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(54) Title: DIFFERENTIAL EXPRESSION OF MOLECULES ASSOCIATED WITH ACUTE STROKE

(57) Abstract: Methods are provided for evaluating a stroke, for example for determining whether a subject has had an ischemic stroke, determining the severity or likely neurological recovery of a subject who has had an ischemic stroke, and determining a treatment regimen for a subject who has had an ischemic stroke, as are arrays and kits that can be used to practice the methods. In particular examples, the method includes screening for expression in ischemic stroke related genes (or proteins), such as white blood cell activation and differentiation genes (or proteins), genes (or proteins) related to hypoxia, genes (or proteins) involved in vascular repair, and genes (or proteins) related to a specific peripheral blood mononuclear cell (PBMC) response to the altered cerebral microenvironment.

WO 2005/116268 A2

DIFFERENTIAL EXPRESSION OF MOLECULES ASSOCIATED WITH ACUTE STROKE

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application No. 60/575,279 filed May 27, 2004, herein incorporated by reference in its entirety.

FIELD

This application relates to methods of evaluating an ischemic stroke, and methods of identifying a treatment modality for a subject who has had an ischemic stroke, as well as arrays and kits that can be used to practice the disclosed methods.

BACKGROUND

Stroke is the third leading cause of death and the leading cause of adult disability in developed countries (Simons *et al.*, *Stroke* 29:1341-6, 1998; Adams *et al.*, *Ischemic Cerebrovascular Disease*. New York: Oxford, 2001). Strokes are caused by an interruption of blood flow to the brain, by either an intravascular occlusion (such as an arterial thrombus) or a hemorrhage. The American Heart Association estimates that there are approximately three million stroke survivors in the United States, most of whom are disabled. Despite the prevalence and burden of this disease, stroke precipitants and pathophysiological mechanisms in individual patients are often unknown. It is also difficult to accurately predict whether a stroke will lead to only minor neurological sequelae or more serious medical consequences.

Gene expression profiling involves the study of mRNA levels in a tissue sample to determine the expression levels of genes that are expressed or transcribed from genomic DNA. Animal experiments in focally ischemic brain tissue have indicated that there are alterations in gene expression following a stroke (Stenzel-Poore *et al.*, *Lancet* 362:1028-37, 2003; Lu *et al.*, *J. Cereb. Blood Flow. Metab.* 23:786-810, 2003; Tang *et al.*, *Eur J Neurosci* 15:1937-52, 2002; Tang *et al.*, *Ann. Neurol.* 50:699-707, 2001; and Tang *et al.*, *J Cereb Blood Flow Metab* 23:310-9, 2003). However, gene expression profiling has not yet been applied to clinical human stroke, primarily because brain tissue samples are inaccessible and rarely justified. Therefore, an assay that can allow one to determine the genetic expression profile of ischemic stroke without the need for brain tissue samples is needed.

Currently, there is no specific blood marker of acute stroke. Following a stroke, released brain antigens can be detected in the blood. Such antigens include S100B, neuron specific enolase (NSE), and glial fibrillary acid protein (GFAP), although S100B and GFAP are of low sensitivity for early stroke diagnosis, and NSE and myelin basic protein (MBP) MBP are non-specific (Lamers *et al.*, *Brain. Res. Bull.* 61:261-4, 2003). Four soluble factors that have demonstrated moderate sensitivity and specificity for the diagnosis of stroke include two markers of inflammation (matrix metalloproteinase-9 and vascular cell adhesion molecule), one marker of glial activation (S100beta) and one thrombosis marker (von Willebrand factor) (Lynch *et al.*, *Stroke* 35:57-63, 2004). However,

a panel of markers which allow one to diagnose and prognose ischemic stroke with high diagnostic sensitivity and specificity is still needed.

SUMMARY

Although stroke is one of the leading causes of morbidity and mortality in developed countries, methods for rapidly and accurately determining whether a subject has had a stroke are expensive and invasive. Therefore, new methods are needed for evaluating a stroke, for example for determining whether an ischemic stroke has occurred, for determining the severity of the stroke or the likely neurological recovery of the subject who had an ischemic stroke, or combinations thereof. In particular examples, the disclosed methods offer a potentially lower cost alternative to expensive imaging modalities (such as MRI and CT scans), can be used in instances where those imaging modalities are not available (such as in field hospitals), and can be more convenient than placing individuals in scanners (for example for subjects who can not be subjected to MRI, such as those having certain types of metallic implants in their bodies).

Using these methods, appropriate therapy protocols for subjects who have had an ischemic stroke can be identified and administered. For example, because the results of the disclosed methods are highly reliable predictors of the ischemic nature of the stroke, the results can also be used (alone or in combination with other clinical evidence and brain scans) to determine whether thrombolytic therapy designed to lyse a neurovascular occlusion such as a thrombus (for example by using tissue plasminogen activator or streptokinase) should be administered to the subject. In certain examples, thrombolytic therapy is given to the subject once the results of the differential expression assay are known if the assay provides an indication that the stroke is ischemic in nature.

The inventors have identified changes in gene expression in peripheral blood mononuclear cells (PBMCs) that allow one to evaluate a stroke, for example to determine whether a subject has had an ischemic stroke, to determine the severity of an ischemic stroke, to determine the likely neurological recovery of the subject, or combinations thereof. The disclosed methods allow one to screen many genes simultaneously and serially and only a relatively small amount of cell or tissue sample is needed. Changes in gene expression were observed in at least 22 genes, at least 82 genes, at least 190 genes, or even at least 637 genes depending on sensitivity and specificity used. In particular examples, subjects who had an ischemic stroke showed increased gene expression in CD163; hypothetical protein FLJ22662 Laminin A motif; bone marrow stromal cell antigen 1 (BST-1, also known as CD157); Fc fragment of IgG, high affinity Ia, receptor for (FcγRI, also known as CD64); baculoviral IAP repeat-containing protein 1 (also referred to in the literature as neuronal apoptosis inhibitory protein); or KIAA0146, or any combinations thereof, such as a change in expression in at least 1, at least 2, at least 3, at least 4, at least 5, or all 6 of these genes. In some examples, subjects who had an ischemic stroke showed increased gene expression in four classes of genes: genes involved in white blood cell activation and differentiation, genes related to hypoxia, genes involved in vascular repair, and genes related to a PBMC response to the altered cerebral microenvironment.

The disclosed gene expression fingerprint of ischemic stroke enables methods of evaluating a stroke, for example determining whether a subject had an ischemic stroke, determining the prognosis of a subject who had an ischemic stroke, as well as determining an appropriate treatment regimen for a subject who had an ischemic stroke. In some examples, the disclosed methods are at least 78% sensitive and at least 80% specific for identifying those subjects who have suffered an ischemic stroke, for example within the past 72 hours. In other examples, the disclosed methods are at least 80% sensitive (such as at least 85% sensitive or at least 90% sensitive) and at least 80% specific (such as at least 85% specific or at least 90% specific) for identifying those subjects who have suffered an ischemic stroke, for example within the past 72 hours. In particular examples, the disclosed methods are at least 80% sensitive for predicting the likelihood of neurological recovery of the subject.

In some examples, the method involves detecting patterns of increased protein expression, decreased protein expression, or both. Such patterns of expression can be detected either at the nucleic acid level (such as quantitation of mRNAs associated with protein expression) or the protein level (such as quantitative spectroscopic detection of proteins). Certain methods involve not only detection of patterns of expression, but detection of the magnitude of expression (increased, decreased, or both), wherein such patterns are associated with the subject having had an ischemic stroke, or is associated with predicted clinical sequelae, such as neurological recovery following an ischemic stroke.

The disclosed methods are the first that permit accurate diagnosis of an ischemic stroke using PBMCs with high sensitivity and specificity. PBMCs infiltrate the evolving cerebral infarct as part of the tissue remodeling process. Release of brain antigens from damaged neural cells may allow sensitization of PBMCs followed by changes in functional gene expression.

The disclosed methods can be performed on a subject who is suspected of having had a stroke, for example prior to radiographic investigation. In another example, the method is performed on a subject known to have had a stroke, as the disclosed assays permit early and accurate stratification of risk of long-lasting neurological impairment.

In one example, the method of evaluating a stroke includes determining whether a subject has changes in expression in four or more ischemic stroke-associated molecules that comprise, consist essentially of, or consist of, sequences (such as a DNA, RNA or protein sequence) involved in white blood cell activation and differentiation, sequences related to hypoxia, sequences involved in vascular repair, and sequences related to a PBMC response to the altered cerebral microenvironment, such as those listed in Table 5.

In other examples, ischemic stroke-associated molecules comprise, consist essentially of, or consist of, CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, or any 1, 2, 3, 4, 5, or 6 of these molecules. For example, ischemic stroke-associated molecules can comprise, consist essentially of, or consist of, 4 or more, such as 5 or more, 10 or more, 20 or more, 22 or more, 50 or more, 75 or more, 80 or more, 82 or more, 100 or more, 150 or more, 190 or more, 200 or more, 300 or more, 500 or more, 600 or

more, or 637 or more of the nucleic acid or protein sequences listed in Tables 2-5. Any of the identified sequences can be used in combination with such sets or subsets of sequences.

In a particular example, evaluating a stroke includes detecting differential expression in at least four ischemic stroke-related molecules of the subject, such as any combination of at least four genes (or the corresponding proteins) listed in any of Tables 2-5, wherein the presence of differential expression of at least four ischemic-stroke related molecules indicates that the subject has had an ischemic stroke. Therefore, such methods can be used to diagnose an ischemic stroke. In particular examples, the at least four ischemic-stroke related molecules include at least one of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, such as at least 2, at least 3, at least 4, at least 5 or at least 6 of such molecules. For example, the method can include determining if the subject has increased gene (or protein) expression of at least one of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, optionally in combination with determining if the subject has altered gene (or protein) expression of any other combination of other ischemic stroke-associated molecules, such as any combination of at least 3 other genes (for example any combination of at least 5, at least 10, at least 20, at least 50, at least 100, at least 200, or even at least 500 genes) listed in Tables 2-5.

In a particular example, differential expression is detected by determining if if the subject has increased gene (or protein) expression of at least one of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, and determining if the subject has decreased gene (or protein) expression of at least one of intercellular adhesion molecule 2, protein kinase D2, GATA binding protein 3, hypothetical protein FLJ20257, or protein kinase C, theta. For example, differential expression can be detected by determining if the subject has increased gene (or protein) expression of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, and determining if the subject has decreased gene (or protein) expression of intercellular adhesion molecule 2, protein kinase D2, GATA binding protein 3, hypothetical protein FLJ20257, and protein kinase C, theta.

In one example, the method includes determining if the subject has an increase in gene expression in any combination of at least four of the genes listed in Table 5, for example an increase in at least 5, at least 10, at least 15, at least 20, or at least 22 of the genes listed in Table 5. An increase in expression in any combination of four or more of the genes listed in Table 5 (or the corresponding proteins), and particularly any combination of at least one gene (or protein) from each of the four classes of genes listed in Table 5 (such as any combination of at least 2 or at least 3 sequences from each of the four classes of genes listed in Table 5) indicates that the subject has had an ischemic stroke. Any one of the set of genes (or proteins) can be identified by a single one or the genes (or proteins) listed in Table 5. Any one of the genes (or proteins) in Table 5 can be combined with any other combination of the genes (or proteins) in Table 5 to produce a combination or subcombination of genes (or proteins).

In one example, the method of evaluating a stroke includes determining if the subject has a change in gene expression (such as an increase or decrease) in any combination of at least 150 of the 190 of the genes listed in Table 3, for example a change in expression in at least 160, at least 170, at least 175, at least 180, or at least 185 of the genes listed in Table 3. Any one of the set of genes can be identified by a single one or the genes listed in Table 3. Any one of the genes (or proteins) in Table 3 can be combined with any other combination of the genes (or proteins) in Table 3 to produce a combination or subcombination of genes. A change in expression in any combination of 150 or more of the genes listed in Table 3 (or the corresponding proteins) indicates that the subject has had an ischemic stroke.

In another example, the method of evaluating a stroke includes determining if the subject has a change in gene expression (such as an increase or decrease) in any combination of at least 510 of the 637 of the genes listed in Table 2, for example an increase or decrease in any combination of at least 510, at least 550, at least 575, at least 600, at least 620, or at least 630 of the genes listed in Table 2. Any one of the set of genes (or proteins) can be identified by a single one or the genes (or proteins) listed in Table 2. Any one of the genes (or proteins) in Table 2 can be combined with any other combination of the genes (or proteins) in Table 2 to produce a combination or subcombination of genes. A change in expression in any combination of 510 or more of the genes listed in Table 2 (or the corresponding proteins) indicates that the subject has had an ischemic stroke.

In some examples, the amount of gene (or protein) expression in the subject is compared to a control, such as the gene (or protein) expression of a subject who has not had an ischemic stroke, wherein an increase or decrease in expression in any combination of four or more ischemic stroke related genes listed in Tables 2-5 compared to the control indicates that the subject has experienced an ischemic stroke. For example, an increase in expression in any combination of four or more ischemic stroke related genes (or the corresponding proteins) listed in Table 5, such as at least one gene (or the corresponding protein) from each class listed in Table 5, compared to the control indicates that the subject has experienced an ischemic stroke.

In particular examples evaluating the stroke includes predicting a likelihood of severity of neurological sequelae of the ischemic stroke. In some examples, evaluating the stroke includes predicting a likelihood of neurological recovery of the subject. For example, if there is differential expression (such as increased expression) in at least the 22 ischemic-stroke related molecules listed in Table 5, indicates that the subject has a higher risk of long-term adverse neurological sequelae and therefore a lower likelihood of neurological recovery. In another example, detecting a change in expression in any combination of 150 or more of the genes listed in Table 2 or 3 (or the corresponding proteins) indicates that the subject has a higher risk of long-term adverse neurological sequelae and therefore a lower likelihood of neurological recovery. In yet another example, detecting a change in expression in any combination of at least 500 of the 637 of the genes listed in Table 2, for example an increase or decrease in any combination of at least 510, at least 550, at least 575, at least 600, at least 620, or at least 630 of the genes listed in Table 2 indicates that the subject has a higher risk of long-term adverse neurological sequelae and therefore a lower likelihood of neurological

recovery. In some examples, differential expression in the subject is compared to differential expression of a subject who has not had an ischemic stroke, wherein a change in expression in at least the 22 ischemic-stroke related molecules listed in Table 5, such as any combination of 150 or more of the genes listed in Tables 2 or 3 (or the corresponding proteins) compared to the control indicates that the subject has a higher risk of long-term adverse neurological sequelae and therefore a lower likelihood of neurological recovery.

The disclosed methods can further include administering to a subject a treatment to avoid or reduce ischemic injury if the presence of differential expression indicates that the subject has had an ischemic stroke. For example, a change in expression in at least four ischemic stroke related molecules, such as a combination that includes at least four of the molecules listed in Tables 2-5, indicates that the subject has had an ischemic stroke (and not a hemorrhagic stroke) and is in need of thrombolytic therapy (such as t-PA or heparin), anticoagulant therapy (such as coumadin), or combinations thereof. Therefore, the disclosed methods differentiate ischemic from hemorrhagic stroke, and allow one to administer the appropriate therapy to the subject. In some examples, the amount of differential expression in the subject is compared to the expression of a subject who has not had an ischemic stroke, wherein a change in expression in at least four ischemic stroke related molecules listed in Table 2-5 (or the corresponding proteins), such as at least those 22 listed in Table 5, compared to the control indicates that the subject would benefit from thrombolytic therapy, anticoagulant therapy, or combinations thereof.

In some examples the presence of differential expression is evaluated by determining a t-statistic value that indicates whether a gene or protein is up- or down-regulated. For example, an absolute t-statistic value can be determined. In some examples, a negative t-statistic indicates that the gene or protein is downregualted, while a positive t-statistic indicates that the gene or protein is upregulated. In particular examples, a t-statistic less than -3 indicates that the gene or protein is downregualted, such as less than -3.5, less than -3.6, less than -3.7 or even less than -3.8, while a t-statistic of at least 3, such as at least 3.5, at least 3.7, or at least 3.8 indicates that the gene or protein is upregulated.

Differential expression can be detected at any time following the onset of clinical signs and symptoms that indicate a potential stroke, such as within 24 hours, within 7-14 days, or within 90 days of onset of clinical signs and symptoms that indicate a potential stroke. Examples of such signs and symptoms include, but are not limited to: headache, sensory loss (such as numbness, particularly confined to one side of the body or face), paralysis (such as hemiparesis), pupillary changes, blindness (including bilateral blindness), ataxia, memory impairment, dysarthria, somnolence, and other effects on the central nervous system recognized by those of skill in the art.

In particular examples, the disclosed methods include isolating nucleic acid molecules from PBMCs of a subject suspected of having had an ischemic stroke (or known to have had an ischemic stroke), such as mRNA molecules. The isolated nucleic acid molecules are contacted with or applied to an array, for example an array that includes oligonucleotide probes capable of hybridizing to ischemic stroke-associated genes. In another particular example, the disclosed methods include

purifying proteins from PBMCs of a subject suspected of having had an ischemic stroke (or known to have had an ischemic stroke). The isolated proteins are contacted with or applied to an array, for example an array that includes antibody probes capable of hybridizing to ischemic stroke-associated proteins. In some examples, PBMCs are obtained within at least the previous 72 hours of a time when the stroke is suspected of occurring, such as within the previous 24 hours.

Also provided herein are arrays that include molecules that permit evaluation of a stroke. Such arrays in particular examples permit quantitation of ischemic stroke-related nucleic acid or protein sequences present in a sample, such as a sample that includes PBMC nucleic acid molecules or proteins.

In one example, the array includes oligonucleotide probes capable of hybridizing to nucleic acid molecues (such as gene, cDNA or mRNA sequences) involved in white blood cell activation and differentiation, nucleic acid molecues related to hypoxia, nucleic acid molecues involved in vascular repair, and nucleic acid molecues related to a PBMC response to the altered cerebral microenvironment, such as at least those listed in Table 5. Examples of particular genes are provided in Tables 2-5. In particular examples, the array includes probes that recognize any combination of at least 4 of the genes listed in any of Tables 2-5, for example at least 10, at least 20, at least 50, at least 100, at least 150, at least 160, at least 170, at least 175, at least 180, at least 185, at least 200, at least 400, at least 500, at least 510, at least 550, at least 575, at least 600, at least 620, or at least 630 of the genes listed in any of Tables 2-5. For example, the array can include oligonucleotide probes capable of hybridizing to a sequence that encodes at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, or any one of these. In one example, the array includes oligonucleotide probes capable of hybridizing to a sequence that encodes one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, such as at least 2, at least 3, at least 4, at least 5 or at least 6 of such molecules. In some examples, the array includes probes that recognize any combination of at least one gene from each of the four classes listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each class.

The isolated nucleic acid molecules are incubated with the array for a time sufficient to allow hybridization between the isolated nucleic acid molecules and oligonucleotide probes, thereby forming isolated nucleic acid molecules:oligonucleotide probe complexes. The isolated nucleic acid molecules:oligonucleotide probe complexes are then analyzed to determine if there are changes in gene expression (such as increases or decreases), for example changes in expression of any combination of four or more of the genes listed in Table 5, such as 20 or more of the genes listed in Tables 2-5, or such as 150 or more of the genes listed in Tables 2-4. In particular examples, changes in gene expression are quantitated. The presence of increased expression of four or more genes listed in Tables 2-5 with a positive t-statistic value, or decreased expression of four or more genes listed in Tables 2-4 with a negative t-statistic value (or any combination thereof, such as decreased expression of at least one gene and increased expression of at least 3 genes listed in Tables 2-4), after multiple comparison correction, indicates that the subject has had an ischemic stroke.

In another example, the method includes isolating proteins from PBMCs of a subject suspected of having had an ischemic stroke, or known to have had an ischemic stroke. In particular examples the assay is performed on substantially purified or isolated PBMCs that have been separated, for example, for other leukocytes in the blood. The isolated proteins are contacted with or applied to an array.

Arrays that can be used to detect and quantitate proteins for evaluating stroke are also provided. For examples, the array, such as a protein-binding array, can include probes (such as an oligonucleotide probes or antibodies) capable of hybridizing to ischemic-stroke related proteins, such as proteins involved in white blood cell activation and differentiation, proteins related to hypoxia, proteins involved in vascular repair, and proteins related to a PBMC response to the altered cerebral microenvironment. Examples of particular ischemic-stroke related proteins are provided in Tables 2-5. The isolated proteins are incubated with the array for a time sufficient to allow hybridization between the proteins and probes on the array, thereby forming protein:probe complexes.

The protein:probe complexes are then analyzed and in some examples quantitated to determine if there are changes in gene expression (such as increases or decreases) in any combination of four or more of the molecules listed in any of Tables 2-5, such as changes in expression of one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, or 2, 3, 4, or 5 of these. In a specific example, protein:probe complexes are analyzed (for example quantitated) to determine if there are increases in expression in any combination of at least one protein from each of the four classes listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each of the classes listed in Table 5. The presence of increased or decreased expression of any combination of four or more proteins listed in Tables 2-4 (or increased expression of any combination of four or more proteins listed in Table 5), indicates that the subject has had an ischemic stroke.

In particular examples, the disclosed arrays are capable of evaluating a stroke, for example for determining whether a subject has had an ischemic stroke, determining the severity of the ischemic stroke, determining the likelihood of neurological recovery of a subject who had an ischemic stroke, determining how to treat a subject who had an ischemic stroke, or combinations thereof. Such arrays include oligonucleotides that are complementary to ischemic stroke-related genes, such as those involved in white blood cell activation and differentiation, genes related to hypoxia, genes involved in vascular repair, and genes related to a PBMC response to the altered cerebral microenvironment. Examples of particular genes are provided in Tables 2-5. Kits including such arrays are also disclosed.

In one example, proteins a biological sample are quantitated, for instance by quantitative mass spectroscopy, to determine whether proteins associated with ischemic stroke or prognosis of ischemic stroke are upregulated, downregulated, or both.

The foregoing and other features and advantages of the disclosure will become more apparent from the following detailed description of a several embodiments.

BRIEF DESCRIPTION OF THE FIGURE

FIG. 1 is a graph showing the effects of various multiple comparison correction techniques on the ischemic stroke microarrays.

SEQUENCE LISTING

The nucleic acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand.

SEQ ID NOS: 1-2 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of adrenomedullin.

SEQ ID NOS: 3-4 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of CD14.

SEQ ID NOS: 5-6 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of CD36.

SEQ ID NOS: 7-8 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of caspase 1.

SEQ ID NOS: 9-10 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of a-Catenin.

SEQ ID NOS: 11-12 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of FcR2a.

SEQ ID NOS: 13-14 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of FcER1a.

SEQ ID NOS: 15-16 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of cathepsin B.

SEQ ID NOS: 17-18 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of TRL2.

SEQ ID NOS: 19-20 are oligonucleotide sequences used to perform RT-PCR to determine expression levels of INFGR1.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

Abbreviations and Terms

The following explanations of terms and methods are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. The singular forms "a," "an," and "the" refer to one or more than one, unless the context clearly dictates otherwise. For example, the term "comprising a nucleic acid molecule" includes single or plural

nucleic acid molecules and is considered equivalent to the phrase "comprising at least one nucleic acid molecule." The term "or" refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise. As used herein, "comprises" means "includes." Thus, "comprising A or B," means "including A, B, or A and B," without excluding additional elements.

Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting.

PBMC: peripheral blood mononuclear cell

Real time-PCR: real time polymerase chain reaction

Amplifying a nucleic acid molecule: To increase the number of copies of a nucleic acid molecule, such as a gene or fragment of a gene, for example a region of a ischemic stroke-associated gene. The resulting products are called amplification products.

An example of *in vitro* amplification is the polymerase chain reaction (PCR), in which a biological sample obtained from a subject (such as a sample containing PBMCs) is contacted with a pair of oligonucleotide primers, under conditions that allow for hybridization of the primers to a nucleic acid molecule in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid molecule. Other examples of *in vitro* amplification techniques include quantitative real-time PCR, strand displacement amplification (see USPN 5,744,311); transcription-free isothermal amplification (see USPN 6,033,881); repair chain reaction amplification (see WO 90/01069); ligase chain reaction amplification (see EP-A-320 308); gap filling ligase chain reaction amplification (see USPN 5,427,930); coupled ligase detection and PCR (see USPN 6,027,889); and NASBA™ RNA transcription-free amplification (see USPN 6,025,134).

Quantitative real-time PCR is another form of *in vitro* amplifying nucleic acid molecules, enabled by Applied Biosystems (TaqMan PCR). Real-time quantitative TaqMan PCR has reduced the variability traditionally associated with quantitative PCR, thus allowing the routine and reliable quantification of PCR products to produce sensitive, accurate, and reproducible measurements of levels of gene expression. The 5' nuclease assay provides a real-time method for detecting only specific amplification products. During amplification, annealing of the probe to its target sequence generates a substrate that is cleaved by the 5' nuclease activity of Taq DNA polymerase when the enzyme extends from an upstream primer into the region of the probe. This dependence on polymerization ensures that cleavage of the probe occurs only if the target sequence is being amplified. The use of fluorogenic probes makes it possible to eliminate post-PCR processing for the analysis of probe degradation. The probe is an oligonucleotide with both a reporter fluorescent dye

and a quencher dye attached. While the probe is intact, the proximity of the quencher greatly reduces the fluorescence emitted by the reporter dye by Förster resonance energy transfer (FRET) through space. Probe design and synthesis has been simplified by the finding that adequate quenching is observed for probes with the reporter at the 5' end and the quencher at the 3' end.

Anti-coagulants: Agents that decrease or prevent blood clotting. Anticoagulants can avoid the formation of new clots, and prevent existing clots from growing (extending), for example by decreasing or stopping the production of proteins necessary for blood to clot. Examples include, but are not limited to, aspirin, heparin, ximelagatran, and warfarin (Coumadin). Administration of anticoagulants is one treatment for ischemic stroke, for example to prevent further strokes. A particular type of anti-coagulant are **anti-platelet agents**, which can also be used to prevent further strokes from occurring and include aspirin, clopidogrel (Plavix), aspirin/dipyridamole combination (Aggrenox), and ticlopidine (Ticlid). Other agents used to prevent stroke recurrence are **antihypertensive drugs** and **lipid-lowering agents** such as statins.

Array: An arrangement of molecules, such as biological macromolecules (such as peptides or nucleic acid molecules) or biological samples (such as tissue sections), in addressable locations on or in a substrate. A "microarray" is an array that is miniaturized so as to require or be aided by microscopic examination for evaluation or analysis. Arrays are sometimes called DNA chips or biochips.

The array of molecules ("features") makes it possible to carry out a very large number of analyses on a sample at one time. In certain example arrays, one or more molecules (such as an oligonucleotide probe) will occur on the array a plurality of times (such as twice), for instance to provide internal controls. The number of addressable locations on the array can vary, for example from at least four, to at least 10, at least 20, at least 30, at least 50, at least 75, at least 100, at least 150, at least 200, at least 300, at least 500, least 550, at least 600, at least 800, at least 1000, at least 10,000, or more. In particular examples, an array includes nucleic acid molecules, such as oligonucleotide sequences that are at least 15 nucleotides in length, such as about 15-40 nucleotides in length. In particular examples, an array includes oligonucleotide probes or primers which can be used to detect ischemia stroke-associated sequences, such as any combination of at least four of those listed in Table 5, such as at least 10, at least 20, at least 50, at least 100, at least 150, at least 160, at least 170, at least 175, at least 180, at least 185, at least 200, at least 400, at least 500, at least 510, at least 550, at least 575, at least 600, at least 620, or at least 630 of the sequences listed in any of Tables 2-5. In some examples, an array includes oligonucleotide probes or primers which can be used to detect at least one gene from each of the four classes of genes listed in Table 5, such as at least 2, at least 3, at least 5, or even at least 10 genes from each of the four classes of genes listed in Table 5.

Within an array, each arrayed sample is addressable, in that its location can be reliably and consistently determined within at least two dimensions of the array. The feature application location on an array can assume different shapes. For example, the array can be regular (such as arranged in uniform rows and columns) or irregular. Thus, in ordered arrays the location of each sample is

assigned to the sample at the time when it is applied to the array, and a key may be provided in order to correlate each location with the appropriate target or feature position. Often, ordered arrays are arranged in a symmetrical grid pattern, but samples could be arranged in other patterns (such as in radially distributed lines, spiral lines, or ordered clusters). Addressable arrays usually are computer readable, in that a computer can be programmed to correlate a particular address on the array with information about the sample at that position (such as hybridization or binding data, including for instance signal intensity). In some examples of computer readable formats, the individual features in the array are arranged regularly, for instance in a Cartesian grid pattern, which can be correlated to address information by a computer.

Protein-based arrays include probe molecules that are or include proteins, or where the target molecules are or include proteins, and arrays including nucleic acids to which proteins are bound, or vice versa. In some examples, an array contains antibodies to ischemic stroke-associated proteins, such as any combination of at least four of those listed in Table 5, such as at least 10, at least 20, at least 50, at least 100, at least 150, at least 160, at least 170, at least 175, at least 180, at least 185, at least 200, at least 400, at least 500, at least 510, at least 550, at least 575, at least 600, at least 620, or at least 630 of the sequences listed in any of Tables 2-5. In particular examples, an array includes antibodies or proteins that can detect at least one protein from each class listed in Table 5, such as at least 2, at least 3, at least 5, or even at least 10 genes from each class listed in Table 5.

Baculoviral IAP repeat-containing protein 1 (Birc1): A protein that includes one or more baculoviral IAP repeat (BIR) domains, which is capable of decreasing (an in some examples inhibiting) the biological activity of caspases, and in some examples thereby decreasing or inhibiting apoptosis. The term baculoviral IAP repeat-containing protein 1 includes any Birc1 gene, cDNA, mRNA, or protein from any organism and that is a Birc1 that can decrease or inhibit caspase biological activity. Also referred to in the literature as neuronal apoptosis inhibitory protein (Naip).

Birc1 sequences are publicly available. For example, GenBank Accession Nos: NM_004536 and NP_004527 disclose human Birc1 nucleic acid and protein sequences, respectively and GenBank Accession Nos: NM_010870 and NP_035000 disclose mouse Birc1 nucleic acid and proteins sequences, respectively.

In one example, a Birc1 sequence includes a full-length wild-type (or native) sequence, as well as Birc1 allelic variants, variants, fragments, homologs or fusion sequences that retain the ability to decrease or inhibit caspase biological activity. In certain examples, Birc1 has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native Birc1. In other examples, Birc1 has a sequence that hybridizes under very high stringency conditions to a sequence set forth in GenBank Accession No. NM_010870 or NM_004536, and retains Birc1 activity.

Binding or stable binding: An association between two substances or molecules, such as the hybridization of one nucleic acid molecule to another (or itself), the association of an antibody with a peptide, or the association of a protein with another protein or nucleic acid molecule. An oligonucleotide molecule binds or stably binds to a target nucleic acid molecule if a sufficient amount

of the oligonucleotide molecule forms base pairs or is hybridized to its target nucleic acid molecule, to permit detection of that binding.

