foundation. Dr. Bushnell serves on a scientific advisory board for Boehringer Ingelheim and has received research support from Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership, the NIH/NINDS, the American Heart Association/Bugher Foundation, and the Hazel K Goddess Fund for Research on Stroke in Women. Dr. Johnston is co-holder of patent re: The RNA panel to identify TIA and risk stratify and receives research support from sanofi-aventis, Strkyer Neurovascular, Boston Scientific, the NIH (NCRR, NINDS), Kaiser-Permanente, and AHA/ASA, Bugher Award. Dr. Sharp serves as Senior Editor for *Brain Research*; holds a patent re: Gene expression profiles for the diagnosis of ischemic stroke; and receives research support from the NIH/NINDS and the American Heart Association.

Received February 16, 2011. Accepted in final form May 4, 2011.

REFERENCES

1. Johnston SC, Nguyen-Huynh MN, Schwarz ME, et al. National Stroke Association guidelines for the management of transient ischemic attacks. *Ann Neurol* 2006;60:301–313.
2. Rothwell PM, Buchan A, Johnston SC. Recent advances in management of transient ischaemic attacks and minor ischaemic strokes. *Lancet Neurol* 2006;5:323–331.
3. Easton JD, Saver JL, Albers GW, et al. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease: the American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. *Stroke* 2009;40:2276–2293.
4. Zhan X, Ander BP, Jickling G, et al. Brief focal cerebral ischemia that simulates transient ischemic attacks in humans regulates gene expression in rat peripheral blood. *J Cereb Blood Flow Metab* 2010;30:110–118.
5. Zhan X, Kim C, Sharp FR. Very brief focal ischemia simulating transient ischemic attacks (TIAs) can injure brain and induce Hsp70 protein. *Brain Res* 2008;1234:183–197.
6. Elneihoum AM, Falke P, Axelsson L, Lundberg E, Lindgarde F, Ohlsson K. Leukocyte activation detected by increased plasma levels of inflammatory mediators in

- patients with ischemic cerebrovascular diseases. *Stroke* 1996;27:1734–1738.
7. Ross AM, Hurn P, Perrin N, Wood L, Carlini W, Potempa K. Evidence of the peripheral inflammatory response in patients with transient ischemic attack. *J Stroke Cerebrovasc Dis* 2007;16:203–207.
 8. McArdle N, Riha RL, Vennelle M, et al. Sleep-disordered breathing as a risk factor for cerebrovascular disease: a case-control study in patients with transient ischemic attacks. *Stroke* 2003;34:2916–2921.
 9. Fon EA, Mackey A, Cote R, et al. Hemostatic markers in acute transient ischemic attacks. *Stroke* 1994;25:282–286.
 10. Bigalke B, Stellos K, Geisler T, et al. Expression of platelet glycoprotein VI is associated with transient ischemic attack and stroke. *Eur J Neurol* 2010;17:111–117.
 11. Arenillas JF, Alvarez-Sabin J, Molina CA, et al. C-reactive protein predicts further ischemic events in first-ever transient ischemic attack or stroke patients with intracranial large-artery occlusive disease. *Stroke* 2003;34:2463–2468.
 12. Cucchiara BL, Messe SR, Sansing L, et al. Lipoprotein-associated phospholipase A2 and C-reactive protein for risk-stratification of patients with TIA. *Stroke* 2009;40:2332–2336.
 13. Moore DF, Li H, Jeffries N, et al. Using peripheral blood mononuclear cells to determine a gene expression profile of acute ischemic stroke: a pilot investigation. *Circulation* 2005;111:212–221.
 14. Tang Y, Xu H, Du X, 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* 2006;26:1089–1102.
 15. Xu H, Tang Y, Liu DZ, et al. Gene expression in peripheral blood differs after cardioembolic compared with large-vessel atherosclerotic stroke: biomarkers for the etiology of ischemic stroke. *J Cereb Blood Flow Metab* 2008;28:1320–1328.
 16. Chong PH, Kezele B. Periodontal disease and atherosclerotic cardiovascular disease: confounding effects or epiphenomenon? *Pharmacotherapy* 2000;20:805–818.
 17. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol* 1996;67:1123–1137.
 18. Elter JR, Offenbacher S, Toole JF, Beck JD. Relationship of periodontal disease and edentulism to stroke/TIA. *J Dent Res* 2003;82:998–1001.
 19. Andersohn F, Waring M, Garbe E. Risk of ischemic stroke in patients with Crohn's disease: a population-based nested case-control study. *Inflamm Bowel Dis* 2010;16:1387–1392.
 20. Freilinger T, Riedel E, Holtmannspotter M, Dichgans M, Peters N. Ischemic stroke and peripheral arterial thromboembolism in a patient with Crohn's disease: a case presentation. *J Neurol Sci* 2008;266:177–179.
 21. Karacostas D, Mavromatis J, Artemis K, Milonas I. Hemorrhagic cerebral infarct and ulcerative colitis: a case report. *Funct Neurol* 1991;6:181–184.
 22. Schneiderman JH, Sharpe JA, Sutton DM. Cerebral and retinal vascular complications of inflammatory bowel disease. *Ann Neurol* 1979;5:331–337.
 23. Shelat SG, Ai J, Zheng XL. Molecular biology of ADAMTS13 and diagnostic utility of ADAMTS13 proteolytic activity and inhibitor assays. *Semin Thromb Hemost* 2005;31:659–672.
 24. Zhao BQ, Chauhan AK, Canault M, et al. von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. *Blood* 2009;114:3329–3334.
 25. Reid MJ, Cross AK, Haddock G, et al. ADAMTS-9 expression is up-regulated following transient middle cerebral artery occlusion (tMCAo) in the rat. *Neurosci Lett* 2009;452:252–257.
 26. Just S. Methodologies and clinical utility of ADAMTS-13 activity testing. *Semin Thromb Hemost* 2010;36:82–90.
 27. Uesu CT, Eisenman JI, Stemmer EA. The problem of dizziness and syncope in old age: transient ischemic attacks versus hypersensitive carotid sinus reflex. *J Am Geriatr Soc* 1976;24:126–135.

Editor's Note to Authors and Readers: Levels of Evidence in *Neurology*®

Effective January 15, 2009, authors submitting Articles or Clinical/Scientific Notes to *Neurology*® that report on clinical therapeutic studies must state the study type, the primary research question(s), and the classification of level of evidence assigned to each question based on the AAN classification scheme requirements. While the authors will initially assign a level of evidence, the final level will be adjudicated by an independent team prior to publication. Ultimately, these levels can be translated into classes of recommendations for clinical care. For more information, please access the articles and the editorial on the use of classification of levels of evidence published in *Neurology*.^{1–3}

1. French J, Gronseth G. Lost in a jungle of evidence: we need a compass. *Neurology* 2008;71:1634–1638.
2. Gronseth G, French J. Practice parameters and technology assessments: what they are, what they are not, and why you should care. *Neurology* 2008;71:1639–1643.
3. Gross RA, Johnston KC. Levels of evidence: taking *Neurology*® to the next level. *Neurology* 2009;72:8–10.