Binding can be detected by any procedure known to one skilled in the art, such as by physical or functional properties of the target:oligonucleotide complex. For example, binding can be detected functionally by determining whether binding has an observable effect upon a biosynthetic process such as expression of a gene, DNA replication, transcription, translation, and the like.

Physical methods of detecting the binding of complementary strands of nucleic acid molecules, include but are not limited to, such methods as DNase I or chemical footprinting, gel shift and affinity cleavage assays, Northern blotting, dot blotting and light absorption detection procedures. For example, one method involves observing a change in light absorption of a solution containing an oligonucleotide (or an analog) and a target nucleic acid at 220 to 300 nm as the temperature is slowly increased. If the oligonucleotide or analog has bound to its target, there is a sudden increase in absorption at a characteristic temperature as the oligonucleotide (or analog) and target disassociate from each other, or melt. In another example, the method involves detecting a signal, such as a detectable label, present on one or both nucleic acid molecules (or antibody or protein as appropriate).

The binding between an oligomer and its target nucleic acid is frequently characterized by the temperature (T_m) at which 50% of the oligomer is melted from its target. A higher (T_m) means a stronger or more stable complex relative to a complex with a lower (T_m).

Bone marrow stromal cell antigen 1 (BST-1): A glycosylphosphatidylinositol (GPI)-anchored protein involved in adhesion to extracellular matrix proteins and in chemotaxis induced in vitro by formyl-methionyl-leucyl-phenylalanine (fMLP), as well as activation of white blood cells. Also known in the art as CD157. The term bone marrow stromal cell antigen 1 (BST-1) includes any BST-1 gene, cDNA, mRNA, or protein from any organism and that is a BST-1 that has BST-1 biological activity. BST-1 sequences are publicly available. For example, GenBank Accession Nos: BT019502 and AAV38309 disclose human BST-1 nucleic acid and proteins sequences, respectively.

In one example, a BST-1 sequence includes a full-length wild-type (or native) sequence, as well as BST-1 allelic variants, variants, fragments, homologs or fusion sequences that retain the ability to function in adhesion and chemotaxis. In certain examples, BST-1 has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native BST-1. In other examples, BST-1 has a sequence that hybridizes under very high stringency conditions to a sequence set forth in GenBank Accession No. BT019502, and retains BST-1 activity.

CD163: A hemoglobin scavenger receptor. The term CD163 includes any CD163 gene, cDNA, mRNA, or protein from any organism and that is a CD163 that can function as a hemoglobin scavenger receptor. CD163 sequences are publicly available. For example, GenBank Accession Nos: Y18388 and CAB45233 disclose human CD163 nucleic acid and protein sequences, respectively and GenBank Accession Nos: NM_053094 and NP_444324 disclose mouse CD163 nucleic acid and proteins sequences, respectively.

In one example, a CD163 sequence includes a full-length wild-type (or native) sequence, as well as CD163 allelic variants, variants, fragments, homologs or fusion sequences that retain the ability to function as a hemoglobin scavenger receptor. In certain examples, CD163 has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native CD163. In other examples, CD163 has a sequence that hybridizes under very high stringency conditions to a sequence set forth in GenBank Accession No. Y18388 or NM_053094, and retains CD163 activity.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and regulatory sequences which determine transcription. cDNA can be synthesized by reverse transcription from messenger RNA extracted from cells.

Clinical indications of stroke: One or more signs or symptoms that are associated with a subject having (or had) a stroke, such as an ischemic stroke. Particular examples include, but are not limited to: headache, sensory loss (such as numbness, particularly confined to one side of the body or face), paralysis (such as hemiparesis), pupillary changes, blindness (including bilateral blindness), ataxia, memory impairment, dysarthria, somnolence, and other effects on the central nervous system recognized by those of skill in the art.

Complementarity and percentage complementarity: Molecules with complementary nucleic acids form a stable duplex or triplex when the strands bind, (hybridize), to each other by forming Watson-Crick, Hoogsteen or reverse Hoogsteen base pairs. Stable binding occurs when an oligonucleotide molecule remains detectably bound to a target nucleic acid sequence under the required conditions.

Complementarity is the degree to which bases in one nucleic acid strand base pair with the bases in a second nucleic acid strand. Complementarity is conveniently described by percentage, that is, the proportion of nucleotides that form base pairs between two strands or within a specific region or domain of two strands. For example, if 10 nucleotides of a 15-nucleotide oligonucleotide form base pairs with a targeted region of a DNA molecule, that oligonucleotide is said to have 66.67% complementarity to the region of DNA targeted.

In the present disclosure, "sufficient complementarity" means that a sufficient number of base pairs exist between an oligonucleotide molecule and a target nucleic acid sequence (such as an ischemic stroke-related sequence, for example any of the sequences listed in Tables 2-5) to achieve detectable binding. When expressed or measured by percentage of base pairs formed, the percentage complementarity that fulfills this goal can range from as little as about 50% complementarity to full (100%) complementary. In general, sufficient complementarity is at least about 50%, for example at least about 75% complementarity, at least about 90% complementarity, at least about 95% complementarity, at least about 98% complementarity, or even at least about 100% complementarity.

A thorough treatment of the qualitative and quantitative considerations involved in establishing binding conditions that allow one skilled in the art to design appropriate oligonucleotides for use under the desired conditions is provided by Beltz *et al. Methods Enzymol.* 100:266-285, 1983, and by Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

DNA (deoxyribonucleic acid): A long chain polymer which includes the genetic material of most living organisms (some viruses have genes including ribonucleic acid, RNA). The repeating units in DNA polymers are four different nucleotides, each of which includes one of the four bases, adenine, guanine, cytosine and thymine bound to a deoxyribose sugar to which a phosphate group is attached. Triplets of nucleotides, referred to as codons, in DNA molecules code for amino acid in a polypeptide. The term codon is also used for the corresponding (and complementary) sequences of three nucleotides in the mRNA into which the DNA sequence is transcribed.

Deletion: The removal of one or more nucleotides from a nucleic acid sequence (or one or more amino acids from a protein sequence), the regions on either side of the removed sequence being joined together.

Differential expression: A difference, such as an increase or decrease, in the conversion of the information encoded in a gene (such as an ischemic stroke related gene) into messenger RNA, the conversion of mRNA to a protein, or both. In some examples, the difference is relative to a control or reference value, such as an amount of gene expression that is expected in a subject who has not had an ischemic stroke or an amount expected in a subject who has had an ischemic stroke. Detecting differential expression can include measuring a change in gene expression.

Downregulated or inactivation: When used in reference to the expression of a nucleic acid molecule, such as a gene, refers to any process which results in a decrease in production of a gene product. A gene product can be RNA (such as mRNA, rRNA, tRNA, and structural RNA) or protein. Therefore, gene downregulation or deactivation includes processes that decrease transcription of a gene or translation of mRNA.

Examples of processes that decrease transcription include those that facilitate degradation of a transcription initiation complex, those that decrease transcription initiation rate, those that decrease transcription elongation rate, those that decrease processivity of transcription and those that increase transcriptional repression. Gene downregulation can include reduction of expression above an existing level. Examples of processes that decrease translation include those that decrease translational initiation, those that decrease translational elongation and those that decrease mRNA stability.

Gene downregulation includes any detectable decrease in the production of a gene product. In certain examples, production of a gene product decreases by at least 2-fold, for example at least 3-fold or at least 4-fold, as compared to a control (such an amount of gene expression in a normal cell). In one example, a control is a relative amount of gene expression or protein expression in a PBMC in a subject who has not suffered an ischemic stroke.

Evaulating a stroke: To determine whether an ischemic stroke has occurred in a subject, to determine the severity of an ischemic stroke, to determine the likely neurological recovery of a subject who has had an ischemic stroke, or combinations thereof.

Expression: The process by which the coded information of a gene is converted into an operational, non-operational, or structural part of a cell, such as the synthesis of a protein. Gene expression can be influenced by external signals. For instance, exposure of a cell to a hormone may

stimulate expression of a hormone-induced gene. Different types of cells can respond differently to an identical signal. Expression of a gene also can be regulated anywhere in the pathway from DNA to RNA to protein. Regulation can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

The expression of a nucleic acid molecule can be altered relative to a normal (wild type) nucleic acid molecule. Alterations in gene expression, such as differential expression, includes but is not limited to: (1) overexpression; (2) underexpression; or (3) suppression of expression. Alternations in the expression of a nucleic acid molecule can be associated with, and in fact cause, a change in expression of the corresponding protein.

Protein expression can also be altered in some manner to be different from the expression of the protein in a normal (wild type) situation. This includes but is not necessarily limited to: (1) a mutation in the protein such that one or more of the amino acid residues is different; (2) a short deletion or addition of one or a few (such as no more than 10-20) amino acid residues to the sequence of the protein; (3) a longer deletion or addition of amino acid residues (such as at least 20 residues), such that an entire protein domain or sub-domain is removed or added; (4) expression of an increased amount of the protein compared to a control or standard amount; (5) expression of a decreased amount of the protein compared to a control or standard amount; (6) alteration of the subcellular localization or targeting of the protein; (7) alteration of the temporally regulated expression of the protein (such that the protein is expressed when it normally would not be, or alternatively is not expressed when it normally would be); (8) alteration in stability of a protein through increased longevity in the time that the protein remains localized in a cell; and (9) alteration of the localized (such as organ or tissue specific or subcellular localization) expression of the protein (such that the protein is not expressed where it would normally be expressed or is expressed where it normally would not be expressed), each compared to a control or standard. Controls or standards for comparison to a sample, for the determination of differential expression, include samples believed to be normal (in that they are not altered for the desired characteristic, for example a sample from a subject who has not had an ischemic stroke) as well as laboratory values, even though possibly arbitrarily set, keeping in mind that such values can vary from laboratory to laboratory.

Laboratory standards and values may be set based on a known or determined population value and can be supplied in the format of a graph or table that permits comparison of measured, experimentally determined values.

Fc fragment of IgG, high affinity Ia, receptor for (high affinity immunoglobulin G receptor Fc gamma RI, FcγRI): One of three classes of receptors for the Fc fragment of IgG (FcγR) that participates in immune complex clearance. Binding of ligand to FcγRI initiates multiple immune activation events, such as phagocytosis, expression of proinflammatory cytokines, and cytotoxicity against Ig-coated target cells. Also known in the art as CD64. The term FcγRI includes any FcγRI gene, cDNA, mRNA, or protein from any organism and that is a FcγRI that can function in

immune complex clearance. FcγRI sequences are publicly available. For example, GenBank Accession Nos: NM_000566 (nucleic acid) and CAI12557 (protein) and NP_000557 (protein) disclose human FcγRI sequences.

In one example, a FcγRI sequence includes a full-length wild-type (or native) sequence, as well as FcγRI allelic variants, variants, fragments, homologs or fusion sequences that retain the ability to function in immune complex clearance. In certain examples, FcγRI has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native FcγRI. In other examples, FcγRI has a sequence that hybridizes under very high stringency conditions to a sequence set forth in GenBank Accession No. NM_000566 and retains FcγRI activity.

Gene expression profile (or fingerprint): Differential or altered gene expression can be detected by changes in the detectable amount of gene expression (such as cDNA or mRNA) or by changes in the detectable amount of proteins expressed by those genes. A distinct or identifiable pattern of gene expression, for instance a pattern of high and low expression of a defined set of genes or gene-indicative nucleic acids such as ESTs; in some examples, as few as one or two genes provides a profile, but more genes can be used in a profile, for example at least 3, at least 4, at least 5, at least 10, at least 20, at least 25, at least 50, at least 80, at least 100, at least 190, at least 200, at least 300, at least 400, at least 500, at least 550, at least 600, or at least 630 or more. A gene expression profile (also referred to as a **fingerprint**) can be linked to a tissue or cell type (such as PBMCs), to a particular stage of normal tissue growth or disease progression (such as ischemic stroke), or to any other distinct or identifiable condition that influences gene expression in a predictable way. Gene expression profiles can include relative as well as absolute expression levels of specific genes, and can be viewed in the context of a test sample compared to a baseline or control sample profile (such as a sample from a subject who has not had an ischemic stroke). In one example, a gene expression profile in a subject is read on an array (such as a nucleic acid or protein array).

Hybridization: To form base pairs between complementary regions of two strands of DNA, RNA, or between DNA and RNA, thereby forming a duplex molecule. Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (such as the Na+ concentration) of the hybridization buffer will determine the stringency of hybridization. Calculations regarding hybridization conditions for attaining particular degrees of stringency are discussed in Sambrook *et al.*, (1989) Molecular Cloning, second edition, Cold Spring Harbor Laboratory, Plainview, NY (chapters 9 and 11). The following is an exemplary set of hybridization conditions and is not limiting:

<u>Very High Stringency (detects sequences that share 90% identity)</u>

Hybridization:	5x SSC at 65°C for 16 hours
Wash twice:	2x SSC at room temperature (RT) for 15 minutes each
Wash twice:	0.5x SSC at 65°C for 20 minutes each

High Stringency (detects sequences that share 80% identity or greater)

Hybridization: 5x-6x SSC at 65°C-70°C for 16-20 hours

Wash twice: 2x SSC at RT for 5-20 minutes each

Wash twice: 1x SSC at 55°C-70°C for 30 minutes each

Low Stringency (detects sequences that share greater than 50% identity)

Hybridization: 6x SSC at RT to 55°C for 16-20 hours

Wash at least twice: 2x-3x SSC at RT to 55°C for 20-30 minutes each.

Hypothetical protein FLJ22662 Laminin A motif: The term hypothetical protein FLJ22662 Laminin A motif sequence includes any hypothetical protein FLJ22662 Laminin A motif sequence gene, cDNA, mRNA, or protein from any organism and that is a hypothetical protein FLJ22662 Laminin A motif sequence. In particular examples, hypothetical protein FLJ22662 Laminin A motif is part of a basement membrane.

Hypothetical protein FLJ22662 Laminin A motif sequences are publicly available. For example, GenBank Accession Nos: BC063561 (nucleic acid), BC000909 (nucleic acid), AAH00909 (protein) and AAH63561 (protein) disclose human sequences. In one example, a hypothetical protein FLJ22662 Laminin A motif sequence includes a full-length wild-type (or native) sequence, as well as hypothetical protein FLJ22662 Laminin A motif allelic variants, variants, fragments, homologs or fusion sequences. In certain examples, CD163 has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native hypothetical protein FLJ22662 Laminin A motif. In other examples, hypothetical protein FLJ22662 Laminin A motif has a sequence that hybridizes under very high stringency conditions to a sequence set forth in GenBank Accession No. BC063561 or BC000909, and retains FLJ22662 activity.

Insertion: The addition of one or more nucleotides to a nucleic acid sequence, or the addition of one or more amino acids to a protein sequence.

Ischemic stroke: An ischemic stroke occurs when a blood vessel that supplies blood to the brain is blocked or narrowed (as contrasted with a hemorrhagic stroke which develops when an artery in the brain leaks or ruptures and causes bleeding inside the brain tissue or near the surface of the brain). The blockage can be a blood clot that forms or lodges inside the blood vessel (thrombus) or an object (such as an air bubble or piece of tissue) that moves through the blood from another part of the body (embolus).

Ischemic Stroke-related (or associated) molecule: A molecule whose expression is affected by an ischemic stroke. Such molecules include, for instance, nucleic acid sequences (such as DNA, cDNA, or mRNAs) and proteins. Specific examples include those listed in Tables 2-5, as well as fragments of the full-length genes, cDNAs, or mRNAs (and proteins encoded thereby) whose expression is altered (such as upregulated or downregulated) in response to an ischemic stroke.

Examples of ischemic stroke-related molecules whose expression is upregulated following an ischemic stroke include sequences involved in white blood cell activation and differentiation, sequences related to hypoxia, sequences involved in vascular repair, and sequences related to a specific PBMC response to the altered cerebral microenvironment, such as those genes listed in Table 5. Specific examples of ischemic stroke-related molecules whose expression is upregulated following an ischemic stroke include CD163; hypothetical protein FLJ22662 Laminin A motif; bone marrow stromal cell antigen 1 (also known as CD157); Fc fragment of IgG, high affinity Ia, receptor for (CD64); baculoviral IAP repeat-containing protein 1 (also known as neuronal apoptosis inhibitory protein); and KIAA0146, or any one of these.

Ischemic stroke-related molecules can be involved in or influenced by an ischemic stroke in different ways, including causative (in that a change in an ischemic stroke-related molecule leads to development of or progression to an ischemic stroke) or resultive (in that development of or progression to an ischemic stroke causes or results in a change in the ischemic stroke-related molecule).

Isolated: An "isolated" biological component (such as a nucleic acid molecule, protein, or cell) has been substantially separated or purified away from other biological components in the cell of the organism, or the organism itself, in which the component naturally occurs, such as other chromosomal and extra-chromosomal DNA and RNA, proteins and cells. Nucleic acid molecules and proteins that have been "isolated" include nucleic acid molecules and proteins purified by standard purification methods. The term also embraces nucleic acid molecules and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acid molecules and proteins. For example, an isolated cell, such as an isolated PBMC is one that is substantially separated from other cells, such as other blood cells.

KIAA0146: The term KIAA0146 includes any KIAA0146 gene, cDNA, mRNA, or protein from any organism and that is a KIAA0146 sequence. KIAA0146 sequences are publicly available. For example, GenBank Accession Nos: AAH15561 (protein), BAA09767 (protein), D63480 (nucleic acid), and BC015561 (nucleic acid) disclose human KIAA0146 sequences.

In one example, a KIAA0146 sequence includes a full-length wild-type (or native) sequence, as well as KIAA0146 allelic variants, variants, fragments, homologs or fusion sequences. In certain examples, KIAA0146 has at least 80% sequence identity, for example at least 85%, 90%, 95%, or 98% sequence identity to a native KIAA0146. In other examples, KIAA0146 has a sequence that hybridizes under very high stringency conditions to a sequence set forth in GenBank Accession No. D63480 or BC015561, and retains KIAA0146 activity.

Label: An agent capable of detection, for example by ELISA, spectrophotometry, flow cytometry, or microscopy. For example, a label can be attached to a nucleic acid molecule or protein, thereby permitting detection of the nucleic acid molecule or protein. Examples of labels include, but are not limited to, radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent agents, fluorophores, haptens, enzymes, and combinations thereof. Methods for labeling and guidance in the choice of labels appropriate for various purposes are discussed for example in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York, 1989)

and Ausubel *et al.* (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1998).

Neurological sequelae: Any abnormality of the nervous system (such as the central nervous system) following or resulting from a disease or injury or treatment, for example following an ischemic stroke.

Nucleic acid array: An arrangement of nucleic acids (such as DNA or RNA) in assigned locations on a matrix, such as that found in cDNA arrays, or oligonucleotide arrays.

Nucleic acid molecules representing genes: Any nucleic acid, for example DNA (intron or exon or both), cDNA, or RNA (such as mRNA), of any length suitable for use as a probe or other indicator molecule, and that is informative about the corresponding gene.

Nucleic acid molecules: A deoxyribonucleotide or ribonucleotide polymer including, without limitation, cDNA, mRNA, genomic DNA, and synthetic (such as chemically synthesized) DNA. The nucleic acid molecule can be double-stranded or single-stranded. Where single-stranded, the nucleic acid molecule can be the sense strand or the antisense strand. In addition, nucleic acid molecule can be circular or linear.

The disclosure includes isolated nucleic acid molecules that include specified lengths of an ischemic stroke-related nucleotide sequence, for example those listed in Tables 2-5. Such molecules can include at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45 or at least 50 consecutive nucleotides of these sequences or more, and can be obtained from any region of an ischemic stroke-related nucleic acid molecule.

Nucleotide: Includes, but is not limited to, a monomer that includes a base linked to a sugar, such as a pyrimidine, purine or synthetic analogs thereof, or a base linked to an amino acid, as in a peptide nucleic acid (PNA). A nucleotide is one monomer in a polynucleotide. A nucleotide sequence refers to the sequence of bases in a polynucleotide.

Oligonucleotide: A plurality of joined nucleotides joined by native phosphodiester bonds, between about 6 and about 300 nucleotides in length. An oligonucleotide analog refers to moieties that function similarly to oligonucleotides but have non-naturally occurring portions. For example, oligonucleotide analogs can contain non-naturally occurring portions, such as altered sugar moieties or inter-sugar linkages, such as a phosphorothioate oligodeoxynucleotide.

Particular oligonucleotides and oligonucleotide analogs can include linear sequences up to about 200 nucleotides in length, for example a sequence (such as DNA or RNA) that is at least 6 nucleotides, for example at least 8, at least 10, at least 15, at least 20, at least 21, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 100 or even at least 200 nucleotides long, or from about 6 to about 50 nucleotides, for example about 10-25 nucleotides, such as 12, 15 or 20 nucleotides.

Oligonucleotide probe: A short sequence of nucleotides, such as at least 8, at least 10, at least 15, at least 20, at least 21, at least 25, or at least 30 nucleotides in length, used to detect the presence of a complementary sequence by molecular hybridization. In particular examples, oligonucleotide probes include a label that permits detection of oligonucleotide probe:target sequence hybridization complexes.

Open reading frame (ORF): A series of nucleotide triplets (codons) coding for amino acids without any internal termination codons. These sequences are usually translatable into a peptide.

Peripheral blood mononuclear cells (PBMCs): Cells present in the blood that have one round nucleus. Examples include lymphocytes, monocytes, and natural killer cells. PBMCs do not include neutrophils, eosinophils or basophils.

Primers: Short nucleic acid molecules, for instance DNA oligonucleotides 10 -100 nucleotides in length, such as about 15, 20, 25, 30 or 50 nucleotides or more in length. Primers can be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand. Primer pairs can be used for amplification of a nucleic acid sequence, such as by PCR or other nucleic acid amplification methods known in the art.

Methods for preparing and using nucleic acid primers are described, for example, in Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual*, CSHL, New York, 1989), Ausubel *et al.* (ed.) (In *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998), and Innis *et al.* (*PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990). PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA). One of ordinary skill in the art will appreciate that the specificity of a particular primer increases with its length. Thus, for example, a primer including 30 consecutive nucleotides of an ischemic stroke-related nucleotide molecule will anneal to a target sequence, such as another homolog of the designated ischemic stroke-related protein, with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, primers can be selected that include at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50 or more consecutive nucleotides of a ischemic stroke-related nucleotide sequence.

Purified: The term "purified" does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified protein preparation is one in which the protein referred to is more pure than the protein in its natural environment within a cell. For example, a preparation of a protein is purified such that the protein represents at least 50% of the total protein content of the preparation. Similarly, a purified oligonucleotide preparation is one in which the oligonucleotide is more pure than in an environment including a complex mixture of oligonucleotides. In addition, a purified cell, such as a purified PBMC, is one that is substantially separated from other cells, such as other blood cells. In one example, purified PBMCs are at least 90% pure, such as at least 95% pure, or even at least 99% pure.

Recombinant: A recombinant nucleic acid molecule is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or by the artificial manipulation of isolated segments of nucleic acid molecules, such as by genetic engineering techniques.

Sample: A biological specimen containing genomic DNA, RNA (including mRNA), protein, or combinations thereof, obtained from a subject. Examples include, but are not limited to, peripheral blood, urine, saliva, tissue biopsy, surgical specimen, amniocentesis samples and autopsy material. In one example, a sample includes peripheral blood mononuclear cells (PBMCs).

Sequence identity/similarity: The identity/similarity between two or more nucleic acid sequences, or two or more amino acid sequences, is expressed in terms of the identity or similarity between the sequences. Sequence identity can be measured in terms of percentage identity; the higher the percentage, the more identical the sequences are. Sequence similarity can be measured in terms of percentage similarity (which takes into account conservative amino acid substitutions); the higher the percentage, the more similar the sequences are. Homologs or orthologs of nucleic acid or amino acid sequences possess a relatively high degree of sequence identity/similarity when aligned using standard methods. This homology is more significant when the orthologous proteins or cDNAs are derived from species which are more closely related (such as human and mouse sequences), compared to species more distantly related (such as human and *C. elegans* sequences).

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman & Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson & Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins & Sharp, *Gene*, 73:237-44, 1988; Higgins & Sharp, *CABIOS* 5:151-3, 1989; Corpet *et al.*, *Nuc. Acids Res.* 16:10881-90, 1988; Huang *et al. Computer Appls. in the Biosciences* 8, 155-65, 1992; and Pearson *et al.*, *Meth. Mol. Bio.* 24:307-31, 1994. Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403-10, 1990) is available from several sources, including the National Center for Biological Information (NCBI, National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. Additional information can be found at the NCBI web site.

BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. To compare two nucleic acid sequences, the options can be set as follows: -i is set to a file containing the first nucleic acid sequence to be compared (such as C:\seq1.txt); -j is set to a file containing the second nucleic acid sequence to be compared (such as C:\seq2.txt); -p is set to blastn; -o is set to any desired file name (such as C:\output.txt); -q is set to –1; -r is set to 2; and all other options are left at their default setting. For example, the following command can be used to generate an output file containing a comparison between two sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastn –o c:\output.txt –q –1 –r 2.

To compare two amino acid sequences, the options of Bl2seq can be set as follows: -i is set to a file containing the first amino acid sequence to be compared (such as C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (such as C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (such as C:\output.txt); and all other options are left at their

default setting. For example, the following command can be used to generate an output file containing a comparison between two amino acid sequences: C:\Bl2seq –i c:\seq1.txt –j c:\seq2.txt –p blastp –o c:\output.txt. If the two compared sequences share homology, then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology, then the designated output file will not present aligned sequences.

Once aligned, the number of matches is determined by counting the number of positions where an identical nucleotide or amino acid residue is presented in both sequences. The percent sequence identity is determined by dividing the number of matches either by the length of the sequence set forth in the identified sequence, or by an articulated length (such as 100 consecutive nucleotides or amino acid residues from a sequence set forth in an identified sequence), followed by multiplying the resulting value by 100. For example, a nucleic acid sequence that has 1166 matches when aligned with a test sequence having 1154 nucleotides is 75.0 percent identical to the test sequence (1166÷1554*100=75.0). The percent sequence identity value is rounded to the nearest tenth. For example, 75.11, 75.12, 75.13, and 75.14 are rounded down to 75.1, while 75.15, 75.16, 75.17, 75.18, and 75.19 are rounded up to 75.2. The length value will always be an integer. In another example, a target sequence containing a 20-nucleotide region that aligns with 20 consecutive nucleotides from an identified sequence as follows contains a region that shares 75 percent sequence identity to that identified sequence (that is, 15÷20*100=75).

```
                          1                  20
Target Sequence:          AGGTCGTGTACTGTCAGTCA
                          | ||  |||  ||||  |||| |
Identified Sequence:      ACGTGGTGAACTGCCAGTGA
```

For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). Homologs are typically characterized by possession of at least 70% sequence identity counted over the full-length alignment with an amino acid sequence using the NCBI Basic Blast 2.0, gapped blastp with databases such as the nr or swissprot database. Queries searched with the blastn program are filtered with DUST (Hancock and Armstrong, 1994, *Comput. Appl. Biosci.* 10:67-70). Other programs use SEG. In addition, a manual alignment can be performed. Proteins with even greater similarity will show increasing percentage identities when assessed by this method, such as at least about 75%, 80%, 85%, 90%, 95%, 98%, or 99% sequence identity.

When aligning short peptides (fewer than around 30 amino acids), the alignment is be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequence will show increasing percentage identities when assessed by this method, such as at least about 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs will typically possess at least 75% sequence identity over short windows of 10-20 amino acids, and can possess sequence identities of at least 85%, 90%,

95% or 98% depending on their identity to the reference sequence. Methods for determining sequence identity over such short windows are described at the NCBI web site.

One indication that two nucleic acid molecules are closely related is that the two molecules hybridize to each other under stringent conditions, as described above. Nucleic acid sequences that do not show a high degree of identity may nevertheless encode identical or similar (conserved) amino acid sequences, due to the degeneracy of the genetic code. Changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid molecules that all encode substantially the same protein. Such homologous nucleic acid sequences can, for example, possess at least about 60%, 70%, 80%, 90%, 95%, 98%, or 99% sequence identity determined by this method. An alternative (and not necessarily cumulative) indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

One of skill in the art will appreciate that the particular sequence identity ranges are provided for guidance only; it is possible that strongly significant homologs could be obtained that fall outside the ranges provided.

Sequences involved in (or related to) white blood cell activation and differentiation: Nucleic acid molecules (such as mRNA, cDNA, gene) and the corresponding protein, whose expression is altered (such as upregulated or downregulated) in connection with the specialization, mobilization, or proliferation of white blood cells, or combinations thereof, for example sequences that are differentially expressed to cause (or are differentially expressed as a result of) specialization, mobilization, and/or proliferation of white blood cells.

Exemplary sequences involved in white blood cell activation and differentiation include genes involved in cell adhesion, enzymes involved in the cell membrane remodeling allowing preparation for change to a more differentiated state, and genes related to cell-cell interactions. Particular examples include, but are not limited to, CD163; hypothetical protein FLJ22662 Laminin A motif; amyloid beta (A4) precursor-like protein 2; N-acetylneuraminate pyruvate lysase; v-fos FBJ murine osteosarcoma viral oncogene homolog; toll-like receptor 2; chondroitin sulfate proteoglycan 2 (versican); interleukin 13 receptor, alpha 1; CD14 antigen; bone marrow stromal cell antigen 1 (also known as CD157); complement component 1, q subcomponent, receptor 1; and paired immunoglobin-like type 2 receptor alpha; and Fc fragment of IgG, high affinity Ia, receptor for (CD64).

Sequences involved in (or related to) hypoxia: Nucleic acid molecules (such as mRNA, cDNA, gene) and the corresponding protein, whose expression is altered (such as upregulated or downregulated) in response to decreased available oxygen in the blood and tissues. For example, the brain is hypoxic following an ischemic stroke. Particular examples include, but are not limited to, adrenomedullin; dual specificity phosphatase 1; cytochrome b-245, beta polypeptide (chronic granulomatous disease); ;eukotriene A4 hydrolase; erythroblastosis virus E26 oncogene homolog 2 (avian); and neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2).

Sequences involved in (or related to) vascular repair: Nucleic acid molecules (such as mRNA, cDNA, gene) and the corresponding protein, whose expression is altered (such as upregulated or downregulated) in response to injury to a blood vessel. Particular examples include, but are not limited to, thrombomodulin; ectonucleoside triphosphate diphosphohydrolase 1; and CD36 antigen (collagen type I receptor, thrombospondin receptor).

Sequences involved in (or related to) a specific PBMC response to the altered cerebral microenvironment: Nucleic acid molecules (such as mRNA, cDNA, gene) and the corresponding protein, whose expression is altered (such as upregulated or downregulated) in PBMCs in response to changes in the brain microenvironment.

Examples include those potentially associated with enhanced neurotransmitter degradation (such as catechol-o-methyl transferase and glutamine ligase), those that permit increased modulation of Ca^{2+} homeostasis in the cerebral environment, genes involved in the inhibition of neuronal apoptosis (such as the neuronal apoptosis inhibitory protein and Ets2), genes involved in proNGF-induced neuronal cell death (such as sortilin), genes involved in apoptotic cell death in the hippocampus after global cerebral ischemic injury (such as phospholipid scramblase 1), and genes involved in neurite growth in neuronal development (such as growth arrest-specific 7).

Particular examples include, but are not limited to, catechol-O-methyltransferase; glutamate-ammonia ligase (glutamine ligase); S100 calcium binding protein A8 (calgranulin A); neuronal apoptosis inhibitory protein: Homo sapiens transcribed sequence with strong similarity to protein sp:Q13075 (H.sapiens) BIR1_HUMAN Baculoviral IAP repeat-containing protein 1; sortilin; phospholipid scramblase 1; growth-arrest-specific 7; and GLI pathogenesis-related 1 (glioma).

Subject: Living multi-cellular vertebrate organisms, a category that includes human and non-human mammals.

Target sequence: A sequence of nucleotides located in a particular region in the human genome that corresponds to a desired sequence, such as ischemic stroke related sequence. The target can be for instance a coding sequence; it can also be the non-coding strand that corresponds to a coding sequence. Examples of target sequences include those sequences associated with ischemic stroke, such as any of those listed in Tables 2-5.

Therapeutically effective amount: An amount of a pharmaceutical preparation that alone, or together with a pharmaceutically acceptable carrier or one or more additional therapeutic agents, induces the desired response. A therapeutic agent, such as an anticoagulant or a thrombolytic agent, is administered in therapeutically effective amounts.

Effective amounts a therapeutic agent can be determined in many different ways, such as assaying for a reduction in atherosclerotic disease or improvement of physiological condition of a subject having vascular disease. Effective amounts also can be determined through various *in vitro*, *in vivo* or *in situ* assays.

Therapeutic agents can be administered in a single dose, or in several doses, for example daily, during a course of treatment. However, the effective amount of can be dependent on the source

applied, the subject being treated, the severity and type of the condition being treated, and the manner of administration.

In one example, it is an amount sufficient to partially or completely alleviate symptoms of ischemic stroke within a subject. Treatment can involve only slowing the progression of the ischemic stroke temporarily, but can also include halting or reversing the progression of the ischemic stroke permanently. For example, a pharmaceutical preparation can decrease one or more symptoms of ischemic stroke, for example decrease a symptom by at least 20%, at least 50%, at least 70%, at least 90%, at least 98%, or even at least 100%, as compared to an amount in the absence of the pharmaceutical preparation.

Thrombolytics: Agents that promote lysis of thrombi that occlude a cerebral vessel. Examples include, but are not limited to, tissue plasminogen activator (tPA), urokinase, and pro-urokinase. Administration of antithrombotics is one treatment for ischemic stroke, and is often a first line treatment for ischemic stroke. For example, intravenous t-PA can be administered within 3 hours of ischemic stroke onset. Intra-arterial thrombolytic therapy and mechanical clot-retrieval devices can be used to promote rapid lysis of thrombi.

Treating a disease: "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition, such a sign or symptom of vascular disease. Treatment can also induce remission or cure of a condition, such as an ischemic stroke. In particular examples, treatment includes preventing a disease, for example by inhibiting the full development of a disease, such as preventing development of a disease or disorder that results from an ischemic stroke. Prevention of a disease does not require a total absence of disease. For example, a decrease of at least 50% can be sufficient.

Upregulated or activation: When used in reference to the expression of a nucleic acid molecule, such as a gene, refers to any process which results in an increase in production of a gene product. A gene product can be RNA (such as mRNA, rRNA, tRNA, and structural RNA) or protein. Therefore, gene upregulation or activation includes processes that increase transcription of a gene or translation of mRNA.

Examples of processes that increase transcription include those that facilitate formation of a transcription initiation complex, those that increase transcription initiation rate, those that increase transcription elongation rate, those that increase processivity of transcription and those that relieve transcriptional repression (for example by blocking the binding of a transcriptional repressor). Gene upregulation can include inhibition of repression as well as stimulation of expression above an existing level. Examples of processes that increase translation include those that increase translational initiation, those that increase translational elongation and those that increase mRNA stability.

Gene upregulation includes any detectable increase in the production of a gene product. In certain examples, production of a gene product increases by at least 2-fold, for example at least 3-fold or at least 4-fold, as compared to a control (such an amount of gene expression in a normal cell). In

one example, a control is a relative amount of gene expression in a PBMC in a subject who has not suffered an ischemic stroke.

Ischemic Stroke-Related Molecules

The inventors have identified 22-637 genes whose expression is altered (such as upregulated or downregulated) following an ischemic stroke. The number of genes identified depended on the specificity and sensitivity of the algorithm used. For example, using the PAM dataset 22 ischemic stroke related genes were identified (Table 5), using the Westfall and Young dataset 82 ischemic stroke related genes were identified (Table 4), using the Bonferroni correction set 190 ischemic stroke related genes were identified (Table 3), and using the Benjamini & Yekutieli set 637 ischemic stroke related genes were identified (Table 2). One skilled in the art will appreciate that changes in protein expression can be detected as an alternative to detecting gene expression.

Several genes not previously associated with ischemic stroke were identified, such as at least CD163; hypothetical protein FLJ22662 Laminin A motif; bone marrow stromal cell antigen 1 (BST-1, also referred to in the literature as CD157); Fc fragment of IgG, high affinity Ia, receptor for (FcγRI, also known as CD64); baculoviral IAP repeat-containing protein 1; and KIAA0146. In particular examples, all of these genes were upregulated following an ischemic stroke. In one example, four classes of genes whose expression was upregulated following an ischemic stroke were identified: sequences involved in activation and differentiation of white blood cells, sequences related to hypoxia, sequences involved in vascular repair, and sequences related to altered cerebral microenvironment. Particular examples of such genes (and their corresponding proteins) are provided in Table 5.

Based on the identification of these ischemic stroke-related molecules, methods were developed to evaluate a stroke. For example, the disclosed methods can be used to diagnose an ischemic stroke, determine the severity of an ischemic stroke, determine the likely neurological recovery of a subject who had an ischemic stroke, or combinations thereof. The method can further include determining an appropriate therapy for a subject found to have experienced an ischemic stroke using the disclosed assays.

The disclosed methods provide a rapid, straightforward, and accurate genetic screening method performed in one assay for evaluating ischemic stroke. It allows identification of subjects who may require anticoagulant therapy following an ischemic stroke. For example, by establishing that an individual has had an ischemic stroke, effective therapeutic measures, such as the emergent administration of a thrombolytic agent or of treatments to prevent stroke recurrence and extension, can be instituted.

Evaluation of an Ischemic Stroke

Provided herein are methods of evaluating a stroke. Particular examples of evaluating a stroke include determining whether a subject, such as an otherwise healthy subject, or a subject suspected or at risk of having an ischemic stroke, has had an ischemic stroke, assessing the severity

of an ischemic stroke, predicting the likelihood of neurological recovery of a subject who has had an ischemic stroke, or combinations thereof. The identification of a subject who has had an ischemic stroke can help to evaluate other clinical data (such as neurological impairment or brain imaging information) to determine whether an ischemic stroke has occurred. In particular examples, the method can determine with a reasonable amount of sensitivity and specificity whether a subject has suffered an ischemic stroke within the previous 72 hours, such as within the previous 48 hours, previous 24 hours, or previous 12 hours. In some examples, isolated or purified PBMCs obtained from the subject are used to determine whether a subject has had an ischemic stroke.

In particular examples, the method also includes administering an appropriate treatment therapy for subjects who have had an ischemic stroke. For example, subjects identified or evaluated as having had an ischemic stroke can then be provided with appropriate treatments, such as anti-platelet agents (for example aspirin) that would be appropriate for a subject identified as having had an ischemic stroke but not as appropriate for a subject who has had a hemorrhagic stroke. It is helpful to be able to classify a subject as having had an ischemic stroke, because the treatments for ischemic stroke are often distinct from the treatments for hemorrhagic stroke. In fact, treating a hemorrhagic stroke with a therapy designed for an ischemic stroke (such as a thrombolytic agent) can have devastating clinical consequences. Hence using the results of the disclosed assays to help distinguish ischemic from hemorrhagic stroke offers a substantial clinical benefit, and allows subjects to be selected for treatments appropriate to ischemic stroke but not hemorrhagic stroke.

In particular examples, methods of evaluating a stroke involve detecting differential expression (such as an increase or decrease in gene or protein expression) in any combination of at least four ischemic stroke-related molecules of the subject, such as any combination of at least four of the genes (or proteins) listed in any of Tables 2-5. In one example, the method includes screening expression of one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, or a combination of ischemic stroke-related molecules that includes at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 of these molecules. For example, the method can include screening expression of CD163, along with other ischemic stroke-related molecules (such as any combination that includes at least 3 additional molecules listed in Tables 2-5).

Differential expression can be represented by increased or decreased expression in the at least one ischemic stroke-related molecule (for instance, a nucleic acid or a protein). For example, differential expression includes, but is not limited to, an increase or decrease in an amount of a nucleic acid molecule or protein, the stability of a nucleic acid molecule or protein, the localization of a nucleic acid molecule or protein, or the biological activity of a nucleic acid molecule or protein. Specific examples include evaulative methods in which changes in gene expression in at least four ischemic stroke-related nucleic acid molecules (or corresponding protein) are detected (for example nucleic acids or proteins obtained from a subject thought to have had or known to have had an ischemic stroke), such as changes in gene (or protein) expression in any combination of at least 5, at least 10, at least 15, at least 20, at least 25, at least 50, at least 100, at least 150, at least 160, at least

170, at least 175, at least 180, at least 185, at least 200, at least 250, at least 300, at least 400, at least 500, at least 510, at least 550, at least 575, at least 600, at least 620, at least 630, or at least 637 ischemic stroke-related molecules. Exemplary ischemic stroke-related molecules are provided in Tables 2-5.

In particular examples a change in expression is detected in a subset of ischemic stroke-related molecules (such as nucleic acid sequences or protein sequences) that selectively evaluate a stroke, for example to determine if a subject has had an ischemic stroke. In a particular example, the subset of molecules can include a set of any combination of four ischemic stroke-related genes listed in Table 5, or a set of any combination of 22 ischemic stroke-related genes listed in Table 5. In a particular example, the subset of molecules includes any combination of at least one gene (or protein) from each class of the four classes listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each class listed in Table 5.

In a particular example, differential expression is detected in ischemic stroke-related molecules that are both upregulated and down regulated. For example, increased expression of one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, and decreased gene (or protein) expression of one or more of intercellular adhesion molecule 2, protein kinase D2, GATA binding protein 3, hypothetical protein FLJ20257, or protein kinase C, theta, indicates that the subject has had a stroke, has had a severe stroke, has a lower likelihood of neurological recovery, or combinations thereof. For example, differential expression can be detected by determining if if the subject has increased gene (or protein) expression of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, and determining if the subject has decreased gene (or protein) expression of intercellular adhesion molecule 2, protein kinase D2, GATA binding protein 3, hypothetical protein FLJ20257, and protein kinase C, theta.

In particular examples, the number of ischemic stroke-related genes screened is at least 5, at least 10, at least 15, at least 20, at least 25, at least 50, at least 100, at least 150, at least 160, at least 170, at least 175, at least 180, at least 185, at least 200, at least 250, at least 300, at least 400, at least 500, at least 510, at least 550, at least 575, at least 600, at least 620, at least 630, or at least 637 ischemic stroke-related molecules. In other examples, the methods employ screening no more than 637, no more than 630, no more than 620, no more than 600, no more than 575, no more than 550, no more than 510, no more than 500, no more than 400, no more than 300, no more than 250, no more than 200, no more than 185, no more than 180, no more than 175, no more than 170, no more than 160, no more than 150, no more than 100, no more than 50, no more than 25, no more than 20, no more than 15, no more than 10, no more than 5, or no more than 4 ischemic stroke-related genes. Examples of particular ischemic stroke-related genes are shown in Tables 2-5. In one example, the number of ischemic stroke-related genes screened includes at least one gene from each class listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each class listed in Table 5.

In certain methods, differential expression includes over- or under-expression of an ischemic stroke-related molecule. For instance, differential expression can include overexpression, for

instance overexpression of any combination of at least 4 molecules (such at least 10 or at least 20 molecules) shown in Table 5, or any combination of at least 4 molecules in any of Tables 2-4 with a positive t-statistic value, such as a t-statistic value of at least 3, such as at least 3.5, at least 3.6 or even at least 3.7. In a particular example, differential expression includes overexpression of any combination of at least one gene from each class listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each of the classes listed in Table 5. In another example, differential expression includes underexpression, for instance underexpression of any combination of at least 5 molecules (such at least 50 or at least 150 molecules) shown in Tables 2-4 with a negative t-statistic value, such as a t-statistic value of less than -3, such as less than - 3.5, less than -3.6 or even less than - 3.7. In a specific example, differential expression includes any combination of underexpression or overexpression of at least 4 ischemic stroke-related molecules shown in Tables 2-4, such as overexpression of at least 3 ischemic stroke-related molecules shown in Tables 2-5 with a positive t-statistic value and underexpression of at least one ischemic stroke related molecule shown in Tables 2-4 with a negative t-statistic value, or for example overexpression of at least 4 ischemic stroke-related molecules shown in Tables 2-5 with a positive t-statistic value, or for example, overexpression of at least 2 ischemic stroke-related molecules shown in Tables 2-5 with a positive t-statistic value and underexpression of at least 2 ischemic stroke related molecules shown in Tables 2-4 with a negative t-statistic value.

In some examples, differential expression of proteins that are associated with ischemic stroke includes detecting patterns of such expression, such as detecting upregulation of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, detecting downregulation of intercellular adhesion molecule 2, protein kinase D2, GATA binding protein 3, hypothetical protein FLJ20257, or protein kinase C, theta, or combinations thereof. For example, detecting upregulation or downreguation can include a magnitude of change of at least 25%, at least 50%, at least 100%, or even at least 200%, such as a magnitude of change of at least 25% for CD163; at least 25% for hypothetical protein FLJ22662 Laminin A motif; at least 25% for BST-1; FcγRI; at least 25% for baculoviral IAP repeat-containing protein 1; at least 25% for KIAA0146; at least 25% for intercellular adhesion molecule 2; at least 25% for protein kinase D2; at least 25% for GATA binding protein 3; at least 25% for hypothetical protein FLJ20257; and at least 25% for protein kinase C, theta. Alternatively, upregulation is detected by a level having a t-value of at least 3 and downregulation is detected by a level having a t-value value of no more than -3.

In particular examples, the disclosed method of evaluating a stroke is at least 78% sensitive (such as at least 80% sensitive, at least 85% sensitive, at least 90% sensitive, or at least 95% sensitive) and at least 80% specific (such as at least 85% specific, at least 90% specific, at least 95% specific, or at least 98% specific) for determining whether a subject has had an ischemic stroke.

As used herein, the term "ischemic stroke-related molecule" includes ischemic stroke-related nucleic acid molecules (such as DNA, RNA, for example cDNA or mRNA) and ischemic stroke-related proteins. The term is not limited to those molecules listed in Tables 2-5 (and

molecules that correspond to those listed), but also includes other nucleic acid molecules and proteins that are influenced (such as to level, activity, localization) by or during an ischemic stroke, including all of such molecules listed herein. Examples of particular ischemic stroke-related genes are listed in Tables 2-5, such as CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146. In examples where the ischemia-related molecule is an ischemia -related nucleic acid sequence, methods of detecting differential expression can include *in vitro* nucleic acid amplification, nucleic acid hybridization (which can include quantified hybridization), RT-PCR, real time PCR, or combinations thereof. In examples where the ischemia-related molecule is an ischemia -related protein sequence, methods of detecting differential expression can include *in vitro* hybridization (which can include quantified hybridization) such as hybridization to a protein-specific binding agent for example an antibody, quantitative spectroscopic methods (for example mass spectrometry, such as surface-enhanced laser desorption/ionization (SELDI)-based mass spectrometry) or combinations thereof.

In particular examples, methods of evaulating a subject who has had or is thought to have had an ischemic stroke includes determining a level of expression (for example in a PBMC) of any combination of at least 4 of the genes (or proteins) listed in Tables 2-5, such as at least 10, at least 15, at least 20, or at least 22 of the genes listed in Table 5, such as at least 150, at least 180, or at least 185 of the gene listed in Table 3, or any combination of at least 500, at least 600, or at least 630 of the genes listed in Table 2. In one example, the method includes determining a level of expression of at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146, or any combination of ischemic stroke related molecules that includes 1, 2, 3, 4, 5, or 6 of these molecules. In one example, the method includes determining a level of expression of at least one gene from each class listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each class.

Methods of evaluating a stroke can include diagnosing a stroke, stratifying the seriousness of a cerebral ischemic event, and predicting neurological recovery. Similarly, methods of evaluating a stroke can include determining the severity of a stroke, predicting neurological recovery, or combinations thereof. For example, a change in expression in any combination of at least 4 of the genes listed in Tables 2-5 indicates that the subject has had an ischemic stroke. For example, an increase in expression in one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146 in particular examples indicates that the subject has had an ischemic stroke.

Determining the level of expression can involve measuring an amount of the ischemia-related molecules in a sample derived from the subject, such as a purified PBMC sample. Such an amount can be compared to that present in a control sample (such as a sample derived from a subject who has not had an ischemic stroke or a standard ischemic stroke-related molecule level in analogous samples from a subject not having ischemia or not having a predisposition developing ischemia), wherein a difference (such as an increase or a decrease reflecting an upregulation or downregulation, respectively) in the level of any combination of at least 4 ischemia-related molecules listed in Tables

2-5, such as any combination of at least 4 ischemia-related molecules listed in Table 5, in the subject relative to the control sample is diagnostic for ischemic stroke.

In other examples, the method includes determining a level of expression of any combination of at least four sequences listed in Table 5, such as at least 10, or at least 22 of the sequences listed in Table 5, for example at least 150 of the genes listed in Table 3, such as at least 160, at least 170, at least 175, at least 180, or at least 185 of the genes listed in Table 3, or at least 500 of the ischemic stroke-related molecules listed in Table 2, such as at least 600 of the ischemic stroke-related molecules listed in Table 2. A change in expression in at least four genes listed in Table 5 (or the corresponding proteins), such as at least 22 of the genes (or the corresponding proteins) listed in Table 5, such as 150 or more of the genes listed in Table 3 (or the corresponding proteins), such as 500 or more more of the genes listed in Table 2 (or the corresponding proteins, indicates that the subject has had a more severe stroke, has a higher risk of long term adverse neurological sequelae, or combinations thereof, than a subject having a change in expression in less than 500 of the molecules listed in Table 3, less than 150 of the molecules listed in Table 3 or less than 22 (or less than four) of the molecules listed in Table 5. Determining the level of expression can involve measuring an amount of the ischemia-related molecules in a sample derived from the subject. Such an amount can be compared to that present in a control sample (such as a sample derived from a subject who has not had an ischemic stroke or a sample derived from the subject at an earlier time), wherein a difference (such as an increase or a decrease reflecting an upregulation or downregulation, respectively) in the level of at least 4 or at least 22 of the ischemia-related molecules listed in Table 5 (such as at least 150 of the ischemia-related molecules listed in Table 3 or such as at least 500 of the ischemia-related molecules listed in Table 2) in the subject relative to the control sample indicates that the subject has had a more severe stroke, has a higher risk of long term adverse neurological sequelae, or both.

The disclosed methods can further include administering to the subject an appropriate treatment to avoid or reduce ischemic injury, if the presence of differential expression indicates that the subject has had an ischemic stroke. Since the results of the disclosed assays are reliable predictors of the ischemic nature of the stroke, the results of the assay can be used (alone or in combination with other clinical evidence and brain scans) to determine whether thrombolytic therapy designed to lyse a neurovascular occlusion such as a thrombus (for example by using tissue plasminogen activator or streptokinase) should be administered to the subject. In certain example, thrombolytic therapy is given to the subject once the results of the differential gene assay are known if the assay provides an indication that the stroke is ischemic in nature. Such methods can reduce brain damage following an ischemic stroke.

In particular examples, the method includes determining if there is an alteration in the expression of at least four sequences listed in Table 5, such as at least 10, or at least 22 of the sequences listed in Table 5, for example at least 150 of the genes listed in Table 3, such as at least 160, at least 170, at least 175, at least 180, or at least 185 of the genes listed in Table 3, or at least 500 of the ischemic stroke-related molecules listed in Table 2, such as at least 600 of the ischemic stroke-related molecules listed in Table 2. In some examples, detecting differential expression of at least 4

ischemic stroke-related molecules involves quantitatively or qualitatively analyzing a DNA, mRNA, cDNA, protein, or combinations thereof.

If differenential expression is detected in at least four, at least 22, at least 150, or at least 500 ischemic stroke-related molecules is identified, this indicates that the subject has experienced an ischemic stroke (and not a hemorrhagic stroke), and a treatment is selected to prevent or reduce brain damage or to provide protection from the onset of brain damage. Examples of such treatment include administration of an anticoagulant, an antithrombotic, or combinations thereof. A particular example includes administration of a thrombolytic agent such as t-PA to lyse the blood clot, alone or in combination with one or more agents that prevent further strokes, such as anticoagulants (such as antiplatelet agents), antihypertensive agents, or lipid lowering agents. In particular examples, the level of expression of a protein in a subject can be appropriately increased or decreased by expressing in the subject a recombinant genetic construct that includes a promoter operably linked to a nucleic acid molecule, wherein the nucleic acid molecule includes at least 10 consecutive nucleotides of an ischemic stroke-related nucleic acid sequence (such as any of the sequences listed in Tables 2-5). Expression of the nucleic acid molecule will change expression of the ischemic stroke-related protein. The nucleic acid molecule can be in an antisense orientation relative to the promoter or in sense orientation relative to the promoter. In some examples, the recombinant genetic construct expresses an ssRNA corresponding to an ischemic stroke-related nucleic acid sequence.

In examples of the methods described herein, detecting differential expression of at least four ischemic stroke-related molecules involves determining whether a gene expression profile from the subject indicates development or progression of brain injury.

In particular examples, the disclosed methods are performed following the onset of signs and symptoms associated with ischemic stroke. Examples of such symptoms include, but are not limited to headache, sensory loss (such as numbness, particularly confined to one side of the body or face), paralysis (such as hemiparesis), pupillary changes, blindness (including bilateral blindness), ataxia, memory impairment, dysarthria, somnolence, and other effects on the central nervous system recognized by those of skill in the art. In particular examples, the method of evaluating a stroke is performed after a sufficient period of time for the differential regulation of the genes (or proteins) to occur, for example at least 24 hours after onset of the symptom or constellation of symptoms that have indicated a potential cerebral ischemic event. In other examples, the method is performed prior to performing any diagnostics imaging tests (such as those that can find anatomic evidence of ischemic stroke). For example, it can be difficult for imaging modalities (such as CT and MRI) to detect acute ischemic strokes, at least until brain changes (such as edema) have taken place in response to the ischemia. Hence the assay described herein is able to detect the stroke even before definitive brain imaging evidence of the stroke is known.

The neurological sequelae of an ischemic event in the central nervous system can have consequences that range from the insignificant to devastating, and the disclosed assays permit early and accurate stratification of risk of long-lasting neurological impairment. For example, a test performed as early as within the first 24 hours of onset of signs and symptoms of a stroke, and even

as late as 7-14 days or even as late as 90 days or more after the event can provide clinical data that is highly predictive of the eventual care needs of the subject.

The disclosed assay is also able to identify subjects who have had an ischemic stroke in the past, for example more than 2 weeks ago, or even more than 90 days ago. The identification of such subjects helps evaluate other clinical data (such as neurological impairment or brain imaging information) to determine whether an ischemic stroke has occurred.

In particular examples, the disclosed methods provide a lower cost alternative to expensive imaging modalities (such as MRI and CT scans), can be used in instances where those imaging modalities are not available (such as in field hospitals), can be more convenient than placing people in scanners (especially considering that some people are not able to fit in the scanner, or can not be subjected to MRI if they have certain types of metallic implants in their bodies), or combinations thereof.

Clinical Specimens

Appropriate specimens for use with the current disclosure in diagnosing and prognosing ischemic stroke include any conventional clinical samples, for instance blood or blood-fractions (such as serum). Techniques for acquisition of such samples are well known in the art (for example see Schluger *et al. J. Exp. Med.* 176:1327-33, 1992, for the collection of serum samples). Serum or other blood fractions can be prepared in the conventional manner. For example, about 200 μL of serum can be used for the extraction of DNA for use in amplification reactions. However, if DNA is not amplified, larger amounts of blood can be collected. For example, if at least 5 μg of mRNA is desired, about 20-30 mls of blood can be collected.

In one example, PBMCs are used as a source of isolated nucleic acid molecules or proteins. The inflammatory response from peripheral blood borne white blood cells, in particular monocytes, are also a component of the evolving ischemic lesion (Kochanek *et al.*, *Stroke* 23:1367-79, 1992). One advantage of using blood (for example instead of brain tissue) is that it is easily available can be drawn serially.

Once a sample has been obtained, the sample can be used directly, concentrated (for example by centrifugation or filtration), purified, amplified, or combinations thereof. For example, rapid DNA preparation can be performed using a commercially available kit (such as the InstaGene Matrix, BioRad, Hercules, CA; the NucliSens isolation kit, Organon Teknika, Netherlands. In one example, the DNA preparation method yields a nucleotide preparation that is accessible to, and amenable to, nucleic acid amplification. Similarly, RNA can be prepared using a commercially available kit (such as the RNeasy Mini Kit, Qiagen, Valencia, CA).

Arrays for Detecting Nucleic Acid and Protein Sequences

In particular examples, methods for detecting a change in expression in the disclosed ischemic stroke-related genes listed in Tables 2-5 use the arrays disclosed herein. Arrays can be used to detect the presence of sequences whose expression is upregulated or downregulated in

response to an ischemic stroke, such as sequences listed in Tables 2-5, for example using specific oligonucleotide probes or antibody probes. The arrays herein termed "ischemic stroke detection arrays," are used to evaluate a stroke, for example to determine whether a subject has had an ischemic stroke, determine the severity of the stroke, predict the likelihood of neurological recovery of a subject who has had an ischemic stroke, to identify an appropriate therapy for a subject who has had an ischemic stroke, or combinations thereof. In particular examples, the disclosed arrays can include nucleic acid molecules, such as DNA or RNA molecules, or antibodies.

Nucleic acid arrays

In one example, the array includes nucleic acid oligonucleotide probes that can hybridize to any combination of at least four of the ischemic stroke-related gene sequences listed in Table 5, at least 150 of the ischemic stroke-related gene sequences listed in Table 3, or at least 500 of the ischemic stroke-related gene sequences listed in Table 2. In particular examples, an array includes oligonucleotides that can recognize all 22 ischemic stroke-associated genes listed in Table 5, all 82 of the ischemic stroke-related gene sequences listed in Table 4, all 190 of the ischemic stroke-related gene sequences listed in Table 3, or all 637 of the ischemic stroke-related gene sequences listed in Table 2. Certain of such arrays (as well as the methods described herein) can include ischemic stroke-related molecules that are not listed in Tables 2-5.

In a specific example, an array includes oligonucleotide probes that can recognize at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, or a probe that can recognize any one of these molecules. For example, the array can include oligonucleotide probes that can recognize at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 of the following, CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146. In another example, the array includes an oligonucleotide probe that can recognize at least CD163, for example in combination with other oligonucleotide probes that recognize other ischemic stroke related molecules (such as any combination of at least 3 of those listed in Tables 2-5).

In another specific example, an array includes oligonucleotide probes that can recognize at least one white blood cell activation and differentiation gene, at least one gene related to hypoxia, at least one gene involved in vascular repair, and at least one gene related to a specific PBMC response to the altered cerebral microenvironment, or at least 2, at least 3, at least 5, or at least 10 genes from each of these families.

In one example, a set of oligonucleotide probes is attached to the surface of a solid support for use in detection of ischemic stroke-associated sequences, such as those nucleic acid sequences (such as cDNA or mRNA) obtained from the subject. Additionally, if an internal control nucleic acid sequence is used (such as a nucleic acid sequence obtained from a PBMC from a subject who has not had an ischemic stroke) an oligonucleotide probe can be included to detect the presence of this control nucleic acid molecule.

The oligonucleotide probes bound to the array can specifically bind sequences obtained from the subject, or amplified from the subject (such as under high stringency conditions). Thus, sequences of use with the method are oligonucleotide probes that recognize ischemic stroke-related sequences, such as gene sequences (or corresponding proteins) listed in Tables 2-5. Such sequences can be determined by examining the sequences of the different species, and choosing oligonucleotide sequences that specifically anneal to a particular ischemic stroke-related sequence (such as those listed in Tables 2-5 or represented by those listed in Tables 2-5), but not others. One of skill in the art can identify other ischemic stroke-associated oligonucleotide molecules that can be attached to the surface of a solid support for the detection of other ischemic stroke-associated nucleic acid sequences.

The methods and apparatus in accordance with the present disclosure takes advantage of the fact that under appropriate conditions oligonucleotides form base-paired duplexes with nucleic acid molecules that have a complementary base sequence. The stability of the duplex is dependent on a number of factors, including the length of the oligonucleotides, the base composition, and the composition of the solution in which hybridization is effected. The effects of base composition on duplex stability can be reduced by carrying out the hybridization in particular solutions, for example in the presence of high concentrations of tertiary or quaternary amines.

The thermal stability of the duplex is also dependent on the degree of sequence similarity between the sequences. By carrying out the hybridization at temperatures close to the anticipated T_m's of the type of duplexes expected to be formed between the target sequences and the oligonucleotides bound to the array, the rate of formation of mis-matched duplexes may be substantially reduced.

The length of each oligonucleotide sequence employed in the array can be selected to optimize binding of target ischemic stroke-associated nucleic acid sequences. An optimum length for use with a particular ischemic stroke-associated nucleic acid sequence under specific screening conditions can be determined empirically. Thus, the length for each individual element of the set of oligonucleotide sequences including in the array can be optimized for screening. In one example, oligonucleotide probes are from about 20 to about 35 nucleotides in length or about 25 to about 40 nucleotides in length.

The oligonucleotide probe sequences forming the array can be directly linked to the support. Alternatively, the oligonucleotide probes can be attached to the support by non-ischemic stroke-associated sequences such as oligonucleotides or other molecules that serve as spacers or linkers to the solid support.

Protein arrays

In another example, an array includes protein sequences (or a fragment of such proteins, or antibodies specific to such proteins or protein fragments), which include at least four of the ischemic stroke-related protein sequences listed in Table 5, at least 150 of the ischemic stroke-related protein sequences listed in Table 3, or at least 500 of the ischemic stroke-related protein sequences listed in

Table 2. In particular examples, an array includes proteins that can recognize all 22 ischemic stroke-associated proteins listed in Table 5, all 82 of the ischemic stroke-related protein sequences listed in Table 4, all 190 of the ischemic stroke-related proteins listed in Table 3, or all 637 of the ischemic stroke-related proteins listed in Table 2. Such arrays can also contain any particular subset of the sequences listed in Tables 2-5. For example, an array can include probes that can recognize at least one white blood cell activation and differentiation protein, at least one protein related to hypoxia, at least one protein involved in vascular repair, and at least one protein related to a specific PBMC response to the altered cerebral microenvironment, or at least 2, at least 3, at least 5, or at least 10 proteins from each of these families. In another specific example, the array includes probes that recognize one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146. For example, the array can include a probe that recognizes CD163 and additional probes that recognize other ischemic stroke related proteins (such as any combination of at least 3 or at least 22 of those listed in Tables 2-5).

The proteins or antibodies forming the array can be directly linked to the support. Alternatively, the proteins or antibodies can be attached to the support by spacers or linkers to the solid support.

Changes in expression of ischemic stroke-related proteins can be detected using, for instance, an ischemic stroke protein-specific binding agent, which in some instances is labeled with an agent that can be detected. In certain examples, detecting a change in protein expression includes contacting a protein sample obtained from the PBMCs of a subject with an ischemic stroke protein-specific binding agent (which can be for example present on an array); and detecting whether the binding agent is bound by the sample and thereby measuring the levels of the ischemic stroke-related protein present in the sample. A difference in the level of an ischemic stroke-related protein in the sample, relative to the level of an ischemic stroke-related protein found an analogous sample from a subject who has not had an ischemic stroke, in particular examples indicates that the subject has suffered an ischemic stroke.

Array substrate

The solid support can be formed from an organic polymer. Suitable materials for the solid support include, but are not limited to: polypropylene, polyethylene, polybutylene, polyisobutylene, polybutadiene, polyisoprene, polyvinylpyrrolidine, polytetrafluroethylene, polyvinylidene difluroide, polyfluoroethylene-propylene, polyethylenevinyl alcohol, polymethylpentene, polycholorotrifluoroethylene, polysulfornes, hydroxylated biaxially oriented polypropylene, aminated biaxially oriented polypropylene, thiolated biaxially oriented polypropylene, etyleneacrylic acid, thylene methacrylic acid, and blends of copolymers thereof (see U.S. Patent No. 5,985,567, herein incorporated by reference).

In general, suitable characteristics of the material that can be used to form the solid support surface include: being amenable to surface activation such that upon activation, the surface of the support is capable of covalently attaching a biomolecule such as an oligonucleotide thereto;

amenability to "in situ" synthesis of biomolecules; being chemically inert such that at the areas on the support not occupied by the oligonucleotides are not amenable to non-specific binding, or when non-specific binding occurs, such materials can be readily removed from the surface without removing the oligonucleotides.

In one example, the solid support surface is polypropylene. Polypropylene is chemically inert and hydrophobic. Non-specific binding is generally avoidable, and detection sensitivity is improved. Polypropylene has good chemical resistance to a variety of organic acids (such as formic acid), organic agents (such as acetone or ethanol), bases (such as sodium hydroxide), salts (such as sodium chloride), oxidizing agents (such as peracetic acid), and mineral acids (such as hydrochloric acid). Polypropylene also provides a low fluorescence background, which minimizes background interference and increases the sensitivity of the signal of interest.

In another example, a surface activated organic polymer is used as the solid support surface. One example of a surface activated organic polymer is a polypropylene material aminated via radio frequency plasma discharge. Such materials are easily utilized for the attachment of nucleotide molecules. The amine groups on the activated organic polymers are reactive with nucleotide molecules such that the nucleotide molecules can be bound to the polymers. Other reactive groups can also be used, such as carboxylated, hydroxylated, thiolated, or active ester groups.

Array formats

A wide variety of array formats can be employed in accordance with the present disclosure. One example includes a linear array of oligonucleotide bands, generally referred to in the art as a dipstick. Another suitable format includes a two-dimensional pattern of discrete cells (such as 4096 squares in a 64 by 64 array). As is appreciated by those skilled in the art, other array formats including, but not limited to slot (rectangular) and circular arrays are equally suitable for use (see U.S. Patent No. 5,981,185, herein incorporated by reference). In one example, the array is formed on a polymer medium, which is a thread, membrane or film. An example of an organic polymer medium is a polypropylene sheet having a thickness on the order of about 1 mil. (0.001 inch) to about 20 mil., although the thickness of the film is not critical and can be varied over a fairly broad range. Particularly disclosed for preparation of arrays at this time are biaxially oriented polypropylene (BOPP) films; in addition to their durability, BOPP films exhibit a low background fluorescence.

The array formats of the present disclosure can be included in a variety of different types of formats. A "format" includes any format to which the solid support can be affixed, such as microtiter plates, test tubes, inorganic sheets, dipsticks, and the like. For example, when the solid support is a polypropylene thread, one or more polypropylene threads can be affixed to a plastic dipstick-type device; polypropylene membranes can be affixed to glass slides. The particular format is, in and of itself, unimportant. All that is necessary is that the solid support can be affixed thereto without affecting the functional behavior of the solid support or any biopolymer absorbed thereon, and that the format (such as the dipstick or slide) is stable to any materials into which the device is introduced (such as clinical samples and hybridization solutions).

The arrays of the present disclosure can be prepared by a variety of approaches. In one example, oligonucleotide or protein sequences are synthesized separately and then attached to a solid support (see U.S. Patent No. 6,013,789, herein incorporated by reference). In another example, sequences are synthesized directly onto the support to provide the desired array (see U.S. Patent No. 5,554,501, herein incorporated by reference). Suitable methods for covalently coupling oligonucleotides and proteins to a solid support and for directly synthesizing the oligonucleotides or proteins onto the support are known to those working in the field; a summary of suitable methods can be found in Matson *et al.*, *Anal. Biochem.* 217:306-10, 1994. In one example, the oligonucleotides are synthesized onto the support using conventional chemical techniques for preparing oligonucleotides on solid supports (such as see PCT applications WO 85/01051 and WO 89/10977, or U.S. Patent No. 5,554,501, herein incorporated by reference).

A suitable array can be produced using automated means to synthesize oligonucleotides in the cells of the array by laying down the precursors for the four bases in a predetermined pattern. Briefly, a multiple-channel automated chemical delivery system is employed to create oligonucleotide probe populations in parallel rows (corresponding in number to the number of channels in the delivery system) across the substrate. Following completion of oligonucleotide synthesis in a first direction, the substrate can then be rotated by 90° to permit synthesis to proceed within a second (2°) set of rows that are now perpendicular to the first set. This process creates a multiple-channel array whose intersection generates a plurality of discrete cells.

The oligonucleotides can be bound to the polypropylene support by either the 3' end of the oligonucleotide or by the 5' end of the oligonucleotide. In one example, the oligonucleotides are bound to the solid support by the 3' end. However, one of skill in the art can determine whether the use of the 3' end or the 5' end of the oligonucleotide is suitable for bonding to the solid support. In general, the internal complementarity of an oligonucleotide probe in the region of the 3' end and the 5' end determines binding to the support.

In particular examples, the oligonucleotide probes on the array include one or more labels, that permit detection of oligonucleotide probe:target sequence hybridization complexes.

Detection of Nucleic Acid and Protein Molecules

The nucleic acid molecules and proteins obtained from the subject (for example from a PBMC) can contain altered levels of one or more genes associated with ischemic stroke, such as those listed in Tables 2-5. Changes in expression can be detected to evaluate a stroke, or example to determine if the subject has had an ischemic stroke, to determine the severity of the stroke, to determine the likelihood of neurological recovery of a subject who has had an ischemic stroke, to determine the appropriate therapy for a subject who has had an ischemic stroke, or combinations thereof. The present disclosure is not limited to particular methods of detection. Any method of detecting a nucleic acid molecule or protein can be used, such as physical or functional assays. For example, the level of gene activation can be quantitated utilizing methods well known in the art and those disclosed herein, such as Northern-Blots, RNase protection assays, nucleic acid or antibody

probe arrays, quantitative PCR (such as TaqMan assays), dot blot assays, in-situ hybridization, or combinations thereof. In addition, proteins can be quantitated using antibody probe arrays, quantitative spectroscopic methods (for example mass spectrometry, such as surface-enhanced laser desorption/ionization (SELDI)-based mass spectrometry), or combinations thereof.

Methods for labeling nucleic acid molecules and proteins so that they can be detected are well known. Examples of such labels include non-radiolabels and radiolabels. Non-radiolabels include, but are not limited to enzymes, chemiluminescent compounds, fluorophores, metal complexes, haptens, colorimetric agents, dyes, or combinations thereof. Radiolabels include, but are not limited to, ^{125}I and ^{35}S. Radioactive and fluorescent labeling methods, as well as other methods known in the art, are suitable for use with the present disclosure. In one example, the primers used to amplify the subject's nucleic acids are labeled (such as with biotin, a radiolabel, or a fluorophore). In another example, the amplified nucleic acid samples are end-labeled to form labeled amplified material. For example, amplified nucleic acid molecules can be labeled by including labeled nucleotides in the amplification reactions. In another example, nucleic acid molecules obtained from a subject are labeled, and applied to an array containing oligonucleotides. In a particular example, proteins obtained from a subject are labeled and subsequently analyzed, for example by applying them to an array.

The nucleic acid molecules obtained from the subject that are associated with ischemic stroke are applied to an ischemic stroke detection array under suitable hybridization conditions to form a hybridization complex. In particular examples, the nucleic acid molecules include a label. In one example, a pre-treatment solution of organic compounds, solutions that include organic compounds, or hot water, can be applied before hybridization (see U.S. Patent No. 5,985,567, herein incorporated by reference).

Hybridization conditions for a given combination of array and target material can be optimized routinely in an empirical manner close to the T_m of the expected duplexes, thereby maximizing the discriminating power of the method. Identification of the location in the array, such as a cell, in which binding occurs, permits a rapid and accurate identification of sequences associated with ischemic stroke present in the amplified material (see below).

The hybridization conditions are selected to permit discrimination between matched and mismatched oligonucleotides. Hybridization conditions can be chosen to correspond to those known to be suitable in standard procedures for hybridization to filters and then optimized for use with the arrays of the disclosure. For example, conditions suitable for hybridization of one type of target would be adjusted for the use of other targets for the array. In particular, temperature is controlled to substantially eliminate formation of duplexes between sequences other than exactly complementary ischemic stroke-associated wild-type of mutant sequences. A variety of known hybridization solvents can be employed, the choice being dependent on considerations known to one of skill in the art (see U.S. Patent 5,981,185, herein incorporated by reference).

Once the nucleic acid molecules associated with ischemic stroke from the subject have been hybridized with the oligonucleotides present in the ischemic stroke detection array, the presence of the hybridization complex can be analyzed, for example by detecting the complexes.

Detecting a hybridized complex in an array of oligonucleotide probes has been previously described (see U.S. Patent No. 5,985,567, herein incorporated by reference). In one example, detection includes detecting one or more labels present on the oligonucleotides, the sequences obtained from the subject, or both. In particular examples, developing includes applying a buffer. In one example, the buffer is sodium saline citrate, sodium saline phosphate, tetramethylammonium chloride, sodium saline citrate in ethylenediaminetetra-acetic, sodium saline citrate in sodium dodecyl sulfate, sodium saline phosphate in ethylenediaminetetra-acetic, sodium saline phosphate in sodium dodecyl sulfate, tetramethylammonium chloride in ethylenediaminetetra-acetic, tetramethylammonium chloride in sodium dodecyl sulfate, or combinations thereof. However, other suitable buffer solutions can also be used.

Detection can further include treating the hybridized complex with a conjugating solution to effect conjugation or coupling of the hybridized complex with the detection label, and treating the conjugated, hybridized complex with a detection reagent. In one example, the conjugating solution includes streptavidin alkaline phosphatase, avidin alkaline phosphatase, or horseradish peroxidase. Specific, non-limiting examples of conjugating solutions include streptavidin alkaline phosphatase, avidin alkaline phosphatase, or horseradish peroxidase. The conjugated, hybridized complex can be treated with a detection reagent. In one example, the detection reagent includes enzyme-labeled fluorescence reagents or calorimetric reagents. In one specific non-limiting example, the detection reagent is enzyme-labeled fluorescence reagent (ELF) from Molecular Probes, Inc. (Eugene, OR). The hybridized complex can then be placed on a detection device, such as an ultraviolet (UV) transilluminator (manufactured by UVP, Inc. of Upland, CA). The signal is developed and the increased signal intensity can be recorded with a recording device, such as a charge coupled device (CCD) camera (manufactured by Photometrics, Inc. of Tucson, AZ). In particular examples, these steps are not performed when fluorophores or radiolabels are used.

In particular examples, the method further includes quantification, for instance by determining the amount of hybridization.

Kits

The present disclosure provides for kits that can be used to evaluate a stroke, for example to determine if a subject has had an ischemic stroke, to determine the severity of the stroke, to determine the likelihood of neurological recovery of a subject who has had an ischemic stroke, to determine the appropriate therapy for a subject who has had an ischemic stroke, or combinations thereof. .Such kits allow one to determine if a subject has a differential expression in ischemic stroke-related genes, such as any combination of four or more of those listed in Table 5, any combination of 150 or more of those listed in Table 3, or any combination of 500 or more of those listed in Table 2, for example any

combination of at least one gene from each class of genes listed in Table 5 (such as at least 2 or at least 3 genes from each of the four classes of genes listed in Table 5).

The disclosed kits include a binding molecule, such as an oligonucleotide probe that selectively hybridizes to an ischemic stroke-related molecule that is the target of the kit. In particular examples, the oligonucleotides probes are attached to an array. In one example, the kit includes oligonucleotide probes or primers (or antibodies) that recognize any combination of at least four of the molecules in Table 5, such as at least 5, at least 10, at least 15, at least 20, or at least 22 of the ischemic stroke-related molecules listed in Table 5, such as any combination of at least 150 of the molecules in Table 3, such as at least 160, at least 170, at least 175, at least 180, at least 185, or at least 190 of the sequences listed in Table 3, such as any combination of at least 500 of the molecules in Table 2, such as at least 525, at least 550, at least 575, at least 600, at least 610, or at least 637 of the sequences listed in Table 2. In particular examples, the kit includes oligonucleotide probes or primers (or antibodies) that recognize at least one gene (or protein) from each class listed in Table 5, such as at least 2, at least 3, at least 5, or at least 10 genes from each class.

In one particular example, the kit includes oligonucleotide probes or primers (or antibodies) that recognize at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146. In one particular example, the kit includes oligonucleotide probes or primers (or antibodies) that recognize at least 1, at least 2, at least 3, at least 4, at least 5 or at least 6 of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; or KIAA0146. In another particular example, the kit includes oligonucleotide probes or primers (or antibodies) that recognize CD163, for example in combination with oligonucleotide probes or primers (or antibodies) that recognize any combination of at least three ischemic stroke related molecules listed in Tables 2-5.

In a particular example, kits include antibodies capable of binding to ischemic stroke-related proteins. Such antibodies can be present on an array.

The kit can further include one or more of a buffer solution, a conjugating solution for developing the signal of interest, or a detection reagent for detecting the signal of interest, each in separate packaging, such as a container. In another example, the kit includes a plurality of ischemic stroke-related target nucleic acid sequences for hybridization with an ischemic stroke detection array to serve as positive control. The target nucleic acid sequences can include oligonucleotides such as DNA, RNA, and peptide-nucleic acid, or can include PCR fragments.

Ischemic Stroke Therapy

The present disclosure also provides methods of reducing brain injury in a subject determined to have suffered an ischemic stroke. For example, if using the assays described above a change in expression in at least 4 of the ischemic stroke-related molecules listed in Table 5 is detected in the subject, for example at least 22 of the ischemic stroke-related molecules listed in Table 5 is detected in the subject, a treatment is selected to avoid or reduce brain injury or to delay the onset of brain injury. In another example, if using the screening methods described above a

change in expression in at least 500 of the ischemic stroke-related molecules listed in Table 2 is detected in the subject, a treatment is selected to avoid or reduce brain injury or to delay the onset of brain injury. The subject then can be treated in accordance with this selection, for example by administration of one or more anticoagulant agents. In some examples, the treatment selected is specific and tailored for the subject, based on the analysis of that subject's profile for one or more ischemic stroke-related molecules.

The disclosure is further illustrated by the following non-limiting Examples.

Example 1

Isolation of Samples

This example describes methods used to obtain RNA from control subjects (subjects who had not previously had a stroke) and subjects who suffered an ischemic stroke within the previous 72 hours.

A cohort of elderly volunteers was obtained and their stroke risk factors recorded, including a history of hypertension, smoking, diabetes mellitus and heart disease. Approximately 30 milliliters of blood was drawn into four yellow top ACD A tubes (ACD Acid Citrate Dextrose A, 22.0 g/L Trisodium Citrate, 8.0 g/L Citric Acid, 24.5 g/L Dextrose, BD Franklin Lakes, NJ) by aseptic antecubital fossa venipuncture. PBMC isolation was completed within two hours.

Acute stroke patients admitted to the National Institutes of Health Stroke Program at Suburban Hospital in Bethesda, MD underwent aseptic antebrachial venipuncture followed by withdrawal of 30 ml of blood as described above. Blood samples were drawn within 72 hours of stroke onset. The blood samples were processed for RNA within two hours of collection.

Table 1 lists the demographic features of the patients and controls in the index cohort (n=38) and the patients and controls in the validation (test) cohort (n=19). The two index groups are reasonably comparable in terms of age sex and pre-morbid risk factors consistent with a community based stroke population.

Table 1: Demographics of Patients and Controls

Factor		Index Cohort		Test Cohort	
		Patients	Controls	Patients	Controls
Number		19	19	9	10
Age (years)		75.7 ± 15.1	66.0 ± 11.5	79.6 ± 8.1	67.6 ± 16.1
Sex	Female	7 (37)	13 (68)	4 (44)	6 (60)
Race	Caucasian	18 (95)	13 (68)	8 (89)	7 (70)
	African American	1 (5)	5 (26)	1 (11)	2 (20)
	Asian	0 (0)	1 (5)	0 (0)	1 (10)
Risk Factors	Hypertension	12 (63)	5 (26)	5 (56)	4 (40)
	Diabetes	1 (5)	0 (0)	1 (11)	1 (10)
	Smoking	7 (37)	7 (37)	5 (56)	2 (20)
	Coronary artery disease	4 (21)	1 (5)	3 (33)	1 (10)
	Framingham risk score	16.2 ± 7.4	9.8 ± 5.6	18.6 ± 2.5	12.2 ± 8.4
Stroke-Related	NIHSS score*	3.7 ± 5.1		5.9 ± 6.2	
	Time to blood draw (hours)	32.4 ± 17.8		53.3 ± 39.7	

Figures are numbers (%) for groups and mean ± SD for continuous factors.
*NIHSS – National Institutes of Health Stroke Scale.

Acute stroke was confirmed by magnetic resonance imaging studies including diffusion weighted imaging (DWI) and perfusion imaging. Stroke risk factors were recorded on each patient and volunteer according to the Framingham risk profile (see Wolf *et al.*, *Stroke* 22:312-8, 1991). Stroke severity was determined by serial neurological examination and by the National Institutes of Health Stroke Scale (NIHSS) score (see Brott *et al.*, *Stroke* 20:871-5, 1989).

RNA was isolated from PBMCs as follows. Total RNA (5-15 μg) was extracted from PBMCs separated from whole blood using a Density Gradient tube (Uni-Sep, Novamed, Jerusalem, Israel) as follows: 20-30 ml ACD anti-coagulated blood was diluted with an equal volume of phosphate buffered saline (PBS) and added to the density gradient tube, followed by centrifugation at 1000g for 30 minutes. After centrifugation, the PBMC layer was removed.

RNA was extracted using RNeasy Mini Kit (Qiagen Cat. # 75162, Valencia, CA), as per the manufacturer's protocol. Briefly, harvested PBMCs are diluted 1:1 with PBS and centrifuged for 10 minutes at 4000 rpm. The resulting supernatant was discarded and the pellet resuspended in 600 μl RLT buffer (1 ml buffer + 10 μl 2-β-mercaptoethanol). The sample was homogenized by passing the lysate 5-10 times through 20-G (French) needle fitted to a syringe. Cells were resuspended in 600 μl of DEPC-H_2O diluted in 70% EtOH and was loaded onto an RNeasy mini spin column fitted with a 2-ml collection tube. The sample was twice centrifuged at 14,000 rpm for 15 seconds. The RNeasy column was transferred to a new 2 ml collection tube and 500 μl of RPE buffer added followed by centrifugation at 14,000 rpm for 15 seconds. RPE buffer (500 μl) was added and the sample centrifuged at 10,000 rpm for 2 minutes. The RNeasy column was then transferred into a new 1.5 ml collection tube and RNA free water (30 μl) directly added to the RNase membrane followed by further centrifugation at 10,000 rpm for 1 minute. This was repeated and the extracted RNA stored at –80°C.

Example 2

RNA Labeling

This example describes methods used to label the RNA obtained in Example 1. However, one skilled in the art will appreciate that other labels and methods can be used.

RNA obtained from PBMCs was biotin-labeled and cleaned according to Affymetrix guidelines for Human Genome 133A arrays. Briefly, the Enzo BioArray HighYield RNA Transcript Labeling Kit3 (Affymetrix, P/N 900182) was used for generating labeled cRNA target. Template cDNA and the other reaction components were added to RNase-free microfuge tubes. To avoid precipitation of DTT, reactions were at room temperature while additions were made. After adding all reagents, the tube was incubated are a 37°C for 4 to 5 hours, gently mixing the contents of the tube every 30-45 minutes during the incubation.

To ensure the quality of the initial isolated total RNA, DNase was used to remove contaminant DNA from the sample. In addition, Northern blot followed by optical density analysis was used to determine the concentration of the RNA band.

If the total RNA concentration was >5 μg, the RNA was used for subsequent gene chip hybridization as per the manufacturer's protocol.

Example 3

Microarray Hybridization and Statistical Analysis

Coded mRNA samples were analyzed using the Affymetrix GeneChipR Human Genome U133A chips that include 22,283 gene probes (around 19,000 genes) of the best characterized human genes. Microarrays were scanned (Axon scanner, Axon Instruments Inc, CA), and images were analyzed using GenePix image analysis software (Axon Instruments Inc, CA) allowing for gene spot fluorescent quantification following subtraction of the surrounding background fluorescent signal within the Affymetrix MASS gene chip analysis suite with production of .CEL, and .DAT output files. The .CDF file or annotation file for the Affymetrix HU133A chip and the .CEL files, containing the scanned gene expression information, were the only data files used in all subsequent analyses.

For the data analysis, .CEL files of 19 patients and 19 controls were used following exclusion from analysis of one chip in each of the index patient and control groups due to unsatisfactory hybridization (see Irizarry *et al.*, *The Analysis of Gene Expression Data.* New York: Springer, 2003). The analysis was completed using the Bioconductor applications of the R programming language and implemented on a 64-bit operating system (SGI Octane 14000 MIPS 600 MHz CPU running Irix 6.5.15) due to the large dataset for analysis (Moore *et al.*, 32 bit architecture – a severe bio-informatics limitation. NHLBI Symposium From Genome to Disease. 2003, Bethesda, MD: 64). Sample RNA degradation during processing was tightly distributed and uniform across all chips.

Quantile normalization was performed simultaneously on the .CEL dataset (stroke patients, n=19, controls, n=19).

Following normalization, expression levels for each gene were calculated using the perfect match array probes and a robust median polish technique after background correction and $\log_2$ transformation (Irizarry *et al.*, *The Analysis of Gene Expression Data.* New York: Springer, 2003). The resulting expression set was compared in a univariate manner between the stroke patients and control group using parametric testing (t-test). The uncorrected p-value were assigned a cutoff threshold value of significance of <0.05. Subsequent multiple comparison correction was performed using Bonferroni and false discovery techniques (Benjamini and Yekutieli, *The Annals of Statistics* 29:1165-88, 2001). The effects of various multiple comparison correction techniques are shown in FIG. 1.

The uncorrected significant gene expression set was further analyzed using permutation analysis of Westfall and Young (*Resampling-based multiple testing: Examples and methods for p-*

value adjustment. New York: John Wiley & Sons, 1993). Hierarchical cluster analysis was performed on the gene subset found to be significantly different between stroke patients and controls using the method of Eisen *et al.* where each gene was pair-wise correlated by calculation of a distance matrix using a Euclidean metric (*Proc. Natl. Acad. Sci.* 95:14863-8, 1998). The distance matrix then formed the basis for hierarchical clustering. Gene annotation and ontology were determined using the Affymetrix on-line NetAffix suite together with subsequent literature searches, allowing categorization of a gene listing into molecular function, cellular function and biological function.

Using the PAM algorithm (Prediction Analysis for Microarrays) the ability of the index set to separate prospectively obtained samples from ten stroke patients and ten controls was examined (Tibshirani *et al., Proc. Natl. Acad. Sci.* 90:6567-72, 2002). The arrays of 9 patients and 10 controls were used. In one stroke case, the hybridization was not of sufficient quality to be included.

Without multiple comparison correction, 5060 genes were significantly different in the dataset. The Benjamini and Yekutieli correction resulted in 771 significant gene probes (Table 2), which represent 637 genes. This approach seeks to limit the false discovery rate (the proportion of non-differentiated genes among all those genes declared significantly different) to 5%. As shown in Table 2, several genes were upregulated (positive T-statistic, such as a value that is at least 3.77) or downregulated (negative t-statistic, such as a value that is less than -3.76, such as less than -3.77) following an ischemic stroke. In addition, several genes not previously associated with ischemic stroke, such as CD163; hypothetical protein FLJ22662 Laminin A motif; bone marrow stromal cell antigen 1/CD157; Fc fragment of IgG, high affinity Ia, receptor for (CD64); baculoviral IAP repeat-containing protein 1; and KIAA0146, were identified.

Table 2: Ischemic stroke related-genes using Benjamini and Yekutieli correction.

Affy ID No.	t-statistic*	Gene Name	UniGene ID No.^
218454_at	7.89390463	hypothetical protein FLJ22662	178470
215049_x_at	7.86959913	CD163 antigen	74076
203645_s_at	7.79274287	CD163 antigen	74076
211404_s_at	7.61929825	amyloid beta (A4) precursor-like protein 2	279518
206120_at	7.61303715	CD33 antigen (gp67)	83731
208771_s_at	7.4480951	leukotriene A4 hydrolase	81118
210872_x_at	7.29576739	growth arrest-specific 7	226133
201328_at	7.19607698	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
222173_s_at	7.01811369	TBC1 domain family, member 2	371016
211612_s_at	6.71007614	interleukin 13 receptor, alpha 1	285115
211067_s_at	6.66328089	growth arrest-specific 7	226133
211368_s_at	6.65646046	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
219788_at	6.6357632	paired immunoglobin-like type 2 receptor alpha	122591
202896_s_at	6.63433745	protein tyrosine phosphatase, non-receptor type substrate 1	156114
221210_s_at	6.63079363	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	64896
204924_at	6.60026287	toll-like receptor 2	439608

206488_s_at	6.54747468	CD36 antigen (collagen type I receptor, thrombospondin receptor)	443120
208146_s_at	6.53595206	carboxypeptidase, vitellogenic-like	95594
213006_at	6.50588342	KIAA0146 protein	381058
208923_at	6.46904449	cytoplasmic FMR1 interacting protein 1	26704
208702_x_at	6.46198549	amyloid beta (A4) precursor-like protein 2	279518
204452_s_at	6.45273495	frizzled homolog 1 (Drosophila)	94234
205715_at	6.43160146	bone marrow stromal cell antigen 1	169998
216942_s_at	6.42353873	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
218217_at	6.41930598	likely homolog of rat and mouse retinoid-inducible serine carboxypeptidase	431107
212192_at	6.41402934	hypothetical protein BC013764	109438
200868_s_at	6.39211608	zinc finger protein 313	144949
202912_at	6.38896329	adrenomedullin	441047
207691_x_at	6.37169995	ectonucleoside triphosphate diphosphohydrolase 1	444105
209124_at	6.322399	myeloid differentiation primary response gene (88)	82116
204620_s_at	6.31071007	chondroitin sulfate proteoglycan 2 (versican)	434488
203535_at	6.29981025	S100 calcium binding protein A9 (calgranulin B)	112405
202878_s_at	6.29001183	complement component 1, q subcomponent, receptor 1	97199
204249_s_at	6.28630536	LIM domain only 2 (rhombotin-like 1)	283063
208872_s_at	6.26653125	polyposis locus protein 1	173119
205603_s_at	6.25337908	diaphanous homolog 2 (Drosophila)	226483
208818_s_at	6.20310945	catechol-O-methyltransferase	240013
205158_at	6.20094021	ribonuclease, RNase A family, 4	283749
200765_x_at	6.19288966	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
220615_s_at	6.13260793	hypothetical protein FLJ10462	134497
202897_at	6.1313157	protein tyrosine phosphatase, non-receptor type substrate 1	156114
204222_s_at	6.12453094	GLI pathogenesis-related 1 (glioma)	511765
201743_at	6.11554977	CD14 antigen	75627
211744_s_at	6.05217577	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
207168_s_at	6.04197964	H2A histone family, member Y	75258
220034_at	6.04155844	interleukin-1 receptor-associated kinase 3	268552
204099_at	6.02751709	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	444445
212335_at	6.01677891	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	334534
211135_x_at	6.01231784	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
203127_s_at	5.98628713	serine palmitoyltransferase, long chain base subunit 2	59403
201041_s_at	5.97525939	dual specificity phosphatase 1	171695
209949_at	5.97496326	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	949
203922_s_at	5.95791758	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	88974
200838_at	5.95626946	cathepsin B	135226
210844_x_at	5.93419339	catenin (cadherin-associated protein), alpha 1, 102kDa	254321

200886_s_at	5.905732	phosphoglycerate mutase 1 (brain)	447492
208949_s_at	5.88800393	lectin, galactoside-binding, soluble, 3 (galectin 3)	411701
211284_s_at	5.87237505	granulin	180577
210992_x_at	5.78142217	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	352642
204860_s_at	5.76755994	Homo sapiens transcribed sequence with strong similarity to protein sp:Q13075 (H.sapiens) BIR1_HUMAN Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein)	508565
212788_x_at	5.75081118	ferritin, light polypeptide	433670
211776_s_at	5.7448982	erythrocyte membrane protein band 4.1-like 3	103839
221731_x_at	5.74075036	chondroitin sulfate proteoglycan 2 (versican)	434488
210225_x_at	5.74059556	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
218404_at	5.73126746	sorting nexin 10	418132
214511_x_at	5.7139856	Fc fragment of IgG, high affinity Ia, receptor for (CD64)	77424
200764_s_at	5.67242227	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
210904_s_at	5.66794891	interleukin 13 receptor, alpha 1	285115
201200_at	5.64946077	cellular repressor of E1A-stimulated genes	5710
209189_at	5.64912247	v-fos FBJ murine osteosarcoma viral oncogene homolog	25647
202943_s_at	5.6217726	N-acetylgalactosaminidase, alpha-	75372
201329_s_at	5.60980712	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
200678_x_at	5.59206951	granulin	180577
200839_s_at	5.59110282	cathepsin B	135226
204053_x_at	5.58890981	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	253309
204759_at	5.57510891	chromosome condensation 1-like	27007
217897_at	5.56972714	FXYD domain containing ion transport regulator 6	410748
203973_s_at	5.56911715	KIAA0146 protein	381058
210951_x_at	5.54846557	RAB27A, member RAS oncogene family	298530
216041_x_at	5.5475628	granulin	180577
208454_s_at	5.54191982	plasma glutamate carboxypeptidase	197335
209970_x_at	5.52920792	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
204646_at	5.50217863	dihydropyrimidine dehydrogenase	1602
202990_at	5.49766192	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	282417
218606_at	5.4924926	zinc finger, DHHC domain containing 7	9725
219316_s_at	5.47793995	chromosome 14 open reading frame 58	267566
207574_s_at	5.47094508	growth arrest and DNA-damage-inducible, beta	110571
212807_s_at	5.46295198	sortilin 1	394609
214875_x_at	5.46291913	amyloid beta (A4) precursor-like protein 2	279518
202446_s_at	5.45795408	phospholipid scramblase 1	348478
210784_x_at	5.416225	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
203561_at	5.4154987	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	352642
210152_at	5.40888799	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4	67846
210427_x_at	5.374221	annexin A2	462864

212830_at	5.37395389	EGF-like-domain, multiple 5	236216
204169_at	5.36588724	IMP (inosine monophosphate) dehydrogenase 1	317095
209500_x_at	5.34575265	tumor necrosis factor (ligand) superfamily, member 13	54673
201432_at	5.33693741	catalase	395771
215646_s_at	5.33373927	chondroitin sulfate proteoglycan 2 (versican)	434488
201422_at	5.33217618	interferon, gamma-inducible protein 30	14623
204112_s_at	5.33018103	histamine N-methyltransferase	42151
214318_s_at	5.32431367	hypothetical protein CG003	390874
204588_s_at	5.32319243	solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	194693
211366_x_at	5.32286549	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
217865_at	5.27748545	ring finger protein 130	155718
211133_x_at	5.26677423	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
209091_s_at	5.26607942	SH3-domain GRB2-like endophilin B1	136309
209474_s_at	5.2656896	ectonucleoside triphosphate diphosphohydrolase 1	444105
209514_s_at	5.25717561	RAB27A, member RAS oncogene family	298530
211571_s_at	5.25409403	chondroitin sulfate proteoglycan 2 (versican)	434488
201426_s_at	5.25332759	vimentin	435800
209069_s_at	5.23594128	H3 histone, family 3B (H3.3B)	180877
208130_s_at	5.23289975	thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A)	444510
220990_s_at	5.22930546	likely ortholog of rat vacuole membrane protein 1	166254
210314_x_at	5.22262249	tumor necrosis factor (ligand) superfamily, member 13	54673
203140_at	5.21224928	B-cell CLL/lymphoma 6 (zinc finger protein 51)	155024
205147_x_at	5.20456789	neutrophil cytosolic factor 4, 40kDa	196352
210101_x_at	5.19857938	SH3-domain GRB2-like endophilin B1	136309
205896_at	5.19850838	solute carrier family 22 (organic cation transporter), member 4	441130
206130_s_at	5.19713599	asialoglycoprotein receptor 2	1259
211367_s_at	5.18249106	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
217521_at	5.1760536	histidine ammonia-lyase	190783
212501_at	5.16612621	CCAAT/enhancer binding protein (C/EBP), beta	99029
218013_x_at	5.16025276	dynactin 4 (p62)	328865
209188_x_at	5.1523164	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	348418
202670_at	5.15097523	mitogen-activated protein kinase kinase 1	132311
217492_s_at	5.14879874	phosphatase and tensin homolog (mutated in multiple advanced cancers 1), pseudogene 1	493716
206600_s_at	5.14522932	solute carrier family 16 (monocarboxylic acid transporters), member 5	90911
208959_s_at	5.13849248	thioredoxin domain containing 4 (endoplasmic reticulum)	154023
209073_s_at	5.1251219	numb homolog (Drosophila)	445301
206237_s_at	5.11823604	neuregulin 1	172816
209185_s_at	5.11676697	insulin receptor substrate 2	143648
211702_s_at	5.09810016	ubiquitin specific protease 32	436133
200742_s_at	5.09255723	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease)	429658
214449_s_at	5.08839258	ras homolog gene family, member Q	442989

204834_at	5.07009362	fibrinogen-like 2	351808
204619_s_at	5.06774454	chondroitin sulfate proteoglycan 2 (versican)	434488
208926_at	5.06247837	sialidase 1 (lysosomal sialidase)	118721
201944_at	5.0610548	hexosaminidase B (beta polypeptide)	69293
202727_s_at	5.05203162	interferon gamma receptor 1	180866
211676_s_at	5.0386297	interferon gamma receptor 1	180866
204493_at	5.03178215	BH3 interacting domain death agonist	300825
219015_s_at	5.03010765	uncharacterized hematopoietic stem/progenitor cells protein MDS031	110853
209397_at	5.03002491	malic enzyme 2, NAD(+)-dependent, mitochondrial	75342
217741_s_at	5.02535951	zinc finger protein 216	406096
201044_x_at	5.01624832	dual specificity phosphatase 1	171695
219694_at	5.013375	hypothetical protein FLJ11127	155085
201127_s_at	5.00643448	ATP citrate lyase	387567
209304_x_at	5.00154395	growth arrest and DNA-damage-inducible, beta	110571
211395_x_at	4.99850312	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	126384
205786_s_at	4.99689814	integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)	172631
212268_at	4.99395229	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	381167
202787_s_at	4.99061446	mitogen-activated protein kinase-activated protein kinase 3	234521
203888_at	4.98963325	thrombomodulin	2030
221841_s_at	4.98297365	Kruppel-like factor 4 (gut)	376206
201888_s_at	4.97738085	interleukin 13 receptor, alpha 1	285115
200785_s_at	4.95578962	low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	162757
203167_at	4.9520306	tissue inhibitor of metalloproteinase 2	6441
201193_at	4.94983228	isocitrate dehydrogenase 1 (NADP+), soluble	11223
208018_s_at	4.94368736	hemopoietic cell kinase	89555
216202_s_at	4.91295079	serine palmitoyltransferase, long chain base subunit 2	59403
212820_at	4.91065301	rabconnectin-3	200828
218092_s_at	4.91053386	HIV-1 Rev binding protein	352962
207654_x_at	4.89959607	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	348418
203746_s_at	4.89297035	holocytochrome c synthase (cytochrome c heme-lyase)	211571
207704_s_at	4.89274931	growth arrest-specific 7	226133
222218_s_at	4.89264688	paired immunoglobin-like type 2 receptor alpha	122591
207980_s_at	4.88126247	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	82071
202917_s_at	4.87438447	S100 calcium binding protein A8 (calgranulin A)	416073
207791_s_at	4.86793585	RAB1A, member RAS oncogene family	227327
222148_s_at	4.85805606	ras homolog gene family, member T1	14202
207275_s_at	4.85293013	fatty-acid-Coenzyme A ligase, long-chain 2	511920
202803_s_at	4.84922223	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	375957
211100_x_at	4.84737438	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
208817_at	4.84504478	catechol-O-methyltransferase	240013

203767_s_at	4.83050164	steroid sulfatase (microsomal), arylsulfatase C, isozyme S	79876
212606_at	4.82536301	WD repeat and FYVE domain containing 3	105340
205174_s_at	4.82195934	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	79033
204714_s_at	4.81879712	coagulation factor V (proaccelerin, labile factor)	30054
221060_s_at	4.81814747	toll-like receptor 4	174312
211999_at	4.81797645	H3 histone, family 3B (H3.3B)	180877
211102_s_at	4.81093803	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
216243_s_at	4.80291726	interleukin 1 receptor antagonist	81134
203126_at	4.79908699	inositol(myo)-1(or 4)-monophosphatase 2	5753
210785_s_at	4.79694283	chromosome 1 open reading frame 38	10649
204232_at	4.78915713	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	433300
200648_s_at	4.78637919	glutamate-ammonia ligase (glutamine synthase)	442669
218627_at	4.77005668	hypothetical protein FLJ11259	416393
209555_s_at	4.76938604	CD36 antigen (collagen type I receptor, thrombospondin receptor)	443120
206034_at	4.76674446	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8	368077
221581_s_at	4.75435645	Williams-Beuren syndrome chromosome region 5	56607
203799_at	4.73734337	type I transmembrane C-type lectin receptor DCL-1	2441
203041_s_at	4.73458725	lysosomal-associated membrane protein 2	232432
209004_s_at	4.73446496	F-box and leucine-rich repeat protein 5	5548
217995_at	4.72584361	sulfide quinone reductase-like (yeast)	435468
220326_s_at	4.72372372	hypothetical protein FLJ10357	22451
207104_x_at	4.72227406	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
217889_s_at	4.71506397	cytochrome b reductase 1	31297
215001_s_at	4.71118486	glutamate-ammonia ligase (glutamine synthase)	442669
207761_s_at	4.71005806	DKFZP586A0522 protein	288771
205726_at	4.70850268	diaphanous homolog 2 (Drosophila)	226483
208704_x_at	4.70631847	amyloid beta (A4) precursor-like protein 2	279518
206674_at	4.70459455	fms-related tyrosine kinase 3	385
219582_at	4.70387413	hypothetical protein FLJ21079	16512
207872_s_at	4.70179932	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
200782_at	4.69959007	annexin A5	145741
201301_s_at	4.6939926	annexin A4	422986
202895_s_at	4.68690449	protein tyrosine phosphatase, non-receptor type substrate 1	156114
209835_x_at	4.67551042	CD44 antigen (homing function and Indian blood group system)	306278
201887_at	4.67403802	interleukin 13 receptor, alpha 1	285115
205329_s_at	4.67285443	sorting nexin 4	267812
205863_at	4.64921037	S100 calcium binding protein A12 (calgranulin C)	19413
202902_s_at	4.64873073	cathepsin S	181301
205640_at	4.64661387	aldehyde dehydrogenase 3 family, member B1	274235
204900_x_at	4.64331607	sin3-associated polypeptide, 30kDa	512813
208908_s_at	4.63754102	calpastatin	440961
217868_s_at	4.63345426	DORA reverse strand protein 1	279583
203360_s_at	4.62883239	c-myc binding protein	78221

207677_s_at	4.62647264	neutrophil cytosolic factor 4, 40kDa	196352
206111_at	4.60837517	ribonuclease, RNase A family, 2 (liver, eosinophil-derived neurotoxin)	728
210153_s_at	4.59360403	malic enzyme 2, NAD(+)-dependent, mitochondrial	75342
222231_s_at	4.58618625	hypothetical protein PRO1855	370927
201537_s_at	4.57925877	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)	181046
202201_at	4.57781605	biliverdin reductase B (flavin reductase (NADPH))	76289
203591_s_at	4.5770407	colony stimulating factor 3 receptor (granulocyte)	381027
214366_s_at	4.5700119	arachidonate 5-lipoxygenase	89499
217977_at	4.56856597	selenoprotein X, 1	279623
212527_at	4.55396497	DNA segment, Chr 15, Wayne State University 75, expressed	511996
211286_x_at	4.54820757	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	520937
222303_at	4.54612275	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
216652_s_at	4.54389883	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	348418
210660_at	4.53820668	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
202867_s_at	4.53800788	DnaJ (Hsp40) homolog, subfamily B, member 12	7960
218559_s_at	4.53299564	v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian)	169487
216950_s_at	4.53084661	Fc fragment of IgG, high affinity Ia, receptor for (CD64)	77424
213503_x_at	4.52978946	annexin A2	462864
214084_x_at	4.52713467	Homo sapiens similar to neutrophil cytosolic factor 1 (47kD, chronic granulomatous disease, autosomal 1) (LOC220830), mRNA	397369
201298_s_at	4.52689504	chromosome 2 open reading frame 6	196437
201940_at	4.52465759	carboxypeptidase D	5057
220266_s_at	4.51798181	Kruppel-like factor 4 (gut)	376206
58780_s_at	4.51518692	hypothetical protein FLJ10357	22451
211791_s_at	4.51444942	potassium voltage-gated channel, shaker-related subfamily, beta member 2	440497
31826_at	4.51322023	KIAA0674 protein	522351
206643_at	4.51089381	histidine ammonia-lyase	190783
204227_s_at	4.50226383	thymidine kinase 2, mitochondrial	274701
201590_x_at	4.50092732	annexin A2	462864
207674_at	4.49675798	Fc fragment of IgA, receptor for	193122
210569_s_at	4.49584084	sialic acid binding Ig-like lectin 9	245828
200889_s_at	4.49074615	signal sequence receptor, alpha (translocon-associated protein alpha)	250773
207697_x_at	4.48374293	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 2	306230
212117_at	4.47875698	ras homolog gene family, member Q	442989
213385_at	4.47853745	chimerin (chimaerin) 2	407520
212112_s_at	4.46538788	syntaxin 12	433838
201943_s_at	4.46470107	carboxypeptidase D	5057
210235_s_at	4.45960765	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 1	128312

211336_x_at	4.4529126	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
211509_s_at	4.45275311	reticulon 4	436349
202349_at	4.44313773	dystonia 1, torsion (autosomal dominant; torsin A)	19261
212625_at	4.4411393	syntaxin 10	43812
211101_x_at	4.44083795	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
217764_s_at	4.43853195	RAB31, member RAS oncogene family	223025
212602_at	4.43444968	WD repeat and FYVE domain containing 3	105340
220088_at	4.42977252	complement component 5 receptor 1 (C5a ligand)	2161
204445_s_at	4.42874496	arachidonate 5-lipoxygenase	89499
202593_s_at	4.4273484	membrane interacting protein of RGS16	512607
201235_s_at	4.42419251	BTG family, member 2	75462
217473_x_at	4.42406639	---	---
212271_at	4.42248213	mitogen-activated protein kinase 1	324473
204861_s_at	4.42112414	baculoviral IAP repeat-containing 1	79019
204502_at	4.41495415	SAM domain and HD domain 1	371264
212663_at	4.41324534	KIAA0674 protein	522351
202295_s_at	4.40282943	cathepsin H	114931
207571_x_at	4.40054035	chromosome 1 open reading frame 38	10649
219974_x_at	4.39530706	uncharacterized hypothalamus protein HCDASE	437091
201444_s_at	4.38865234	ATPase, H+ transporting, lysosomal accessory protein 2	183434
204043_at	4.38432768	transcobalamin II; macrocytic anemia	417948
201963_at	4.37921369	fatty-acid-Coenzyme A ligase, long-chain 2	511920
205071_x_at	4.37300052	X-ray repair complementing defective repair in Chinese hamster cells 4	150930
205173_x_at	4.36642735	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
200615_s_at	4.36556565	adaptor-related protein complex 2, beta 1 subunit	370123
211419_s_at	4.36300495	chimerin (chimaerin) 2	407520
205789_at	4.36189551	CD1D antigen, d polypeptide	1799
212124_at	4.35711838	retinoic acid induced 17	438767
202436_s_at	4.35300568	cytochrome P450, family 1, subfamily B, polypeptide 1	154654
203971_at	4.34868301	solute carrier family 31 (copper transporters), member 1	414471
219892_at	4.34634755	transmembrane 6 superfamily member 1	151155
208594_x_at	4.34443555	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
202877_s_at	4.33642974	complement component 1, q subcomponent, receptor 1	97199
214501_s_at	4.33619357	H2A histone family, member Y	75258
201425_at	4.33199642	aldehyde dehydrogenase 2 family (mitochondrial)	331141
203066_at	4.32776894	B cell RAG associated protein	523379
202484_s_at	4.32503279	methyl-CpG binding domain protein 2	25674
211296_x_at	4.31941158	ubiquitin C	183704
213590_at	4.31821109	solute carrier family 16 (monocarboxylic acid transporters), member 5	90911
215990_s_at	4.31739177	B-cell CLL/lymphoma 6 (zinc finger protein 51)	155024
208653_s_at	4.30983843	CD164 antigen, sialomucin	43910
208734_x_at	4.30135956	RAB2, member RAS oncogene family	78305
209005_at	4.29969708	F-box and leucine-rich repeat protein 5	5548

218739_at	4.29917434	abhydrolase domain containing 5	19385
208248_x_at	4.29909709	amyloid beta (A4) precursor-like protein 2	279518
208934_s_at	4.29599303	lectin, galactoside-binding, soluble, 8 (galectin 8)	4082
202820_at	4.28937583	aryl hydrocarbon receptor	170087
210154_at	4.28524889	malic enzyme 2, NAD(+)-dependent, mitochondrial	75342
201311_s_at	4.28104152	SH3 domain binding glutamic acid-rich protein like	14368
210732_s_at	4.27689192	lectin, galactoside-binding, soluble, 8 (galectin 8)	4082
200942_s_at	4.27633661	heat shock factor binding protein 1	250899
201538_s_at	4.27183553	dual specificity phosphatase 3 (vaccinia virus phosphatase VH1-related)	181046
201179_s_at	4.27082196	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3	73799
205418_at	4.2619161	feline sarcoma oncogene	7636
209297_at	4.25619908	intersectin 1 (SH3 domain protein)	66392
206934_at	4.25371303	signal-regulatory protein beta 1	194784
219889_at	4.24708622	frequently rearranged in advanced T-cell lymphomas	126057
212657_s_at	4.24239023	interleukin 1 receptor antagonist	81134
209305_s_at	4.24071078	growth arrest and DNA-damage-inducible, beta	110571
201720_s_at	4.23720249	Lysosomal-associated multispanning membrane protein-5	436200
202100_at	4.23631606	v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)	348024
210422_x_at	4.23603224	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	135163
203574_at	4.2315199	nuclear factor, interleukin 3 regulated	79334
209616_s_at	4.22891755	carboxylesterase 1 (monocyte/macrophage serine esterase 1)	278997
203185_at	4.2285949	Ras association (RalGDS/AF-6) domain family 2	80905
212681_at	4.22845394	erythrocyte membrane protein band 4.1-like 3	103839
221675_s_at	4.22714814	choline phosphotransferase 1	225567
209499_x_at	4.22636014	tumor necrosis factor (ligand) superfamily, member 13	54673
204959_at	4.22614064	myeloid cell nuclear differentiation antigen	153837
204277_s_at	4.22513355	thymidine kinase 2, mitochondrial	274701
204393_s_at	4.22305118	acid phosphatase, prostate	388677
216899_s_at	4.22228511	src family associated phosphoprotein 2	410745
205627_at	4.21755984	cytidine deaminase	72924
220001_at	4.21439779	peptidyl arginine deiminase, type IV	397050
211864_s_at	4.21257919	fer-1-like 3, myoferlin (C. elegans)	362731
213241_at	4.21206935	plexin C1	286229
215708_s_at	4.21183314	Homo sapiens transcribed sequence with strong similarity to protein sp:P49643 (H.sapiens) PRI2_HUMAN DNA primase large subunit (DNA primase 58 kDa subunit) (P58)	356530
205568_at	4.2078814	aquaporin 9	104624
201900_s_at	4.20012284	aldo-keto reductase family 1, member A1 (aldehyde reductase)	372170
216015_s_at	4.19778183	cold autoinflammatory syndrome 1	159483
204908_s_at	4.19238275	B-cell CLL/lymphoma 3	31210
206420_at	4.19184626	immunoglobulin superfamily, member 6	135194
206359_at	4.18559959	suppressor of cytokine signaling 3	436943
216905_s_at	4.18538303	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin)	56937

218439_s_at	4.18516434	PTD002 protein	151458
211816_x_at	4.18499309	Fc fragment of IgA, receptor for	193122
204336_s_at	4.18093971	regulator of G-protein signalling 19	422336
201647_s_at	4.17905107	scavenger receptor class B, member 2	323567
219872_at	4.17884427	hypothetical protein DKFZp434L142	323583
211527_x_at	4.17650619	vascular endothelial growth factor	73793
211749_s_at	4.17531793	vesicle-associated membrane protein 3 (cellubrevin)	66708
219666_at	4.17515543	membrane-spanning 4-domains, subfamily A, member 6A	371612
221858_at	4.17028941	KIAA0608 protein	100960
208351_s_at	4.16464496	mitogen-activated protein kinase 1	324473
218035_s_at	4.1625156	RNA-binding protein	95549
209276_s_at	4.16115658	glutaredoxin (thioltransferase)	28988
202497_x_at	4.16041756	solute carrier family 2 (facilitated glucose transporter), member 3	419240
213988_s_at	4.15957906	spermidine/spermine N1-acetyltransferase	28491
202381_at	4.14084013	a disintegrin and metalloproteinase domain 9 (meltrin gamma)	2442
205936_s_at	4.13559524	hexokinase 3 (white cell)	411695
209287_s_at	4.13557674	CDC42 effector protein (Rho GTPase binding) 3	352554
221194_s_at	4.13485757	PTD016 protein	30154
210648_x_at	4.13480197	sorting nexin 3	12102
205237_at	4.1324867	ficolin (collagen/fibrinogen domain containing) 1	440898
204899_s_at	4.12933139	sin3-associated polypeptide, 30kDa	512813
207085_x_at	4.12207721	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	520937
207266_x_at	4.12058364	RNA binding motif, single stranded interacting protein 1	241567
221492_s_at	4.12038439	autophagy Apg3p/Aut1p-like	26367
207387_s_at	4.11738071	glycerol kinase	1466
204122_at	4.11663505	TYRO protein tyrosine kinase binding protein	9963
207671_s_at	4.11610597	vitelliform macular dystrophy (Best disease, bestrophin)	167344
207857_at	4.10927392	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
201850_at	4.10860435	capping protein (actin filament), gelsolin-like	82422
202934_at	4.10351746	hexokinase 2	406266
206335_at	4.10242677	galactosamine (N-acetyl)-6-sulfate sulfatase (Morquio syndrome, mucopolysaccharidosis type IVA)	159479
221078_s_at	4.09863405	hypothetical protein FLJ10392	292925
201337_s_at	4.09676745	vesicle-associated membrane protein 3 (cellubrevin)	66708
203005_at	4.0932063	lymphotoxin beta receptor (TNFR superfamily, member 3)	1116
203676_at	4.09251365	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	334534
205401_at	4.09190279	alkylglycerone phosphate synthase	407933
218865_at	4.08907346	hypothetical protein FLJ22390	195345
201473_at	4.08893087	jun B proto-oncogene	400124
220000_at	4.08612321	sialic acid binding Ig-like lectin 5	117005
208983_s_at	4.0855044	platelet/endothelial cell adhesion molecule (CD31 antigen)	78146
218424_s_at	4.08296857	dudulin 2	57655
201186_at	4.08234245	low density lipoprotein receptor-related protein	75140

		associated protein 1	
210959_s_at	4.08090496	steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)	552
213160_at	4.07719887	dedicator of cyto-kinesis 2	17211
201463_s_at	4.07608806	transaldolase 1	438678
200078_s_at	4.07300443	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit c"	7476
201506_at	4.07150831	transforming growth factor, beta-induced, 68kDa	421496
217826_s_at	4.07137819	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	184325
38487_at	4.06946114	stabilin 1	301989
217827_s_at	4.06896229	acid cluster protein 33	242458
201642_at	4.06630731	interferon gamma receptor 2 (interferon gamma transducer 1)	409200
211997_x_at	4.06180968	H3 histone, family 3B (H3.3B)	180877
211540_s_at	4.06076677	retinoblastoma 1 (including osteosarcoma)	408528
221036_s_at	4.05925589	anterior pharynx defective 1B-like	42954
208097_s_at	4.049855	thioredoxin domain containing	125221
201828_x_at	4.04673957	CAAX box 1	250708
217853_at	4.04364724	tensin-like SH2 domain-containing 1	12210
207270_x_at	4.04254236	CMRF35 leukocyte immunoglobulin-like receptor	2605
217159_x_at	4.04053417	sialic acid binding Ig-like lectin 7	274470
209901_x_at	4.03786304	allograft inflammatory factor 1	76364
216236_s_at	4.03358432	solute carrier family 2 (facilitated glucose transporter), member 14	401274
204961_s_at	4.03301863	neutrophil cytosolic factor 1 (47kDa, chronic granulomatous disease, autosomal 1)	458275
202101_s_at	4.03192448	v-ral simian leukemia viral oncogene homolog B (ras related; GTP binding protein)	348024
208189_s_at	4.03164659	myosin VIIA (Usher syndrome 1B (autosomal recessive, severe))	370421
201554_x_at	4.03078928	glycogenin	174071
219505_at	4.03062109	cat eye syndrome chromosome region, candidate 1	170310
202445_s_at	4.02993448	Notch homolog 2 (Drosophila)	8121
208071_s_at	4.02784691	leukocyte-associated Ig-like receptor 1	407964
220832_at	4.02284364	toll-like receptor 8	272410
212419_at	4.02284151	hypothetical protein FLJ90798	28264
203857_s_at	4.02278318	for protein disulfide isomerase-related	76901
202122_s_at	4.02240203	cargo selection protein (mannose 6 phosphate receptor binding protein)	140452
208936_x_at	4.02052401	lectin, galactoside-binding, soluble, 8 (galectin 8)	4082
219806_s_at	4.01642219	FN5 protein	416456
205922_at	4.01615381	vanin 2	293130
209311_at	4.01610957	BCL2-like 2	410026
210340_s_at	4.01506832	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	520937
216346_at	4.01350716	SEC14-like 3 (S. cerevisiae)	434140
202944_at	4.00826135	N-acetylgalactosaminidase, alpha-	75372
206877_at	4.00750632	MAX dimerization protein 1	379930
209473_at	4.00302974	ectonucleoside triphosphate diphosphohydrolase 1	444105
208785_s_at	3.99946577	Homo sapiens transcribed sequence with strong similarity to protein ref:NP_073729.1 (H.sapiens) microtubule-associated proteins 1A/1B light chain 3 [Homo sapiens]	419777

202108_at	3.99887308	peptidase D	444207
201926_s_at	3.99380381	decay accelerating factor for complement (CD55, Cromer blood group system)	408864
201413_at	3.990689	hydroxysteroid (17-beta) dehydrogenase 4	356894
210190_at	3.99016278	syntaxin 11	118958
215842_s_at	3.98910601	ATPase, Class VI, type 11A	29189
204361_s_at	3.98317434	src family associated phosphoprotein 2	410745
202826_at	3.97921395	serine protease inhibitor, Kunitz type 1	233950
200798_x_at	3.97812485	myeloid cell leukemia sequence 1 (BCL2-related)	86386
203471_s_at	3.97445343	pleckstrin	77436
213532_at	3.97420929	hypothetical protein LOC285148	509314
206710_s_at	3.97151204	erythrocyte membrane protein band 4.1-like 3	103839
221879_at	3.97046064	ceroid-lipofuscinosis, neuronal 6, late infantile, variant	43654
204446_s_at	3.97025459	arachidonate 5-lipoxygenase	89499
200677_at	3.96789671	pituitary tumor-transforming 1 interacting protein	369026
201118_at	3.96505944	phosphogluconate dehydrogenase	392837
205868_s_at	3.96375543	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)	83572
212252_at	3.96319972	calcium/calmodulin-dependent protein kinase kinase 2, beta	297343
203887_s_at	3.96298525	thrombomodulin	2030
202192_s_at	3.96240466	growth arrest-specific 7	226133
201096_s_at	3.95648407	ADP-ribosylation factor 4	435639
219911_s_at	3.94841313	solute carrier family 21 (organic anion transporter), member 12	235782
200796_s_at	3.94807209	myeloid cell leukemia sequence 1 (BCL2-related)	86386
219890_at	3.94731168	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 5	126355
208724_s_at	3.94528806	RAB1A, member RAS oncogene family	227327
212374_at	3.94463692	fem-1 homolog b (C. elegans)	362733
219104_at	3.94384785	ring finger protein 141	44685
203748_x_at	3.94217815	RNA binding motif, single stranded interacting protein 1	241567
210773_s_at	3.94043935	formyl peptide receptor-like 1	99855
219607_s_at	3.93984379	membrane-spanning 4-domains, subfamily A, member 4	325960
206348_s_at	3.9380531	pyruvate dehydrogenase kinase, isoenzyme 3	193124
215856_at	3.93781449	hypothetical protein LOC284266	287692
200737_at	3.93283779	phosphoglycerate kinase 1	78771
218831_s_at	3.93205147	Fc fragment of IgG, receptor, transporter, alpha	111903
202437_s_at	3.92627151	cytochrome P450, family 1, subfamily B, polypeptide 1	154654
201942_s_at	3.92566977	carboxypeptidase D	5057
219859_at	3.92247489	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 9	236516
212054_x_at	3.91644887	KIAA0676 protein	155829
208540_x_at	3.91561327	---	506947
213119_at	3.91488544	solute carrier family 36 (proton/amino acid symporter), member 1	409314
205119_s_at	3.91440214	formyl peptide receptor 1	753
201576_s_at	3.91382693	galactosidase, beta 1	445183
212014_x_at	3.9085234	CD44 antigen (homing function and Indian blood group system)	306278

210156_s_at	3.90785487	protein-L-isoaspartate (D-aspartate) O-methyltransferase	79137
205540_s_at	3.90407281	Ras-related GTP binding B	50282
212598_at	3.90198488	WD repeat and FYVE domain containing 3	105340
221724_s_at	3.89891636	C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 6	115515
208952_s_at	3.89761036	KIAA0217 protein	192881
200738_s_at	3.89752042	phosphoglycerate kinase 1	78771
206380_s_at	3.89281079	properdin P factor, complement	53155
211287_x_at	3.89139509	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	520937
210953_at	3.89109344	KIAA0669 gene product	52526
201798_s_at	3.88996697	fer-1-like 3, myoferlin (C. elegans)	362731
208885_at	3.88981825	lymphocyte cytosolic protein 1 (L-plastin)	381099
202671_s_at	3.88812247	pyridoxal (pyridoxine, vitamin B6) kinase	284491
202433_at	3.88667317	solute carrier family 35, member B1	154073
220775_s_at	3.88608806	ubiquitin-conjugating enzyme E2-like	407991
202030_at	3.88474448	branched chain alpha-ketoacid dehydrogenase kinase	20644
205639_at	3.88308443	acyloxyacyl hydrolase (neutrophil)	82542
202096_s_at	3.88275079	benzodiazapine receptor (peripheral)	202
202241_at	3.87918071	phosphoprotein regulated by mitogenic pathways	444947
200958_s_at	3.8722837	syndecan binding protein (syntenin)	164067
211689_s_at	3.87053754	transmembrane protease, serine 2	439309
207157_s_at	3.86989748	guanine nucleotide binding protein (G protein), gamma 5	436765
210186_s_at	3.86911308	FK506 binding protein 1A, 12kDa	374638
200987_x_at	3.86682174	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)	152978
208853_s_at	3.8665582	calnexin	155560
212026_s_at	3.86220136	likely ortholog of mouse exocyst component protein 70 kDa homolog (S. cerevisiae) Exo70: exocyst component protein 70 kDa homolog (S. cerevisiae)	511946
201898_s_at	3.861804	ubiquitin-conjugating enzyme E2A (RAD6 homolog)	379466
209615_s_at	3.85852692	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	64056
208488_s_at	3.85497758	complement component (3b/4b) receptor 1, including Knops blood group system	334019
203853_s_at	3.85414745	GRB2-associated binding protein 2	30687
209131_s_at	3.85261742	synaptosomal-associated protein, 23kDa	202308
204150_at	3.85178342	stabilin 1	301989
212188_at	3.85075667	hypothetical protein BC013764	109438
211087_x_at	3.85069595	mitogen-activated protein kinase 14	79107
205920_at	3.8477849	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	1194
219079_at	3.84686335	NADPH cytochrome B5 oxidoreductase	5741
201619_at	3.84148866	peroxiredoxin 3	397062
214438_at	3.84078431	H2.0-like homeo box 1 (Drosophila)	74870
211507_s_at	3.83656731	myotubularin related protein 3	412833
217835_x_at	3.83446552	chromosome 20 open reading frame 24	184062
217825_s_at	3.82162344	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	184325
205681_at	3.81769723	BCL2-related protein A1	227817

200827_at	3.81766405	procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase, Ehlers-Danlos syndrome type VI)	75093
211797_s_at	3.8151505	nuclear transcription factor Y, gamma	285133
204194_at	3.81135547	BTB and CNC homology 1, basic leucine zipper transcription factor 1	154276
201078_at	3.81097664	transmembrane 9 superfamily member 2	298272
206343_s_at	3.81055509	neuregulin 1	172816
218091_at	3.80590961	HIV-1 Rev binding protein	352962
205468_s_at	3.80336547	interferon regulatory factor 5	334450
200929_at	3.79657368	transmembrane trafficking protein	74137
206881_s_at	3.79242916	leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3	113277
209404_s_at	3.79062874	CGI-109 protein	278391
207549_x_at	3.79036164	membrane cofactor protein (CD46, trophoblast-lymphocyte cross-reactive antigen)	83532
219646_at	3.78787522	hypothetical protein FLJ20186	62771
219991_at	3.78398642	solute carrier family 2 (facilitated glucose transporter), member 9	95497
211922_s_at	3.78374234	catalase	395771
210275_s_at	3.78115446	zinc finger protein 216	406096
216883_x_at	3.77914282	phosphodiesterase 6D, cGMP-specific, rod, delta	48291
202833_s_at	3.77713205	serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	297681
217124_at	3.77620277	KIAA1023 protein	446063
209062_x_at	3.77508965	nuclear receptor coactivator 3	382168
208310_s_at	3.77468002	follistatin-like 1	433622
212041_at	3.77461664	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d isoform 1	106876
214500_at	3.77290459	H2A histone family, member Y	75258
217746_s_at	3.77075614	programmed cell death 6 interacting protein	9663
218754_at	-3.7702453	hypothetical protein FLJ23323	59425
214749_s_at	-3.7716173	hypothetical protein FLJ20811	83530
203094_at	-3.7722118	MAD2L1 binding protein	122346
221230_s_at	-3.7758096	retinoblastoma binding protein 1-like 1	17428
212912_at	-3.7760375	ribosomal protein S6 kinase, 90kDa, polypeptide 2	301664
205004_at	-3.7765763	NF-kappa B-repressing factor	437084
205775_at	-3.7769304	DNA segment on chromosome 6(unique) 2654 expressed sequence	140944
219007_at	-3.7819237	nucleoporin 43kDa	53263
206082_at	-3.7826255	HLA complex P5	511759
214022_s_at	-3.7838188	interferon induced transmembrane protein 1 (9-27)	458414
210243_s_at	-3.7882423	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 3	321231
204228_at	-3.7929943	peptidyl prolyl isomerase H (cyclophilin H)	9880
204411_at	-3.793944	KIAA0449 protein	511940
213388_at	-3.7946632	Homo sapiens mRNA; cDNA DKFZp586I1823 (from clone DKFZp586I1823)	448231
205963_s_at	-3.7953901	DnaJ (Hsp40) homolog, subfamily A, member 3	6216
221535_at	-3.8112387	hypothetical protein FLJ11301	436471
209302_at	-3.811649	polymerase (RNA) II (DNA directed) polypeptide H	432574
221867_at	-3.8131693	hypothetical protein FLJ31821	511839
213028_at	-3.8138531	Homo sapiens cDNA FLJ44314 fis, clone	419777

		TRACH2025932	
209870_s_at	-3.8147819	amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like)	26468
210847_x_at	-3.8164631	tumor necrosis factor receptor superfamily, member 25	299558
218955_at	-3.8194881	BRF2, subunit of RNA polymerase III transcription initiation factor, BRF1-like	274136
64418_at	-3.8220874	AP1 gamma subunit binding protein 1	404215
36888_at	-3.8320679	KIAA0841 protein	7426
219971_at	-3.8331453	interleukin 21 receptor	210546
221963_x_at	-3.836647	Homo sapiens transcribed sequence with strong similarity to protein pir:TSHUP1 (H.sapiens) TSHUP1 thrombospondin 1 precursor - human	---
200045_at	-3.8367612	ATP-binding cassette, sub-family F (GCN20), member 1	9573
221135_s_at	-3.8379739	HT001 protein	254124
221940_at	-3.8380758	C18B11 homolog (44.9kD)	173311
203386_at	-3.8411936	TBC1 domain family, member 4	173802
212660_at	-3.8431641	PHD finger protein 15	397990
206240_s_at	-3.8440853	zinc finger protein 136 (clone pHZ-20)	479874
204461_x_at	-3.8452	RAD1 homolog (S. pombe)	7179
49329_at	-3.8471735	hypothetical protein FLJ14360	351563
201763_s_at	-3.8485837	death-associated protein 6	336916
218601_at	-3.8507869	up-regulated gene 4	5131
216309_x_at	-3.8522271	jerky homolog (mouse)	142296
213742_at	-3.8525732	splicing factor, arginine/serine-rich 11	443458
205255_x_at	-3.8532902	transcription factor 7 (T-cell specific, HMG-box)	169294
219123_at	-3.8535773	zinc finger protein 232	279914
39248_at	-3.8602222	aquaporin 3	234642
214351_x_at	-3.8603602	ribosomal protein L13	410817
213360_s_at	-3.8667261	similar to Nuclear envelope pore membrane protein POM 121 (Pore membrane protein of 121 kDa) (P145)	450237
210031_at	-3.8668203	CD3Z antigen, zeta polypeptide (TiT3 complex)	97087
204484_at	-3.8676808	phosphoinositide-3-kinase, class 2, beta polypeptide	343329
217798_at	-3.8703856	CCR4-NOT transcription complex, subunit 2	165725
200957_s_at	-3.8715464	structure specific recognition protein 1	79162
206188_at	-3.8905582	KIAA0628 gene product	43133
221518_s_at	-3.8970401	ubiquitin specific protease 47	441028
221978_at	-3.8977136	major histocompatibility complex, class I, F	411958
218500_at	-3.9056647	mesenchymal stem cell protein DSCD75	25237
219765_at	-3.9104452	hypothetical protein FLJ12586	458377
207339_s_at	-3.9120005	lymphotoxin beta (TNF superfamily, member 3)	376208
218496_at	-3.9220606	ribonuclease H1	511960
204891_s_at	-3.9250376	lymphocyte-specific protein tyrosine kinase	1765
203611_at	-3.9251415	telomeric repeat binding factor 2	63335
213689_x_at	-3.9253484	ribosomal protein L5	469653
38398_at	-3.9258197	MAP-kinase activating death domain	82548
46256_at	-3.9261723	SPRY domain-containing SOCS box protein SSB-3	7247
214692_s_at	-3.9264268	jerky homolog (mouse)	142296
40446_at	-3.9318725	PHD finger protein 1	166204
217802_s_at	-3.9322297	nuclear ubiquitous casein kinase and cyclin-dependent kinase substrate	510265
218573_at	-3.9379843	APR-1 protein	279819

221277_s_at	-3.9418985	hypothetical protein FKSG32	98682
204182_s_at	-3.9459862	zinc finger protein 297B	355581
212653_s_at	-3.94854	KIAA0903 protein	16218
201717_at	-3.9504943	mitochondrial ribosomal protein L49	75859
218700_s_at	-3.9516027	RAB7, member RAS oncogene family-like 1	115325
217950_at	-3.9535594	nitric oxide synthase interacting protein	7236
208758_at	-3.9548631	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	90280
202617_s_at	-3.9570199	methyl CpG binding protein 2 (Rett syndrome)	3239
212935_at	-3.9602659	MCF.2 cell line derived transforming sequence-like	436905
222077_s_at	-3.9734422	Rac GTPase activating protein 1	23900
221087_s_at	-3.9735482	apolipoprotein L, 3	241535
202330_s_at	-3.9749735	uracil-DNA glycosylase	78853
206545_at	-3.9833463	CD28 antigen (Tp44)	1987
218414_s_at	-3.9863793	nudE nuclear distribution gene E homolog 1 (A. nidulans)	263925
209440_at	-3.9905647	phosphoribosyl pyrophosphate synthetase 1	56
219966_x_at	-3.9987967	BTG3 associated nuclear protein	448828
215359_x_at	-4.0024233	zinc finger protein 44 (KOX 7)	501604
215012_at	-4.0067262	zinc finger protein 451	188662
205192_at	-4.0075631	mitogen-activated protein kinase kinase kinase 14	440315
206118_at	-4.0108593	signal transducer and activator of transcription 4	80642
213574_s_at	-4.011904	karyopherin (importin) beta 1	439683
200644_at	-4.015547	MARCKS-like protein	75061
218274_s_at	-4.0156737	hypothetical protein FLJ10415	437647
212037_at	-4.0191262	pinin, desmosome associated protein	409965
203723_at	-4.0201929	inositol 1,4,5-trisphosphate 3-kinase B	78877
202970_at	-4.03346	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	173135
219169_s_at	-4.0376252	transcription factor B1, mitochondrial	279908
202562_s_at	-4.0376994	chromosome 14 open reading frame 1	15106
213648_at	-4.0401386	KIAA0116 protein	254717
205442_at	-4.0500447	KIAA0626 gene product	178121
219658_at	-4.0522935	hypothetical protein FLJ12598	126906
217627_at	-4.0526775	hypothetical protein FLJ30921	290703
202968_s_at	-4.0534127	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	173135
204008_at	-4.0615836	dynein, axonemal, light polypeptide 4	258203
203450_at	-4.065337	chromosome 22 open reading frame 2	334911
219812_at	-4.0673436	stromal antigen 3	323634
219109_at	-4.0689872	PF20	6783
213473_at	-4.0764661	ankyrin repeat domain 13	122764
40016_g_at	-4.0768183	KIAA0303 protein	212787
203556_at	-4.079399	transcription factor ZHX2	30209
209798_at	-4.0837583	nuclear protein, ataxia-telangiectasia locus	89385
219635_at	-4.0865204	hypothetical protein FLJ14260	287629
212589_at	-4.0884147	related RAS viral (r-ras) oncogene homolog 2	206097
204327_s_at	-4.0891305	zinc finger protein 202	112556
216262_s_at	-4.0902501	TGFB-induced factor 2 (TALE family homeobox)	94785
222348_at	-4.0911661	KIAA0303 protein	212787
220035_at	-4.101629	nucleoporin 210	292119
213039_at	-4.1044199	Rho-specific guanine nucleotide exchange factor p114	6150

208858_s_at	-4.1054066	likely ortholog of mouse membrane bound C2 domain containing protein	8309
218805_at	-4.1065589	immune associated nucleotide 4 like 1 (mouse)	412331
209558_s_at	-4.108666	huntingtin interacting protein-1-related	96731
207394_at	-4.1102265	zinc finger protein 137 (clone pHZ-30)	373648
220418_at	-4.1185695	ubiquitin associated and SH3 domain containing, A	183924
219155_at	-4.1208338	phosphatidylinositol transfer protein, cytoplasmic 1	405933
222266_at	-4.121441	chromosome 19 open reading frame 2	7943
214739_at	-4.1236762	hypothetical protein MGC4126	334483
219006_at	-4.1353669	chromosome 6 open reading frame 66	512144
209657_s_at	-4.14338	heat shock transcription factor 2	158195
64064_at	-4.1445869	immune associated nucleotide 4 like 1 (mouse)	412331
205964_at	-4.147438	zinc finger protein 426	324978
204635_at	-4.1496187	ribosomal protein S6 kinase, 90kDa, polypeptide 5	109058
212320_at	-4.151357	beta 5-tubulin	356729
208094_s_at	-4.1580744	hypothetical protein MGC10471	24998
48117_at	-4.1689003	hypothetical protein BC011981	110407
218492_s_at	-4.1719389	THAP domain containing 7	512756
219045_at	-4.1781811	ras homolog gene family, member F (in filopodia)	512618
217152_at	-4.1800035	nuclear receptor co-repressor 1	144904
203159_at	-4.1901598	glutaminase	128410
219700_at	-4.1962225	plexin domain containing 1	125036
213958_at	-4.2016236	CD6 antigen	436949
210763_x_at	-4.2056306	natural cytotoxicity triggering receptor 3	509513
209586_s_at	-4.2061369	TcD37 homolog	78524
202931_x_at	-4.2107515	bridging integrator 1	193163
202741_at	-4.2145218	protein kinase, cAMP-dependent, catalytic, beta	156324
218259_at	-4.2150435	myocardin-related transcription factor B	151076
202724_s_at	-4.2194658	forkhead box O1A (rhabdomyosarcoma)	170133
217912_at	-4.2238457	PP3111 protein	351484
220969_s_at	-4.2271761	---	---
220367_s_at	-4.2316067	mSin3A-associated protein 130	133523
219315_s_at	-4.2333807	hypothetical protein FLJ20898	25549
218510_x_at	-4.237976	hypothetical protein FLJ20152	82273
216983_s_at	-4.2380619	zinc finger protein 224	279855
218735_s_at	-4.2447866	zinc finger protein	438994
213179_at	-4.2560283	RCD1 required for cell differentiation1 homolog (S. pombe)	148767
204020_at	-4.2566815	purine-rich element binding protein A	29117
204630_s_at	-4.2576483	golgi SNAP receptor complex member 1	124436
201853_s_at	-4.2599718	cell division cycle 25B	153752
214771_x_at	-4.2674176	Rho interacting protein 3	430725
213539_at	-4.281273	CD3D antigen, delta polypeptide (TiT3 complex)	95327
202693_s_at	-4.2866716	serine/threonine kinase 17a (apoptosis-inducing)	9075
200953_s_at	-4.2970373	cyclin D2	376071
205590_at	-4.3097591	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	189527
213193_x_at	-4.3143922	Homo sapiens T cell receptor beta chain BV20S1 BJ1-5 BC1 mRNA, complete cds	487862
210915_x_at	-4.342139	Homo sapiens T cell receptor beta chain BV20S1 BJ1-5 BC1 mRNA, complete cds	349283
220176_at	-4.3595778	chromosome 14 open reading frame 127	288981
38340_at	-4.3617132	huntingtin interacting protein-1-related	96731

209246_at	-4.3621957	ATP-binding cassette, sub-family F (GCN20), member 2	438823
204633_s_at	-4.3717038	ribosomal protein S6 kinase, 90kDa, polypeptide 5	109058
202250_s_at	-4.3738264	H326	120904
210538_s_at	-4.3739708	baculoviral IAP repeat-containing 3	127799
219350_s_at	-4.3780164	second mitochondria-derived activator of caspase	169611
209014_at	-4.3855467	melanoma antigen, family D, 1	5258
204642_at	-4.4025727	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1	154210
207892_at	-4.4032046	tumor necrosis factor (ligand) superfamily, member 5 (hyper-IgM syndrome)	652
217957_at	-4.4033051	likely ortholog of mouse gene trap locus 3	279818
212333_at	-4.4079871	DKFZP564F0522 protein	23060
202178_at	-4.4303479	protein kinase C, zeta	407181
210279_at	-4.431287	G protein-coupled receptor 18	88269
202726_at	-4.4373616	ligase I, DNA, ATP-dependent	1770
214298_x_at	-4.4417332	septin 6	
207426_s_at	-4.4487533	tumor necrosis factor (ligand) superfamily, member 4 (tax-transcriptionally activated glycoprotein 1, 34kDa)	181097
212126_at	-4.4504669	Homo sapiens, clone IMAGE:5288883, mRNA	149466
206150_at	-4.4509904	tumor necrosis factor receptor superfamily, member 7	355307
209282_at	-4.4662679	protein kinase D2	205431
212313_at	-4.4669031	hypothetical protein MGC29816	5019
205379_at	-4.4687636	carbonyl reductase 3	154510
217961_at	-4.4688902	hypothetical protein FLJ20551	7994
219843_at	-4.4747042	intracisternal A particle-promoted polypeptide	157180
219826_at	-4.4750415	hypothetical protein FLJ23233	98593
209682_at	-4.4805853	Cas-Br-M (murine) ecotropic retroviral transforming sequence b	436986
221790_s_at	-4.4853949	LDL receptor adaptor protein	184482
203408_s_at	-4.4896587	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)	416026
210389_x_at	-4.4925237	likely ortholog of mouse tubulin, delta 1	270847
221601_s_at	-4.5045431	regulator of Fas-induced apoptosis	58831
202478_at	-4.5256195	tribbles homolog 2	155418
214439_x_at	-4.5286267	bridging integrator 1	193163
36545_s_at	-4.5486784	KIAA0542 gene product	62209
211596_s_at	-4.571525	leucine-rich repeats and immunoglobulin-like domains 1	166697
213587_s_at	-4.5921811	chromosome 7 open reading frame 32	351612
203717_at	-4.6064222	dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	44926
203648_at	-4.6075718	KIAA0218 gene product	75863
218723_s_at	-4.6189582	RGC32 protein	76640
201528_at	-4.6259618	replication protein A1, 70kDa	84318
202107_s_at	-4.6331766	MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)	57101
32259_at	-4.665109	enhancer of zeste homolog 1 (Drosophila)	194669
221211_s_at	-4.6673208	chromosome 21 open reading frame 7	41267
201313_at	-4.6724774	enolase 2, (gamma, neuronal)	511915
221234_s_at	-4.6843954	BTB and CNC homology 1, basic leucine zipper transcription factor 2	88414

46665_at	-4.6856302	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	7188
219590_x_at	-4.6895774	CGI-30 protein	406051
203965_at	-4.6942774	ubiquitin specific protease 20	5452
205042_at	-4.7104355	UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase	5920
205233_s_at	-4.7127287	platelet-activating factor acetylhydrolase 2, 40kDa	477083
209881_s_at	-4.723093	linker for activation of T cells	498997
210201_x_at	-4.7310315	bridging integrator 1	193163
208795_s_at	-4.7315545	MCM7 minichromosome maintenance deficient 7 (S. cerevisiae)	438720
206829_x_at	-4.7446912	zinc finger protein 430	309348
215785_s_at	-4.7595567	cytoplasmic FMR1 interacting protein 2	211201
206337_at	-4.7716012	chemokine (C-C motif) receptor 7	1652
214177_s_at	-4.7755992	pre-B-cell leukemia transcription factor interacting protein 1	505806
204828_at	-4.7856359	RAD9 homolog A (S. pombe)	240457
205013_s_at	-4.8029535	adenosine A2a receptor	197029
203564_at	-4.8064489	Fanconi anemia, complementation group G	434873
202481_at	-4.811268	short-chain dehydrogenase/reductase 1	17144
205310_at	-4.8291386	hypothetical protein 20D7-FC4	128702
215235_at	-4.830164	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	387905
203956_at	-4.8519414	KIAA0852 protein	143840
214833_at	-4.8566377	KIAA0792 gene product	119387
204957_at	-4.8590232	origin recognition complex, subunit 5-like (yeast)	153138
212414_s_at	-4.8647531	septin 6	90998
213164_at	-4.8698375	mitochondrial ribosomal protein S6	268016
211005_at	-4.9017443	linker for activation of T cells	498997
209670_at	-4.9218273	T cell receptor alpha locus	74647
57082_at	-4.9228846	LDL receptor adaptor protein	184482
203846_at	-4.9250454	tripartite motif-containing 32	236218
200965_s_at	-5.0317556	actin binding LIM protein 1	442540
214808_at	-5.0730906	Homo sapiens cDNA FLJ11958 fis, clone HEMBB1000996.	519791
35147_at	-5.0896447	MCF.2 cell line derived transforming sequence-like	436905
206039_at	-5.1020197	RAB33A, member RAS oncogene family	56294
201677_at	-5.1309712	DC12 protein	458320
221011_s_at	-5.1441771	likely ortholog of mouse limb-bud and heart gene	57209
203062_s_at	-5.1456619	mediator of DNA damage checkpoint 1	433653
207231_at	-5.167881	zinc finger DAZ interacting protein 3	409210
207734_at	-5.2935692	hypothetical protein FLJ20340	272794
202423_at	-5.3252148	MYST histone acetyltransferase (monocytic leukemia) 3	93231
201930_at	-5.3290801	MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	444118
213620_s_at	-5.4358397	intercellular adhesion molecule 2	433303
38269_at	-5.5792458	protein kinase D2	205431
209603_at	-5.9506916	GATA binding protein 3	169946
219798_s_at	-5.9710113	hypothetical protein FLJ20257	178011
210038_at	-6.1736133	protein kinase C, theta	408049

*Positive t-statistic indicates that the gene is upregulated following an ischemic stroke. Negative t-statistic indicates that the gene is downregulated following an ischemic stroke.

^ UniGene ID number is system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location. UniGene numbers can be searched on the NCBI website.

Following Bonferroni correction, 231 gene probes, corresponding to 190 genes, were found to be significant (Table 3). Clear separation of the stroke and control gene expression levels were observed. As shown in Table 3, several genes were upregulated (positive T-statistic, such as a value that is at least 4.73) or downregulated (negative t-statistic, such as a value that is less than -4.73) following an ischemic stroke.

Table 3: Ischemic stroke related-genes using Bonferroni correction.

Affy ID No.	t-statistic*	Gene Name	UniGene ID No. ^
218454_at	7.8939046	hypothetical protein FLJ22662	178470
215049_x_at	7.8695991	CD163 antigen	74076
203645_s_at	7.7927429	CD163 antigen	74076
211404_s_at	7.6192982	amyloid beta (A4) precursor-like protein 2	279518
206120_at	7.6130371	CD33 antigen (gp67)	83731
208771_s_at	7.4480951	leukotriene A4 hydrolase	81118
210872_x_at	7.2957674	growth arrest-specific 7	226133
201328_at	7.196077	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
222173_s_at	7.0181137	TBC1 domain family, member 2	371016
211612_s_at	6.7100761	interleukin 13 receptor, alpha 1	285115
211067_s_at	6.6632809	growth arrest-specific 7	226133
211368_s_at	6.6564605	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
219788_at	6.6357632	paired immunoglobin-like type 2 receptor alpha	122591
202896_s_at	6.6343375	protein tyrosine phosphatase, non-receptor type substrate 1	156114
221210_s_at	6.6307936	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	64896
204924_at	6.6002629	toll-like receptor 2	439608
206488_s_at	6.5474747	CD36 antigen (collagen type I receptor, thrombospondin receptor)	443120
208146_s_at	6.5359521	carboxypeptidase, vitellogenic-like	95594
213006_at	6.5058834	KIAA0146 protein	381058
208923_at	6.4690445	cytoplasmic FMR1 interacting protein 1	26704
208702_x_at	6.4619855	amyloid beta (A4) precursor-like protein 2	279518
204452_s_at	6.452735	frizzled homolog 1 (Drosophila)	94234
205715_at	6.4316015	bone marrow stromal cell antigen 1	169998
216942_s_at	6.4235387	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
218217_at	6.419306	likely homolog of rat and mouse retinoid-inducible serine carboxypeptidase	431107
212192_at	6.4140293	hypothetical protein BC013764	109438
200868_s_at	6.3921161	zinc finger protein 313	144949
202912_at	6.3889633	adrenomedullin	441047
207691_x_at	6.3716999	ectonucleoside triphosphate diphosphohydrolase 1	444105
209124_at	6.322399	myeloid differentiation primary response gene (88)	82116

204620_s_at	6.3107101	chondroitin sulfate proteoglycan 2 (versican)	434488
203535_at	6.2998102	S100 calcium binding protein A9 (calgranulin B)	112405
202878_s_at	6.2900118	complement component 1, q subcomponent, receptor 1	97199
204249_s_at	6.2863054	LIM domain only 2 (rhombotin-like 1)	283063
208872_s_at	6.2665313	polyposis locus protein 1	173119
205603_s_at	6.2533791	diaphanous homolog 2 (Drosophila)	226483
208818_s_at	6.2031095	catechol-O-methyltransferase	240013
205158_at	6.2009402	ribonuclease, RNase A family, 4	283749
200765_x_at	6.1928897	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
220615_s_at	6.1326079	hypothetical protein FLJ10462	134497
202897_at	6.1313157	protein tyrosine phosphatase, non-receptor type substrate 1	156114
204222_s_at	6.1245309	GLI pathogenesis-related 1 (glioma)	511765
201743_at	6.1155498	CD14 antigen	75627
211744_s_at	6.0521758	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
207168_s_at	6.0419796	H2A histone family, member Y	75258
220034_at	6.0415584	interleukin-1 receptor-associated kinase 3	268552
204099_at	6.0275171	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	444445
212335_at	6.0167789	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	334534
211135_x_at	6.0123178	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
203127_s_at	5.9862871	serine palmitoyltransferase, long chain base subunit 2	59403
201041_s_at	5.9752594	dual specificity phosphatase 1	171695
209949_at	5.9749633	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	949
203922_s_at	5.9579176	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	88974
200838_at	5.9562695	cathepsin B	135226
210844_x_at	5.9341934	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
200886_s_at	5.905732	phosphoglycerate mutase 1 (brain)	447492
208949_s_at	5.8880039	lectin, galactoside-binding, soluble, 3 (galectin 3)	411701
211284_s_at	5.8723751	granulin	180577
210992_x_at	5.7814222	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	352642
204860_s_at	5.7675599	Homo sapiens transcribed sequence with strong similarity to protein sp:Q13075 (H.sapiens) BIR1_HUMAN Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein)	508565
212788_x_at	5.7508112	ferritin, light polypeptide	433670
211776_s_at	5.7448982	erythrocyte membrane protein band 4.1-like 3	103839
221731_x_at	5.7407504	chondroitin sulfate proteoglycan 2 (versican)	434488
210225_x_at	5.7405956	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
218404_at	5.7312675	sorting nexin 10	418132
214511_x_at	5.7139856	Fc fragment of IgG, high affinity Ia, receptor for (CD64)	77424
200764_s_at	5.6724223	catenin (cadherin-associated protein), alpha 1,	254321

		102kDa	
210904_s_at	5.6679489	interleukin 13 receptor, alpha 1	285115
201200_at	5.6494608	cellular repressor of E1A-stimulated genes	5710
209189_at	5.6491225	v-fos FBJ murine osteosarcoma viral oncogene homolog	25647
202943_s_at	5.6217726	N-acetylgalactosaminidase, alpha-	75372
201329_s_at	5.6098071	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
200678_x_at	5.5920695	granulin	180577
200839_s_at	5.5911028	cathepsin B	135226
204053_x_at	5.5889098	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	253309
204759_at	5.5751089	chromosome condensation 1-like	27007
217897_at	5.5697271	FXYD domain containing ion transport regulator 6	410748
203973_s_at	5.5691171	KIAA0146 protein	381058
210951_x_at	5.5484656	RAB27A, member RAS oncogene family	298530
216041_x_at	5.5475628	granulin	180577
208454_s_at	5.5419198	plasma glutamate carboxypeptidase	197335
209970_x_at	5.5292079	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
204646_at	5.5021786	dihydropyrimidine dehydrogenase	1602
202990_at	5.4976619	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	282417
218606_at	5.4924926	zinc finger, DHHC domain containing 7	9725
219316_s_at	5.47794	chromosome 14 open reading frame 58	267566
207574_s_at	5.4709451	growth arrest and DNA-damage-inducible, beta	110571
212807_s_at	5.462952	sortilin 1	394609
214875_x_at	5.4629191	amyloid beta (A4) precursor-like protein 2	279518
202446_s_at	5.4579541	phospholipid scramblase 1	348478
210784_x_at	5.416225	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
203561_at	5.4154987	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	352642
210152_at	5.408888	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4	67846
210427_x_at	5.374221	annexin A2	462864
212830_at	5.3739539	EGF-like-domain, multiple 5	236216
204169_at	5.3658872	IMP (inosine monophosphate) dehydrogenase 1	317095
209500_x_at	5.3457527	tumor necrosis factor (ligand) superfamily, member 13	54673
201432_at	5.3369374	catalase	395771
215646_s_at	5.3337393	chondroitin sulfate proteoglycan 2 (versican)	434488
201422_at	5.3321762	interferon, gamma-inducible protein 30	14623
204112_s_at	5.330181	histamine N-methyltransferase	42151
214318_s_at	5.3243137	hypothetical protein CG003	390874
204588_s_at	5.3231924	solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	194693
211366_x_at	5.3228655	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
217865_at	5.2774855	ring finger protein 130	155718
211133_x_at	5.2667742	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
209091_s_at	5.2660794	SH3-domain GRB2-like endophilin B1	136309
209474_s_at	5.2656896	ectonucleoside triphosphate diphosphohydrolase 1	444105

209514_s_at	5.2571756	RAB27A, member RAS oncogene family	298530
211571_s_at	5.254094	chondroitin sulfate proteoglycan 2 (versican)	434488
201426_s_at	5.2533276	vimentin	435800
209069_s_at	5.2359413	H3 histone, family 3B (H3.3B)	180877
208130_s_at	5.2328997	thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A)	444510
220990_s_at	5.2293055	likely ortholog of rat vacuole membrane protein 1	166254
210314_x_at	5.2226225	tumor necrosis factor (ligand) superfamily, member 13	54673
203140_at	5.2122493	B-cell CLL/lymphoma 6 (zinc finger protein 51)	155024
205147_x_at	5.2045679	neutrophil cytosolic factor 4, 40kDa	196352
210101_x_at	5.1985794	SH3-domain GRB2-like endophilin B1	136309
205896_at	5.1985084	solute carrier family 22 (organic cation transporter), member 4	441130
206130_s_at	5.197136	asialoglycoprotein receptor 2	1259
211367_s_at	5.1824911	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
217521_at	5.1760536	histidine ammonia-lyase	190783
212501_at	5.1661262	CCAAT/enhancer binding protein (C/EBP), beta	99029
218013_x_at	5.1602528	dynactin 4 (p62)	328865
209188_x_at	5.1523164	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	348418
202670_at	5.1509752	mitogen-activated protein kinase kinase 1	132311
217492_s_at	5.1487987	phosphatase and tensin homolog (mutated in multiple advanced cancers 1), pseudogene 1	493716
206600_s_at	5.1452293	solute carrier family 16 (monocarboxylic acid transporters), member 5	90911
208959_s_at	5.1384925	thioredoxin domain containing 4 (endoplasmic reticulum)	154023
209073_s_at	5.1251219	numb homolog (Drosophila)	445301
206237_s_at	5.118236	neuregulin 1	172816
209185_s_at	5.116767	insulin receptor substrate 2	143648
211702_s_at	5.0981002	ubiquitin specific protease 32	436133
200742_s_at	5.0925572	ceroid-lipofuscinosis, neuronal 2, late infantile (Jansky-Bielschowsky disease)	429658
214449_s_at	5.0883926	ras homolog gene family, member Q	442989
204834_at	5.0700936	fibrinogen-like 2	351808
204619_s_at	5.0677445	chondroitin sulfate proteoglycan 2 (versican)	434488
208926_at	5.0624784	sialidase 1 (lysosomal sialidase)	118721
201944_at	5.0610548	hexosaminidase B (beta polypeptide)	69293
202727_s_at	5.0520316	interferon gamma receptor 1	180866
211676_s_at	5.0386297	interferon gamma receptor 1	180866
204493_at	5.0317822	BH3 interacting domain death agonist	300825
219015_s_at	5.0301077	uncharacterized hematopoietic stem/progenitor cells protein MDS031	110853
209397_at	5.0300249	malic enzyme 2, NAD(+)-dependent, mitochondrial	75342
217741_s_at	5.0253595	zinc finger protein 216	406096
201044_x_at	5.0162483	dual specificity phosphatase 1	171695
219694_at	5.013375	hypothetical protein FLJ11127	155085
201127_s_at	5.0064345	ATP citrate lyase	387567
209304_x_at	5.001544	growth arrest and DNA-damage-inducible, beta	110571
211395_x_at	4.9985031	Fc fragment of IgG, low affinity IIb, receptor for (CD32)	126384

205786_s_at	4.9968981	integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)	172631
212268_at	4.9939523	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	381167
202787_s_at	4.9906145	mitogen-activated protein kinase-activated protein kinase 3	234521
203888_at	4.9896332	thrombomodulin	2030
221841_s_at	4.9829736	Kruppel-like factor 4 (gut)	376206
201888_s_at	4.9773809	interleukin 13 receptor, alpha 1	285115
200785_s_at	4.9557896	low density lipoprotein-related protein 1 (alpha-2-macroglobulin receptor)	162757
203167_at	4.9520306	tissue inhibitor of metalloproteinase 2	6441
201193_at	4.9498323	isocitrate dehydrogenase 1 (NADP+), soluble	11223
208018_s_at	4.9436874	hemopoietic cell kinase	89555
216202_s_at	4.9129508	serine palmitoyltransferase, long chain base subunit 2	59403
212820_at	4.910653	rabconnectin-3	200828
218092_s_at	4.9105339	HIV-1 Rev binding protein	352962
207654_x_at	4.8995961	down-regulator of transcription 1, TBP-binding (negative cofactor 2)	348418
203746_s_at	4.8929704	holocytochrome c synthase (cytochrome c heme-lyase)	211571
207704_s_at	4.8927493	growth arrest-specific 7	226133
222218_s_at	4.8926469	paired immunoglobin-like type 2 receptor alpha	122591
207980_s_at	4.8812625	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	82071
202917_s_at	4.8743845	S100 calcium binding protein A8 (calgranulin A)	416073
207791_s_at	4.8679359	RAB1A, member RAS oncogene family	227327
222148_s_at	4.8580561	ras homolog gene family, member T1	14202
207275_s_at	4.8529301	fatty-acid-Coenzyme A ligase, long-chain 2	511920
202803_s_at	4.8492222	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	375957
211100_x_at	4.8473744	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
208817_at	4.8450448	catechol-O-methyltransferase	240013
203767_s_at	4.8305016	steroid sulfatase (microsomal), arylsulfatase C, isozyme S	79876
212606_at	4.825363	WD repeat and FYVE domain containing 3	105340
205174_s_at	4.8219593	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)	79033
204714_s_at	4.8187971	coagulation factor V (proaccelerin, labile factor)	30054
221060_s_at	4.8181475	toll-like receptor 4	174312
211999_at	4.8179764	H3 histone, family 3B (H3.3B)	180877
211102_s_at	4.810938	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	149924
216243_s_at	4.8029173	interleukin 1 receptor antagonist	81134
203126_at	4.799087	inositol(myo)-1(or 4)-monophosphatase 2	5753
210785_s_at	4.7969428	chromosome 1 open reading frame 38	10649
204232_at	4.7891571	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	433300
200648_s_at	4.7863792	glutamate-ammonia ligase (glutamine synthase)	442669
218627_at	4.7700567	hypothetical protein FLJ11259	416393
209555_s_at	4.769386	CD36 antigen (collagen type I receptor,	443120

		thrombospondin receptor)	
206034_at	4.7667445	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 8	368077
221581_s_at	4.7543565	Williams-Beuren syndrome chromosome region 5	56607
203799_at	4.7373434	type I transmembrane C-type lectin receptor DCL-1	2441
203041_s_at	4.7345873	lysosomal-associated membrane protein 2	232432
209004_s_at	4.734465	F-box and leucine-rich repeat protein 5	5548
210201_x_at	-4.731032	bridging integrator 1	193163
208795_s_at	-4.731554	MCM7 minichromosome maintenance deficient 7 (S. cerevisiae)	438720
206829_x_at	-4.744691	zinc finger protein 430	309348
215785_s_at	-4.759557	cytoplasmic FMR1 interacting protein 2	211201
206337_at	-4.771601	chemokine (C-C motif) receptor 7	1652
214177_s_at	-4.775599	pre-B-cell leukemia transcription factor interacting protein 1	505806
204828_at	-4.785636	RAD9 homolog A (S. pombe)	240457
205013_s_at	-4.802954	adenosine A2a receptor	197029
203564_at	-4.806449	Fanconi anemia, complementation group G	434873
202481_at	-4.811268	short-chain dehydrogenase/reductase 1	17144
205310_at	-4.829139	hypothetical protein 20D7-FC4	128702
215235_at	-4.830164	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	387905
203956_at	-4.851941	KIAA0852 protein	143840
214833_at	-4.856638	KIAA0792 gene product	119387
204957_at	-4.859023	origin recognition complex, subunit 5-like (yeast)	153138
212414_s_at	-4.864753	septin 6	90998
213164_at	-4.869838	mitochondrial ribosomal protein S6	268016
211005_at	-4.901744	linker for activation of T cells	498997
209670_at	-4.921827	T cell receptor alpha locus	74647
57082_at	-4.922885	LDL receptor adaptor protein	184482
203846_at	-4.925045	tripartite motif-containing 32	236218
200965_s_at	-5.031756	actin binding LIM protein 1	442540
214808_at	-5.073091	Homo sapiens cDNA FLJ11958 fis, clone HEMBB1000996.	397369
35147_at	-5.089645	MCF.2 cell line derived transforming sequence-like	436905
206039_at	-5.10202	RAB33A, member RAS oncogene family	56294
201677_at	-5.130971	DC12 protein	458320
221011_s_at	-5.144177	likely ortholog of mouse limb-bud and heart gene	57209
203062_s_at	-5.145662	mediator of DNA damage checkpoint 1	433653
207231_at	-5.167881	zinc finger DAZ interacting protein 3	409210
207734_at	-5.293569	hypothetical protein FLJ20340	272794
202423_at	-5.325215	MYST histone acetyltransferase (monocytic leukemia) 3	93231
201930_at	-5.32908	MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe) (S. cerevisiae)	444118
213620_s_at	-5.43584	intercellular adhesion molecule 2	433303
38269_at	-5.579246	protein kinase D2	205431
209603_at	-5.950692	GATA binding protein 3	169946
219798_s_at	-5.971011	hypothetical protein FLJ20257	178011
210038_at	-6.173613	protein kinase C, theta	408049

*Positive t-statistic indicates that the gene is upregulated following an ischemic stroke. Negative t-statistic indicates that the gene is downregulated following an ischemic stroke.

^ UniGene ID number is system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a

unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location. UniGene numbers can be searched on the NCBI website.

After multiple comparison correction (MCC) using the Westfall and Young permutation approach, 91 gene probes, corresponding to 82 genes were found to be significantly different (Table 4). As shown in Table 4, several genes were upregulated (positive T-statistic, such as a value that is at least 5.3) or downregulated (negative t-statistic, such as a value that is less than -5.4) following an ischemic stroke.

Table 4: Ischemic stroke related-genes using Westfall and Young correction.

Affy ID #	t-statistic*	Gene Name	UniGene ID No.^
218454_at	7.893904631	hypothetical protein FLJ22662	178470
215049_x_at	7.869599129	CD163 antigen	74076
203645_s_at	7.792742866	CD163 antigen	74076
211404_s_at	7.61929825	amyloid beta (A4) precursor-like protein 2	279518
206120_at	7.613037145	CD33 antigen (gp67)	83731
208771_s_at	7.448095101	leukotriene A4 hydrolase	81118
210872_x_at	7.295767389	growth arrest-specific 7	226133
201328_at	7.196076979	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
222173_s_at	7.01811369	TBC1 domain family, member 2	371016
211612_s_at	6.710076137	interleukin 13 receptor, alpha 1	285115
211067_s_at	6.663280893	growth arrest-specific 7	226133
211368_s_at	6.656460461	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
219788_at	6.635763202	paired immunoglobin-like type 2 receptor alpha	122591
202896_s_at	6.634337453	protein tyrosine phosphatase, non-receptor type substrate 1	156114
221210_s_at	6.630793631	N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	64896
204924_at	6.60026287	toll-like receptor 2	439608
206488_s_at	6.547474681	CD36 antigen (collagen type I receptor, thrombospondin receptor)	443120
208146_s_at	6.535952056	carboxypeptidase, vitellogenic-like	95594
213006_at	6.505883417	KIAA0146 protein	381058
208923_at	6.469044495	cytoplasmic FMR1 interacting protein 1	26704
208702_x_at	6.461985493	amyloid beta (A4) precursor-like protein 2	279518
204452_s_at	6.452734953	frizzled homolog 1 (Drosophila)	94234
205715_at	6.431601459	bone marrow stromal cell antigen 1	169998
216942_s_at	6.423538729	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
218217_at	6.419305978	likely homolog of rat and mouse retinoid-inducible serine carboxypeptidase	431107
212192_at	6.414029336	hypothetical protein BC013764	109438
200868_s_at	6.392116081	zinc finger protein 313	144949
202912_at	6.388963292	adrenomedullin	441047
207691_x_at	6.371699946	ectonucleoside triphosphate diphosphohydrolase 1	444105

209124_at	6.322399002	myeloid differentiation primary response gene (88)	82116
204620_s_at	6.310710071	chondroitin sulfate proteoglycan 2 (versican)	434488
203535_at	6.299810247	S100 calcium binding protein A9 (calgranulin B)	112405
202878_s_at	6.290011829	complement component 1, q subcomponent, receptor 1	97199
204249_s_at	6.286305359	LIM domain only 2 (rhombotin-like 1)	283063
208872_s_at	6.266531252	polyposis locus protein 1	173119
205603_s_at	6.253379078	diaphanous homolog 2 (Drosophila)	226483
208818_s_at	6.203109452	catechol-O-methyltransferase	240013
205158_at	6.200940206	ribonuclease, RNase A family, 4	283749
200765_x_at	6.192889656	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
220615_s_at	6.13260793	hypothetical protein FLJ10462	134497
202897_at	6.131315699	protein tyrosine phosphatase, non-receptor type substrate 1	156114
204222_s_at	6.124530943	GLI pathogenesis-related 1 (glioma)	511765
201743_at	6.115549767	CD14 antigen	75627
211744_s_at	6.052175772	CD58 antigen, (lymphocyte function-associated antigen 3)	75626
207168_s_at	6.04197964	H2A histone family, member Y	75258
220034_at	6.041558439	interleukin-1 receptor-associated kinase 3	268552
204099_at	6.027517093	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 3	444445
212335_at	6.016778906	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	334534
211135_x_at	6.012317836	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
203127_s_at	5.986287131	serine palmitoyltransferase, long chain base subunit 2	59403
201041_s_at	5.975259394	dual specificity phosphatase 1	171695
209949_at	5.974963258	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	949
203922_s_at	5.957917579	cytochrome b-245, beta polypeptide (chronic granulomatous disease)	88974
200838_at	5.956269465	cathepsin B	135226
210844_x_at	5.934193387	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
200886_s_at	5.905731995	phosphoglycerate mutase 1 (brain)	447492
208949_s_at	5.888003927	lectin, galactoside-binding, soluble, 3 (galectin 3)	411701
211284_s_at	5.872375053	granulin	180577
210992_x_at	5.781422168	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	352642
204860_s_at	5.767559943	Homo sapiens transcribed sequence with strong similarity to protein sp:Q13075 (H.sapiens) BIR1_HUMAN Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein)	508565
212788_x_at	5.750811183	ferritin, light polypeptide	433670

211776_s_at	5.744898203	erythrocyte membrane protein band 4.1-like 3	
221731_x_at	5.740750361	chondroitin sulfate proteoglycan 2 (versican)	434488
210225_x_at	5.740595562	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
218404_at	5.731267464	sorting nexin 10	418132
214511_x_at	5.713985599	Fc fragment of IgG, high affinity Ia, receptor for (CD64)	77424
200764_s_at	5.672422269	catenin (cadherin-associated protein), alpha 1, 102kDa	254321
210904_s_at	5.667948907	interleukin 13 receptor, alpha 1	285115
201200_at	5.649460774	cellular repressor of E1A-stimulated genes	5710
209189_at	5.649122471	v-fos FBJ murine osteosarcoma viral oncogene homolog	25647
202943_s_at	5.621772605	N-acetylgalactosaminidase, alpha-	75372
201329_s_at	5.609807116	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	292477
200678_x_at	5.592069508	granulin	180577
200839_s_at	5.591102824	cathepsin B	135226
204053_x_at	5.588909808	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	253309
204759_at	5.575108906	chromosome condensation 1-like	27007
217897_at	5.56972714	FXYD domain containing ion transport regulator 6	410748
203973_s_at	5.569117146	KIAA0146 protein	381058
210951_x_at	5.548465566	RAB27A, member RAS oncogene family	298530
216041_x_at	5.547562803	granulin	180577
208454_s_at	5.541919824	plasma glutamate carboxypeptidase	197335
209970_x_at	5.529207916	caspase 1, apoptosis-related cysteine protease (interleukin 1, beta, convertase)	2490
204646_at	5.502178632	dihydropyrimidine dehydrogenase	1602
202990_at	5.497661918	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	282417
218606_at	5.492492596	zinc finger, DHHC domain containing 7	9725
219316_s_at	5.477939952	chromosome 14 open reading frame 58	267566
207574_s_at	5.470945076	growth arrest and DNA-damage-inducible, beta	110571
212807_s_at	5.462951979	sortilin 1	394609
214875_x_at	5.462919125	amyloid beta (A4) precursor-like protein 2	279518
202446_s_at	5.457954078	phospholipid scramblase 1	348478
210784_x_at	5.416225005	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	511766
203561_at	5.415498696	Fc fragment of IgG, low affinity IIa, receptor for (CD32)	352642
210152_at	5.408887988	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4	67846
210427_x_at	5.374221003	annexin A2	462864
212830_at	5.373953889	EGF-like-domain, multiple 5	236216
204169_at	5.36588724	IMP (inosine monophosphate) dehydrogenase 1	317095
213620_s_at	-5.435839683	intercellular adhesion molecule 2	433303

38269_at	-5.579245846	protein kinase D2	205431
209603_at	-5.950691641	GATA binding protein 3	169946
219798_s_at	-5.971011322	hypothetical protein FLJ20257	178011
210038_at	-6.173613284	protein kinase C, theta	408049

*Positive t-statistic indicates that the gene is upregulated following an ischemic stroke. Negative t-statistic indicates that the gene is downregulated following an ischemic stroke.
^ UniGene ID number is system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location. UniGene numbers can be searched on the NCBI website.

In contrast to the Benjamini and Yekutieli approach, the Westfall and Young approach limits the probability of making even one false positive declaration at 5%. There was a predominant up-regulation pattern with 77/82 genes up-regulated and 5 down-regulated (Table 4).

After PAM correction, 28 gene probes, corresponding to 22 genes were found to be significantly different (Table 5). As shown in Table 5, several genes were upregulated following an ischemic stroke.

Table 5: Ischemic stroke related-genes using PAM correction.

Affymetrix Probe ID	Name and Function
	White Blood Cell Activation and Differentiation
215049_x_at	CD163
218454_at	Hypothetical protein FLJ22662 Laminin A motif
211404_s_at	Amyloid beta (A4) precursor-like protein 2
221210_s_at	N-acetylneuraminate pyruvate lysase
209189_at	v-fos FBJ murine osteosarcoma viral oncogene homolog
204924_at	Toll-like receptor 2
211571_s_at	Chondroitin sulfate proteoglycan 2 (versican)
211612_s_at	Interleukin 13 receptor, alpha 1
201743_at	CD14 antigen
205715_at	Bone marrow stromal cell antigen 1/CD157
202878_s_at	Complement component 1, q subcomponent, receptor 1
219788_at	Paired immunoglobin-like type 2 receptor alpha
214511_x_at	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
	Vascular Repair
203888_at	Thrombomodulin
207691_x_at	Ectonucleoside triphosphate diphosphohydrolase 1
206488_s_at	CD36 antigen (collagen type I receptor, thrombospondin receptor)
	Response to Hypoxia
202912_at	Adrenomedullin
201041_s_at	Dual specificity phosphatase 1
203922_s_at	Cytochrome b-245, beta polypeptide (chronic granulomatous disease)
208771_s_at	Leukotriene A4 hydrolase
201328_at	Erythroblastosis virus E26 oncogene homolog 2 (avian)
209949_at	Neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)

	Response to Altered Cerebral Microenvironment
208818_s_at	Catechol-O-methyltransferase
200648_s_at	Glutamate-ammonia ligase (glutamine ligase)
202917_s_at	S100 calcium binding protein A8 (calgranulin A)
204860_s_at	Neuronal apoptosis inhibitory protein: Homo sapiens transcribed sequence with strong similarity to protein sp:Q13075 (H.sapiens) BIR1_HUMAN Baculoviral IAP repeat-containing protein 1
212807_s_at	Sortilin
202446_s_at	Phospholipid scramblase 1
211067_s_at	Growth-arrest-specific 7
204222_s_at	GLI pathogenesis-related 1 (glioma)

Table 6 provides a summary of the number of ischemic-stroke related genes found using different correction methods.

Table 6: Number of genes different between stroke and control subjects by multiple comparison correction filter.*

Multiple comparison filter	No. of genes
PAM dataset	22
Westfall and Young dataset	82
Bonferroni correction set	231
Benjamini & Yekutieli set	771
Raw p value list	5060

*There were 22,283 gene probes on the microarray. The most conservative multiple comparison correction is the PAM dataset, then the Westfall and Young and Bonferroni dataset followed by the Benjamini & Yekutieli dataset.

Example 4

Classes of Gene Expression Increased Following Ischemic Stroke

This example describes the four classes of genes whose expression was increased following ischemic stroke, based on the results obtained in Example 3.

A number of broad classes of gene expression were found (representative examples are shown in Table 5 above). The first were genes that indicated differentiation of monocytes into macrophages and lymphocyte activation (for example, CD14, toll-like receptor 2 and FcR2a). Concomitantly, a number of genes for cell cycle arrest were up-regulated. Some other up-regulated genes were for cytoskeletal proteins (for example, alpha-catenin and galectin 3) involved in anchoring of white blood cells to tissue.

The second main grouping was related to hypoxia, many being inducible by hypoxia inducible factor-1 (for example, adrenomedullin, FcR2a and CD14). There may be a common promoter region for hypoxia inducible factor-1.

A third class of genes is related to vascular repair. For example, up-regulation of ectonucleoside triphosphate diphosphohydrolase 1 results in decreased platelet interaction and aggregation.

The fourth broad class of genes is related to a specific PBMC response to the altered cerebral microenvironment.

Surprisingly, no specific steroid stress-related genes were identified.

In summary, the gene classes demonstrate both specific and non-specific gene expression in PBMCs during acute ischemic stroke. The finding of genes induced by hypoxic stress, vascular repair genes and neuronal specific genes demonstrates a specific response to ischemic stroke.

Example 5

Predicting Severity and Neurological Recovery of Ischemic Stroke

This example describes methods used to analyze PBMCs isolated from 26 subjects at three time-points following ischemic stroke, to demonstrate that there is a correlation between recovery and alterations in gene expression.

Expression of the 22 genes listed in Table 5 was determined using the methods described in the above examples in a second and independent series of 26 patients studied two years after the initial series. These patients had blood samples drawn at day 1 (withing 24 hours of onset of symptoms), day 7-14 and day 90 post stroke (26 subjects had blood draws at day 1, 25 subjects had a blood draw at day 7-14 and 21 subjects had a blood draw at day 90 [some patients were deceased by this time]). At day 1, detecting differential gene expression in the 22 genes accurately classified 81% of subjects (21/26) as having had an ischemic stroke. The 5 subjects classified as control (that is, subjects classified as not having had an ischemic stroke) using the method tended to be younger or to have mild stroke severity scores. These results confirm the diagnostic accuracy of the PAM list (Table 5) for acute stroke diagnosis (shown in Table 9), as this was the second independent series of subjects on which these results have been confirmed.

At days 7-14, detecting differential gene expression in the 22 genes accurately classified 64% of subjects (16/25) as having had an ischemic stroke. At day 90, detecting differential gene expression in the 22 genes accurately classified 62% of subjects (13/21) as having had an ischemic stroke. Without wishing to be bound to a particular theory, it is proposed that the persistent gene changes of ischemic stroke at the day 7-14 and day 90 time points reflects ongoing inflammatory or other processes related to the stroke or a lack of recovery of these processes. Those who remained classified as a stroke at these time points were those with the more severe strokes and worse outcomes (see below).

The recovery of the subjects was compared to their classification determined using the 22 genes listed in Table 5. An excellent recovery was defined as a Barthel score of 100 at three months post stroke (for example see Mahoney *et al.*, *Md. State Med. J.* 14:61-5, 1965). The Barthel score is a measure of 10 activities of daily living such as getting dressed, walking, going to the toilet. The score ranges from 100 (fully independent) to 0 (totally dependent and incapacitated or deceased).

In terms of excellent stroke recovery, all 9/9 (100%) patients who were classified as a control at their last measurement (whether classified as a stroke or a control at the first time point) had excellent recovery. In contrast only 8/17 (47%) patients who remained classified as a stroke at their last follow-up measurement (at day 7-14 in 3 patients who died and day 90 in the remaining)

had excellent stroke recovery (p=0.008). This indicates persistence of the stroke state is related to changes in gene expression.

Therefore, it appears that the reason that some of the subjects were indicated to not have had an ischemic stroke is that they recovered by day 90. Therefore, the disclosed ischemic stroke related molecules, such as those listed in Tables 2-5, for example those listed in Table 5 can be used to be determine the prognosis of a subject who has had an ischemic stroke.

In view of these results, disclosed are methods of stratifying the seriousness of a stroke, and assessing the likely neurological recovery of the subject. For example, stratification or assessing the likely neurological recovery of the subject can be performed as early as one day (or within 24 hours) after the ischemic stroke, 7-14 days after the ischemic stroke, or 90 days after the ischemic stroke. In particular examples, the method includes detecting differential expression in at least four ischemic stroke-related molecules, such as at least the 22 genes (or corresponding proteins) listed in Table 5. Detection of increased expression of at least four ischemic stroke-related molecules, such as at least the 22 genes (or corresponding proteins) listed in Table 5, indicates that the stroke was severe and the subject has a lower probability of neurological recovery (for example as compared to an amount of expected neurological recovery in a subject who did not have increased expression of the 22 genes/proteins listed in Table 5). In particular examples, the increased expression is determined by calculating a t-statistic value, wherein a t-statistic value of at least 3, at least 5.3, or at least 6 indicates that expression is increased.

In particular examples, the assay results can predict a Barthel score of at least 45, for example at least 50, 90 or 100, as an indicatatin of neurological recovery.

Example 6

Temporal Relationship of Evaluating a Stroke

This example describes the temporal relationship of the disclosed methods to the stroke or suspected stroke. The assay can be performed following the onset of signs and symptoms associated with ischemic stroke. Particular examples of signs and symptoms associated with ischemic stroke include but are not limited to: headache, sensory loss (such as numbness, particularly confined to one side of the body or face), paralysis (such as hemiparesis), pupillary changes, blindness (including bilateral blindness), ataxia, memory impairment, dysarthria, somnolence, and other effects on the central nervous system recognized by those of skill in the art.

A sample can be obtained from the subject (such as a PBMC sample) and analyzed using the disclosed methods, for example, within 1 hour, within 6 hours, within 12 hours, or even within 24 hours of having signs or symptoms associated with ischemic stroke, In another example, a sample is obtained at least 7 days later following the onset of signs and symptoms associated with ischemic stroke, such as within 7-14 days of having signs or symptoms associated with ischemic stroke, or within 90 days.

In particular examples, the assay can be performed after a sufficient period of time for the differential regulation of the genes (or proteins) to occur, for example at least 24 hours after onset of

the symptom or constellation of symptoms that have indicated a potential cerebral ischemic event. In other examples it occurs prior to performing any imaging tests are performed to find anatomic evidence of ischemic stroke. Moreover, it is often difficult for imaging modalities (such as CT and MRI) to detect acute ischemic strokes, at least until brain changes (such as edema) have taken place in response to the ischemia. Hence the assay described herein in particular examples is able to detect the ischemic stroke even before definitive brain imaging evidence of the stroke is known.

Since the results of this assay are also highly reliable predictors of the ischemic nature of the stroke, the results of the assay can also be used (for example in combination with other clinical evidence and brain scans) to determine whether thrombolytic therapy designed to lyse a neurovascular occlusion such as a thrombus (for example by using tissue plasminogen activator or streptokinase) should be administered to the subject. In certain example, thrombolytic therapy is given to the subject once the results of the differential gene assay are known if the assay provides an indication that the stroke is ischemic in nature.

Moreover, the neurological sequelae of an ischemic event in the central nervous system can have consequences that range from the insignificant to the devastating, and the disclosed assay permits early and accurate stratification of risk of long-lasting neurological impairment. For example, a test performed as early as within the first 24 hours of onset of signs and symptoms of a stroke, and even as late as 7-14 days or even as late as 90 days or more after the event can provide clinical data that is highly predictive of the eventual care needs of the subject.

The disclosed assay is also able to identify subjects who have had an ischemic stroke in the past, for example more than 2 weeks ago, or even more than 90 days ago. The identification of such subjects helps evaluate other clinical data (such as neurological impairment or brain imaging information) to determine whether an ischemic stroke has occurred. Subjects identified or evaluated in this manner can then be provided with appropriate treatments, such as anti-platelet agents (for example aspirin) that would be appropriate for a subject identified as having had an ischemic stroke but not as appropriate for subject who have had a hemorrhagic stroke. It is helpful to be able to classify subject as having had an ischemic stroke, because the treatments for ischemic stroke are often distinct from the treatments for hemorrhagic stroke. In fact, treating a hemorrhagic stroke with a therapy designed for an ischemic stroke (such as a thrombolytic agent) can have devastating clinical consequences. Hence using the results of the disclosed assay to help distinguish ischemic from hemorrhagic stroke offers substantial clinical benefit, and allows subjects to be selected for treatments appropriate to ischemic stroke but not hemorrhagic stroke.

Example 7

Quantitative real time polymerase chain reaction

This example describes the use of quantitative real time polymerase chain reaction to confirm results obtained using the microarrays.

Quantitative real time polymerase chain reaction of gene expression levels were performed using RNA samples from 10 patients and 9 controls. Nine genes were selected for analysis on the

basis of their significantly high expression in the index set. One further gene, not up-regulated in the permutation dataset was selected as a negative control. Primers were obtained from the published literature and ordered from Invitrogen (Carlsbad, CA) as listed in Table 7.

Table 7: Primers for real time-PCR

Gene	GenBank ID No	Primer Forward*	Primer reverse*
Adreno-medullin	NM_001124	CGAAAGAAGTGGAATAAGTGGGC (1)	CCGCAGTTCCCTCTTCCC (2)
CD14	NM_000591	CAAGGTACTGAGCATTGCCCA (3)	TGTTCGCAGGAAAAGGCAG (4)
CD36	M24795	GATGCAGCCTCATTTCCACCT (5)	AGGCCTTGGATGGAAGAACA (6)
Caspase 1	NM_033292	GACCCGAGCTTTGATTGACTCC (7)	TTGATCTGCTGAGAGTCCCAGC (8
a-Catenin	BC000385	GATGACCGTCGTGAGCGAATT (9)	TTACGTCCAGCATTGCCCA (10)
FcR2a	NM_021642	GACTGTGCTTTCCGAATGGCT (11)	TGACCTTGACCAGAGGCTTGTC (1
FcER1a	NM_002001.	AGATGGCGTGTTAGCAGTCCCT (13)	GCCATTGTGGAACCATTTGG (14)
Cathepsin B	NM_147781	CTGGCTGGTTGCCAACTCC (15)	AAAGAAGCCATTGTCACCCCA (16
TRL2	BC033756.	TCGGCGTTCTCTCAGGTGAC (17)	TGCAACACCAAACACTGGGAG (1
INFGR1	BC005333	AGAATTTGCTGTATGCCGAGATG (19)	TGATATCCAGTTTAGGTGGTCCA (20)

*SEQ ID NOS: shown in parenthesis.

Real time PCR was performed with an Opticon 2 (MJ research). Real time PCR results between patients and controls were compared using non-parametric statistics (Mann Whitney U tests).

As shown in Table 8, expression values derived from the microarrays correlated with RT-PCR for 9 up-regulated genes. Using RT-PCR, higher values for 8/9 genes in the up-regulated list were found, with a significant difference in 7/9 genes between 10 patients and 9 controls. A negative control was also included (gene not up-regulated in the permutation dataset) with no significant difference observed between patients who suffered a stroke and controls.

Table 8: Correlation of expression data with real time-PCR values

Gene Name	*Genbank ID*	*Median Patients n=10*	*Median Controls n=9*	*p*
Up-regulated in Westfall and Young Set				
Adrenomedullin	NM_001124	1.295	0.39	0.0015
CD14	NM_000591	2.207	1.094	0.0003
CD36	M24795	2.08	1.23	0.02
Caspase 1	NM_033292	14.24	6.62	0.0041
a-Catenin	BC000385	2.559	1.5487	0.0789
FcR2a	NM_021642	0.58	0.26	0.003
FcER1a	NM_002001	2.655	2.87	0.9048
Cathepsin B	NM_147781	0.9	0.32	0.0041
Toll-like receptor 2	BC033756	0.4939	0.1561	0.0021
Not Up-regulated in Westfall and Young Set				
INFGR1	BC005333	0.985	0.64	0.1128

Using data from 9 patients and 10 controls and the PAM, stroke was prospectively classified with a sensitivity of 78% and a specificity of 80% (Table 9).

Table 9: Accuracy of training dataset in the prediction of stroke.*

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
No.	7/9	8/10	7/9	8/10
%	78	80	78	80

*An independent cohort of 9 stroke patients and 10 controls was used. Using a nearest shrunken centroid algorithm, stroke was classified with a sensitivity of 78% and a specificity of 80%.

In summary, a distinct genomic profile of acute ischemic stroke in the peripheral blood mononuclear cells was identified. In addition, four broad classes of ischemic stroke related genes were identified that are upregulated following an ischemic stroke: white blood cell activation and differentiation genes, genes associated with hypoxia, vascular repair genes and genes associated with an altered cerebral microenvironment, including neuronal apoptosis inhibitory protein.

Example 8

Array for Evaluating a Stroke

This example describes particular arrays that can be used to evaluate a stroke, for example to diagnose an ischemic stroke.

In one example, the array includes probes (such as an oligonucleotide or antibody) that can recognize at least one gene (or protein) that is upregulated following an ischemic stroke, such as one or more of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, or any 1, 2, 3, 4, 5, or 6 of these. For example, the array can include a probe (such as an oligonucleotide or antibody) recognizes CD163. In yet another example, the array includes probes (such as an oligonucleotide or antibody) that can recognize at least one gene (or protein) that is downregulated following an ischemic stroke, such as one or more of intercellular adhesion molecule 2; protein kinase D2; GATA binding protein 3; hypothetical protein FLJ20257; or protein kinase C, theta. In a particular example, the array includes probes (such as an oligonucleotide or antibody) that can recognize at least one gene (or protein) that is upregulated following an ischemic stroke (such as at least one of CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146) and at least one gene (or protein) that is downregulated following an ischemic stroke (such as one or more of intercellular adhesion molecule 2; protein kinase D2; GATA binding protein 3; hypothetical protein FLJ20257; or protein kinase C, theta).

Other examplary probes that can be used are listed in Tables 2-5 and are identified by their Affymetrix identification number. The disclosed oligonucleotide probes can further include one or more detectable labels, to permit detection of hybridization signals between the probe and a target sequence.

In one example, the array includes probes (such as an oligonucleotide or antibody) that recognize any combination of at least four different genes (or proteins) listed in Tables 2-5. In particular examples, the array includes probes recognize all 22 genes (or proteins) listed in Table 5. The accuracy of the PAM list (Table 5) to diagnose ischemic acute stroke has been confirmed in two independent series of subjects. The ability of the PAM list (Table 5) to provide an indication of the severity of the stroke and to determine the likelihood of neurological recovery has also been demonstrated. In some examples, the array includes oligonucleotides, proteins, or antibodies that recognize any combination of at least one gene from each of the four classes listed in Table 5 (such as at least 2 or at least 3 genes from each class).

In another example, the array includes probes (such as an oligonucleotide or antibody) that recognize any combination of at least 150 different genes listed in Table 3 or all 190 genes listed in Table 3. In yet another example, the array includes probes that recognize at least 500 different genes listed in Table 2. In particular examples, the probes recognize all 637 genes listed in Table 2.

Compilation of "loss" and "gain" of hybridization signals will reveal the genetic status of the individual with respect to the ischemic stroke-associated genes listed in Tables 2-5.

Example 9

Quantitative Spectroscopic Methods

This example describes quantitative spectroscopic approaches methods, such as SELDI, that can be used to detect differential protein expression of ischemic stroke related proteins.

In one example, surface-enhanced laser desorption-ionization time-of-flight (SELDI-TOF) mass spectrometry is used to detect changes in differential protein expression, for example by using the ProteinChip™ (Ciphergen Biosystems, Palo Alto, CA). Such methods are well known in the art (for example see U.S. Pat. No. 5,719,060; U.S. Pat. No. 6,897,072; and U.S. Pat. No. 6,881,586, all herein incorporated by reference). SELDI is a solid phase method for desorption in which the analyte is presented to the energy stream on a surface that enhances analyte capture or desorption.

Briefly, one version of SELDI uses a chromatographic surface with a chemistry that selectively captures analytes of interest, such as ischemic stroke related proteins. Chromatographic surfaces can be composed of hydrophobic, hydrophilic, ion exchange, immobilized metal, or other chemistries. For example, the surface chemistry can include binding functionalities based on oxygen-dependent, carbon-dependent, sulfur-dependent, and/or nitrogen-dependent means of covalent or noncovalent immobilization of analytes. The activated surfaces are used to covalently immobilize specific "bait" molecules such as antibodies, receptors, or oligonucleotides often used for biomolecular interaction studies such as protein-protein and protein-DNA interactions.

The surface chemistry allows the bound analytes to be retained and unbound materials to be washed away. Subsequently, analytes bound to the surface (such as ischemic stroke related proteins) can be desorbed and analyzed by any of several means, for example using mass spectrometry. When the analyte is ionized in the process of desorption, such as in laser desorption/ionization mass spectrometry, the detector can be an ion detector. Mass spectrometers generally include means for

determining the time-of-flight of desorbed ions. This information is converted to mass. However, one need not determine the mass of desorbed ions to resolve and detect them: the fact that ionized analytes strike the detector at different times provides detection and resolution of them. Alternatively, the analyte can be detectably labeled (for example with a fluorophore or radioactive isotope). In these cases, the detector can be a fluorescence or radioactivity detector. A plurality of detection means can be implemented in series to fully interrogate the analyte components and function associated with retained molecules at each location in the array.

Therefore, in a particular example, the chromatographic surface includes antibodies that recognize ischemic stroke related proteins. In one example, antibodies are immobilized onto the surface using a bacterial Fc binding support. The chromatographic surface is incubated with a sample from the subject, such as a sample that includes PMBC proteins (such as a PBMC lysate). The antigens present in the sample can recognize the antibodies on the chromatographic surface. The unbound proteins and mass spectrometric interfering compounds are washed away and the proteins that are retained on the chromatographic surface are analyzed and detected by SELDI-TOF. The MS profile from the sample can be then compared using differential protein expression mapping, whereby relative expression levels of proteins at specific molecular weights are compared by a variety of statistical techniques and bioinformatic software systems.

Example 10

Nucleic Acid-Based Analysis

The ischemic stroke-related nucleic acid molecules provided herein (such as those disclosed in Tables 2-5) can be used in evaluating a stroke, for example for determining whether a subject has had an ischemic stroke, determining the severity or likely neurological recovery of a subject who has had an ischemic stroke, and determining a treatment regimen for a subject who has had an ischemic stroke. For such procedures, a biological sample of the subject is assayed for an increase or decrease in expression of ischemic stroke-related nucleic acid molecules, such as those listed in Tables 2-5. Suitable biological samples include samples containing genomic DNA or RNA (including mRNA) obtained from cells of a subject, such as those present in peripheral blood, urine, saliva, tissue biopsy, surgical specimen, amniocentesis samples and autopsy material. In a particular example, the sample includes PBMCs (or components thereof, such as nucleic acids or proteins isolated from PBMCs).

The detection in the biological sample of increased or decreased expression in four or more ischemic stroke-related nucleic acid molecules, such any combination of four or more molecules listed in Table 5, 150 or more molecules listed in Table 3, or 500 or more molecules listed in Table 2, can be achieved by methods known in the art. In some examples, expression is determined for any combination of at least one gene from each class listed in Table 5 (such as at least 2 or at least 3 genes from each class). In some examples, expression is determined for at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146.

Increased or decreased expression of an ischemic stroke-related molecule also can be detected by measuring the cellular level of ischemic stroke-related nucleic acid molecule-specific mRNA. mRNA can be measured using techniques well known in the art, including for instance Northern analysis, RT-PCR and mRNA *in situ* hybridization. Details of mRNA analysis procedures can be found, for instance, in provided examples and in Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Oligonucleotides specific to ischemic stroke-related sequences can be chemically synthesized using commercially available machines. These oligonucleotides can then be labeled, for example with radioactive isotopes (such as ^{32}P) or with non-radioactive labels such as biotin (Ward and Langer *et al., Proc. Natl. Acad. Sci. USA* 78:6633-57, 1981) or a fluorophore, and hybridized to individual DNA samples immobilized on membranes or other solid supports by dot-blot or transfer from gels after electrophoresis. These specific sequences are visualized, for example by methods such as autoradiography or fluorometric (Landegren *et al., Science* 242:229-37, 1989) or colorimetric reactions (Gebeyehu *et al., Nucleic Acids Res.* 15:4513-34, 1987).

Nucleic acid molecules isolated from PBMCs can be amplified using routine methods to form nucleic acid amplification products. These nucleic acid amplification products can then be contacted with an oligonucleotide probe that will hybridize under stringent conditions with an ischemic stroke-related nucleic acid. The nucleic acid amplification products which hybridize with the probe are then detected and quantified. The sequence of the oligonucleotide probe can bind specifically to a nucleic acid molecule represented by the sequences listed in Tables 2-5.

Example 11

Protein-Based Analysis

This example describes methods that can be used to detect changes in expression of ischemic stroke-related proteins. Ischemic stroke-related protein sequences can be used in methods of evaluating a stroke, for example for determining whether a subject has had an ischemic stroke, determining the severity or likely neurological recovery of a subject who has had an ischemic stroke, and determining a treatment regimen for a subject who has had an ischemic stroke. For such procedures, a biological sample of the subject is assayed for a change in expression (such as an increase or decrease) of any combination of at least four ischemic stroke-related proteins, such as any combination of at least four of those listed in Table 5, at least 150 of those listed in Table 3, or at least 500 of those listed in Table 2. In some examples, protein expression is determined for any combination of at least one gene from each of the four classes of genes listed in Table 5 (such as at least 2 or at least 3 genes from each of the four classes of genes listed in Table 5). In some examples, protein expression is determined for at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146.

Suitable biological samples include samples containing protein obtained from cells of a subject, such as those present in peripheral blood. A change in the amount of four or more ischemic

stroke-related proteins in a subject, such as an increase in four or more ischemic stroke-related proteins listed in Table 5, can indicate that the subject has suffered an ischemic stroke.

The determination of increased or decreased ischemic stroke-related protein levels, in comparison to such expression in a normal subject (such as a subject who has not previously had an ischemic stroke), is an alternative or supplemental approach to the direct determination of the expression level of ischemic stroke-related nucleic acid sequences by the methods outlined above. The availability of antibodies specific to ischemic stroke-related protein(s) will facilitate the detection and quantitation of ischemic stroke-related protein(s) by one of a number of immunoassay methods that are well known in the art, such as those presented in Harlow and Lane (*Antibodies, A Laboratory Manual*, CSHL, New York, 1988). Methods of constructing such antibodies are known in the art.

Any standard immunoassay format (such as ELISA, Western blot, or RIA assay) can be used to measure ischemic stroke-related protein levels. A comparison to wild-type (normal) ischemic stroke-related protein levels and an increase or decrease in ischemic stroke-related polypeptide levels (such as an increase in any combination of at least 4 proteins listed in Table 5 or a decrease in any combination of at least 4 proteins listed in Tables 2-4 with a negative t-statistic) is indicative of ischemic stroke. Immunohistochemical techniques can also be utilized for ischemic stroke-related protein detection and quantification. For example, a tissue sample can be obtained from a subject, and a section stained for the presence of an ischemic stroke-related protein using the appropriate ischemic stroke-related protein specific binding agents and any standard detection system (such as one that includes a secondary antibody conjugated to horseradish peroxidase). General guidance regarding such techniques can be found in Bancroft and Stevens (*Theory and Practice of Histological Techniques*, Churchill Livingstone, 1982) and Ausubel *et al.* (*Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998).

For the purposes of quantitating ischemic stroke-related proteins, a biological sample of the subject that includes cellular proteins can be used. Quantitation of an ischemic stroke-related protein can be achieved by immunoassay and the amount compared to levels of the protein found in cells from a subject who has not had an ischemic stroke. A significant increase in the amount of four or more ischemic stroke-related proteins in the cells of a subject compared to the amount of the same ischemic stroke-related protein found in normal human cells is usually at least 2-fold, at least 3-fold, at least 4-fold or greater difference. Substantial overexpression of four or more ischemic stroke-related protein(s) can be indicative of an ischemic stroke. Similarly, a significant decrease in the amount of four or more ischemic stroke-related proteins in the cells of a subject compared to the amount of the same ischemic stroke-related protein found in normal human cells is usually at least 2-fold, at least 3-fold, at least 4-fold or greater difference. Substantial underexpression of four or more ischemic stroke-related protein(s) can be indicative of an ischemic stroke or poor prognosis.

An alternative method of evaluating a stroke is to quantitate the level of four or more ischemia-related proteins in a subject, for instance in the cells of the subject. This diagnostic tool is useful for detecting reduced or increased levels of ischemia-related proteins, for instance, though specific techniques can be used to detect changes in the size of proteins, for instance. Localization or

coordinated expression (temporally or spatially) of ischemia-related proteins can also be examined using well known techniques.

Example 12

Kits

Kits are provided for evaluating a stroke, for example for determining whether a subject has had an ischemic stroke, determining the severity or likely neurological recovery of a subject who has had an ischemic stroke, and determining a treatment regimen for a subject who has had an ischemic stroke (such as kits containing ischemic stroke detection arrays). Kits are also provided that contain the reagents need to detect hybridization complexes formed between oligonucleotides on an array and ischemic stroke-related nucleic acid molecules obtained from a subject, or between proteins or antibodies on an array and proteins obtained from a subject suspected of having had (or known to have had) an ischemic stroke. These kits can each include instructions, for instance instructions that provide calibration curves or charts to compare with the determined (such as experimentally measured) values. The disclosed kits can include reagents needed to determine gene copy number (genomic amplification or deletion), such as probes or primers specific for an ischemia-related nucleic acid sequence.

Kits are provided to determine the level (or relative level) of expression or of any combination of four or more ischemic stroke-related nucleic acids (such as mRNA) or ischemic stroke-related proteins (such as kits containing nucleic acid probes, proteins, antibodies, or other ischemic stroke-related protein specific binding agents) listed in Tables 2-5.

Kits are provided that permit detection of ischemic stroke-related mRNA expression levels (including over- or under-expression, in comparison to the expression level in a control sample). Such kits include an appropriate amount of one or more of the oligonucleotide primers for use in, for instance, reverse transcription PCR reactions, and can also include reagents necessary to carry out RT-PCR or other *in vitro* amplification reactions, including, for instance, RNA sample preparation reagents (such as an RNAse inhibitor), appropriate buffers (such as polymerase buffer), salts (such as magnesium chloride), and deoxyribonucleotides (dNTPs).

In some examples, kits are provided with the reagents needed to perform quantitative or semi-quantitative Northern analysis of ischemic stroke-related mRNA. Such kits can include at least four ischemic stroke-related sequence-specific oligonucleotides for use as probes. Oligonucleotides can be labeled, for example with a radioactive isotope, enzyme substrate, co-factor, ligand, chemiluminescent or fluorescent agent, hapten, or enzyme.

Kits are provided that permit detection of ischemic stroke-related genomic amplification or deletion. Nucleotide sequences encoding an ischemic stroke-related protein, and fragments thereof, can be supplied in the form of a kit for use in detection of ischemic stroke-related genomic amplification/deletion or diagnosis of an ischemic stroke, progression of an ischemic stroke, or therapy assessment for subjects who have suffered an ischemic stroke. In examples of such a kit, an appropriate amount of one or more oligonucleotide primers specific for an ischemic stroke-related-

sequence (such as those listed in Table 1) is provided in one or more containers. The oligonucleotide primers can be provided suspended in an aqueous solution or as a freeze-dried or lyophilized powder, for instance. The container(s) in which the oligonucleotide(s) are supplied can be any conventional container that is capable of holding the supplied form, for instance, microfuge tubes, ampoules, or bottles. In some applications, pairs of primers are provided in pre-measured single use amounts in individual, typically disposable, tubes, or equivalent containers. With such an arrangement, the sample to be tested for the presence of ischemic stroke-related genomic amplification/deletion can be added to the individual tubes and *in vitro* amplification carried out directly.

The amount of each primer supplied in the kit can be any amount, depending for instance on the market to which the product is directed. For instance, if the kit is adapted for research or clinical use, the amount of each oligonucleotide primer provided is likely an amount sufficient to prime several *in vitro* amplification reactions. Those of ordinary skill in the art know the amount of oligonucleotide primer that is appropriate for use in a single amplification reaction. General guidelines can be found in Innis *et al.* (*PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990), Sambrook *et al.* (In *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor, New York, 1989), and Ausubel *et al.* (In *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, 1998).

A kit can include more than two primers to facilitate the *in vitro* amplification of ischemic stroke-related genomic sequences, such as those listed in Tables 2-5, or the 5' or 3' flanking region thereof.

In some examples, kits also include the reagents needed to perform *in vitro* amplification reactions, such as DNA sample preparation reagents, appropriate buffers (for example polymerase buffer), salts (for example magnesium chloride), and deoxyribonucleotides (dNTPs). Written instructions can also be included. Kits can further include labeled or unlabeled oligonucleotide probes to detect the *in vitro* amplified sequences. The appropriate sequences for such a probe will be any sequence that falls between the annealing sites of two provided oligonucleotide primers, such that the sequence the probe is complementary to is amplified during the *in vitro* amplification reaction (if it is present in the sample).

One or more control sequences can be included in the kit for use in the *in vitro* amplification reactions. The design of appropriate positive and negative control sequences is well known to one of ordinary skill in the art.

In particular examples, a kit includes an array with oligonucleotides (or antibodies) that recognize any combination of at least four ischemic stroke-related sequences, such as any combination of at least four of those listed in Table 5, at least 22 of those listed in Table 5, at least 150 of those listed in Table 3, or at least 500 of those listed in Table 2. In one example, the array includes oligonucleotides (or antibodies) that recognize at least 1, at least 2, at least 3, at least 4, at least 5, or at least 6 of the following: CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146. For example, the array can include oligonucleotides (or antibodies) that recognize at least CD163; hypothetical protein

FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146.

In some examples, the array includes agents (such as oligonucleotides, proteins, or antibodies) that can recognize any combination of at least one gene (or protein) from each class listed in Table 5 (such as at least 2 or at least 3 genes (or proteins) from each class). The array can include other oligonucleotides, for example to serve as negative or positive controls. The oligonucleotides that recognize ischemic stroke-related and control sequences can be on the same array, or on different arrays. Particular arrays are disclosed in Examples 7-9.

Kits are also provided for the detection of ischemic stroke-related protein expression, for instance increased expression of any combination of at least 4 proteins listed in Table 5. Such kits include one or more ischemic stroke-related proteins (full-length, fragments, or fusions) or specific binding agent (such as a polyclonal or monoclonal antibody or antibody fragment), and can include at least one control. The ischemic stroke-related protein specific binding agent and control can be contained in separate containers. The kits can also include agents for detecting ischemic stroke-related protein:agent complexes, for instance the agent can be detectably labeled. If the detectable agent is not labeled, it can be detected by second antibodies or protein A, for example, either of both of which also can be provided in some kits in one or more separate containers. Such techniques are well known.

Additional components in some kits include instructions for carrying out the assay. Instructions permit the tester to determine whether ischemic stroke-linked expression levels are elevated, reduced, or unchanged in comparison to a control sample. Reaction vessels and auxiliary reagents such as chromogens, buffers, enzymes, etc. can also be included in the kits.

Example 13

Gene Expression Profiles (Fingerprints)

With the disclosure of many ischemic stroke-related molecules (as represented for instance by those listed in Tables 2-5), gene expression profiles that provide information on evaluating a stroke, for example for determining whether a subject has had an ischemic stroke, determining the severity or likely neurological recovery of a subject who has had an ischemic stroke, and determining a treatment regimen for a subject who has had an ischemic stroke, are now enabled.

Ischemic stroke-related expression profiles include the distinct and identifiable pattern of expression (or level) of sets of ischemic stroke-related genes, for instance a pattern of increased and decreased expression of a defined set of genes, or molecules that can be correlated to such genes, such as mRNA levels or protein levels or activities. The set of molecules in a particular profile can include any combination of at least four of the sequences listed in any of Tables 2-5.

Another set of molecules that could be used in a profile include any combination of at least four sequences listed in Table 5, each of which is overexpressed following an ischemic stroke. For example, an ischemic stroke-related gene expression profile can include one sequence from each of the following four classes of genes: white blood cell activation and differentiation genes, genes

related to hypoxia, genes involved in vascular repair, and genes related to a specific PBMC response to the altered cerebral microenvironment. In another example, the molecules included in the profile include at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, or any one of these.

Yet another example of a set of molecules that could be used in a profile would include any combination of at least 150 of the sequences listed in Table 3, whose expression is upregulated or downregulated following an ischemic stroke. In a particular example, a set of molecules that could be used in a profile would include any combination of at least 500 of the sequences listed in Table 2, whose expression is upregulated or downregulated following an ischemic stroke.

Particular profiles can be specific for a particular stage or age of normal tissue (such as PMBCs). Thus, gene expression profiles can be established for a pre-ischemic stroke tissue (such as normal tissue not subjected to an ischemic challenge or preconditioning) or an ischemic challenged tissue. Each of these profiles includes information on the expression level of at least four or more genes whose expression is altered following an ischemic stroke. Such information can include relative as well as absolute expression levels of specific genes. Likewise, the value measured can be the relative or absolute level of protein expression or protein activity, which can be correlated with a "gene expression level." Results from the gene expression profiles of an individual subject can be viewed in the context of a test sample compared to a baseline or control sample fingerprint/profile.

The levels of molecules that make up a gene expression profile can be measured in any of various known ways, which may be specific for the type of molecule being measured. Thus, nucleic acid levels (such as direct gene expression levels, such as the level of mRNA expression) can be measured using specific nucleic acid hybridization reactions. Protein levels can be measured using standard protein assays, using immunologic-based assays (such as ELISAs and related techniques), or using activity assays. Examples for measuring nucleic acid and protein levels are provided herein; other methods are well known to those of ordinary skill in the art.

Examples of ischemia-related gene expression profiles can be in array format, such as a nucleotide (such as polynucleotide) or protein array or microarray. The use of arrays to determine the presence and/or level of a collection of biological macromolecules is now well known (see, for example, methods described in published PCT application number WO 99/48916, describing hypoxia-related gene expression arrays). In array-based measurement methods, an array can be contacted with polynucleotides (in the case of a nucleic acid-based array) or polypeptides (in the case of a protein-based array) from a sample from a subject. The amount or position of binding of the subject's polynucleotides or polypeptides then can be determined, for instance to produce a gene expression profile for that subject. Such gene expression profile can be compared to another gene expression profile, for instance a control gene expression profile from a subject known to have suffered a stroke, or known to not have suffered a stroke. Such a method could be used to determine whether a subject had an ischemic stroke or determine the prognosis of a subject who had an ischemic stroke. In addition, the subject's gene expression profile can be correlated with one or more

appropriate treatments, which can be correlated with a control (or set of control) expression profiles for levels of ischemia, for instance.

In view of the many possible embodiments to which the principles of our invention may be applied, it should be recognized that the illustrated embodiment is only a preferred example of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

We claim:

1. A method of evaluating an ischemic stroke in a subject, comprising:

detecting differential expression of at least four ischemic stroke-related molecules of the subject, wherein the at least four ischemic-stroke related molecules are represented by any combination of at least four molecules listed in any of Tables 2-5, and wherein the presence of differential expression of at least four ischemic-stroke related molecules indicates that the subject has had an ischemic stroke.

2. The method of claim 1, wherein detecting differential expression comprises detecting differential expression within 24 hours of onset of clinical signs and symptoms that indicate a potential stroke.

3. The method of claim 2, wherein detecting differential expression comprises detecting differential expression within 7-14 days of onset of clinical signs and symptoms that indicate a potential stroke.

4. The method of claim 2, wherein detecting differential expression comprises detecting differential expression within 90 days of onset of clinical signs and symptoms that indicate a potential stroke.

5. The method of claim 1, wherein the at least four ischemic-stroke related molecules comprise CD163.

6. The method of claim 5, wherein the at least four ischemic-stroke related molecules further comprise hypothetical protein FLJ22662 Laminin A motif; bone marrow stromal cell antigen 1/CD157 (BST-1); Fc fragment of IgG, high affinity Ia, receptor for (CD64) (FcγRI); baculoviral IAP repeat-containing protein 1; and KIAA0146.

7. The method of claim 1, wherein the method comprises determining whether there is an upregulation in any combination of at least CD163; hypothetical protein FLJ22662 Laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146, the method comprises determining whether there is a downregulation in any combination of at least intercellular adhesion molecule 2, protein kinase D2, GATA binding protein 3, hypothetical protein FLJ20257, and protein kinase C, theta.

8. The method of claim 1, wherein differential expression comprises upregulation and wherein the method comprises determining whether there is an upregulation in any combination of at least four ischemic stroke-related genes listed in Table 5, wherein the presence of an increase in expression of at least four ischemic stroke-related molecules indicates that the subject has had an ischemic stroke.

9. The method of claim 1, wherein the method comprises determining whether there is upregulation in all 22 ischemic stroke-related genes listed in Table 5, wherein the presence of an increase in

expression of any combination of at least four ischemic stroke-related molecules indicates that the subject has had an ischemic stroke.

10. The method of claim 1, wherein the method comprises determining whether there is upregulation in all 22 ischemic-stroke related genes listed in Table 5, wherein the presence of an increase in expression in all 22 ischemic stroke-related molecules indicates that the subject has had an ischemic stroke.

11. The method of claim 1, wherein the method comprises determining whether the subject has differential expression of any combination of at least 100 of the ischemic stroke-related genes listed in Tables 2-4, wherein the presence of an increase or decrease in expression of any combination of at least 100 ischemic-stroke related molecules indicates that the subject has had an ischemic stroke.

12. The method of claim 1, wherein the ischemic stroke-related molecules comprise ischemic stroke-related protein molecules.

13. The method of claim 1, wherein the method has a sensitivity of at least 78% and accuracy of at least 80%.

14. The method of claim 1, wherein the subject had an onset of clinical signs and symptoms of an ischemic stroke no more than 72 hours prior to determining whether there is differential expression of at least four ischemic stroke-related molecules.

15. The method of claim 1, wherein the ischemic stroke-related molecules comprise ischemic-stroke related nucleic acid molecules.

16. The method of claim 15, where the nucleic acid molecules comprise mRNA or cDNA.

17. The method of claim 11, wherein the nucleic acid molecules are isolated from the subject, thereby generating isolated nucleic acid molecules, and wherein the isolated nucleic acid molecules are hybridized with oligonucleotides that detect the at least four ischemic stroke-related molecules.

18. The method of claim 17, wherein hybridizing with the oligonucleotides comprises:

incubating the isolated nucleic acid molecules with the oligonucleotides for a time sufficient to allow hybridization between the isolated nucleic acid molecules and oligonucleotides, thereby forming isolated nucleic acid molecule: oligonucleotide complexes; and

analyzing the isolated nucleic acid molecule:oligonucleotide complexes to determine if expression of the isolated nucleic acid molecules was altered, wherein the presence of differential

expression of at least four ischemic stroke-related nucleic acids indicates that the subject has had an ischemic stroke.

19. The method of claim 18, wherein analyzing the isolated nucleic acid molecule:oligonucleotide complexes comprises determining an amount of nucleic acid hybridization, and wherein a greater amount of hybridization to at least four ischemic stroke-related nucleic acids from the subject, as compared to an amount of hybridization to at least four ischemic stroke-related nucleic acids from a subject who has not had an ischemic stroke, indicates that the subject has had an ischemic stroke.

20. The method of claim 18, wherein analyzing the isolated nucleic acid molecule:oligonucleotide complexes includes detecting and quantifying the complexes.

21. The method of claim 17, wherein the oligonucleotides are present on an array substrate.

22. The method of claim 17, wherein the nucleic acid molecules isolated from the subject are obtained from peripheral blood mononuclear cells (PBMCs).

23. The method of claim 17, wherein the isolated nucleic acid molecules are labeled with a detectable label.

24. The method of claim 17, wherein the oligonucleotides are labeled with a detectable label.

25. The method of claim 17, wherein the oligonucleotides are complementary to any combination of at least 4 genes listed in Table 5.

26. The method of claim 17, wherein the oligonucleotides are complementary to all genes listed in Table 5.

27. The method of claim 17, wherein the oligonucleotides are complementary to any combination of at least 500 genes listed in Table 2.

28. The method of claim 17, wherein the oligonucleotides are complementary to all genes listed in Table 2.

29. The method of claim 1, wherein determining whether there is differential expression of at least four ischemic stroke-related molecules comprises:

measuring a level of at least four ischemic stroke-related nucleic acid molecules in a sample derived from the subject, wherein a difference in the level of the at least four ischemic stroke-related nucleic acid molecules in the sample, relative to a level of the at least four ischemic stroke-related

nucleic acid molecules in an analogous sample from a subject not having had an ischemic stroke is differential expression in those at least four ischemic stroke-related molecules.

30. The method of claim 1, wherein determining whether there is differential expression of at least four ischemic stroke-related molecules comprises:

measuring a quantity of at least four ischemic stroke-related proteins in a sample derived from the subject, wherein a difference in the quantity in the quantity of at least four ischemic stroke-related proteins in the sample, relative to a functional activity level of the at least four ischemic stroke-related proteins in an analogous sample from a subject not having had an ischemic stroke, is a differential expression in those at least four ischemic stroke-related molecules.

31. The method of claim 1, determining whether there is differential expression of at least four ischemic stroke-related molecules comprises determining whether a gene expression profile from the subject indicates ischemic stroke.

32. The method of claim 31, wherein the gene expression profile is generated using an array of molecules comprising an ischemic stroke expression profile.

33. The method of claim 31, comprising comparing the ischemic stroke expression profile from the subject to at least one control gene expression profile for a subject who has not had an ischemic stroke.

34. The method of claim 1, wherein differential expression comprises upregulation and wherein the method comprises determining whether there is an upregulation in any combination of at least one gene from each class of genes listed in Table 5, wherein the class of genes comprise: sequences involved in white blood cell activation and differentiation, sequences involved in vascular repair, sequences involved in a response to hypoxia, and sequences involved in a response to altered cerebral microenvironment.

35. A method of evaluating ischemic stroke in a subject, comprising:

applying isolated nucleic acid molecules obtained from PBMCs of the subject to an array, wherein the array consists of oligonucleotides complementary to all 22 genes listed in Table 5;

incubating the isolated nucleic acid molecules with the array for a time sufficient to allow hybridization between the isolated nucleic acid molecules and oligonucleotide probes, thereby forming isolated nucleic acid molecule:oligonucleotide complexes; and

analyzing the isolated nucleic acid molecule:oligonucleotide complexes to determine if expression of the isolated nucleic acid molecules is altered, wherein the presence of differential expression in at least 15 of the 22 genes indicates that the subject has had an ischemic stroke.

36. The method of claim 1, wherein evaluating the ischemic stroke comprises predicting a likelihood of severity of neurological sequelae of the ischemic stroke.

37. The method of claim 36, wherein differential expression is detected in a sample obtained from the subject within 24 hours of onset of clinical indications of stroke.

38. The method of claim 36, wherein differential expression is detected in at least 22 ischemic-stroke related molecules of the subject, wherein the at least 22 ischemic-stroke related molecules are represented by molecules listed in Table 5, and wherein the presence of differential expression of at least 22 ischemic-stroke related molecules indicates that the subject has a higher risk of long-term adverse neurological sequelae.

39. The method of claim 38, wherein the method comprises determining whether there is differential expression of at least 150 ischemic-stroke related molecules of the subject, wherein the at least 150 ischemic-stroke related molecules are represented by 150 genes listed in Table 3, and wherein the presence of differential expression of at least 150 ischemic-stroke related molecules indicates that the subject has a higher risk of long-term adverse neurological sequelae.

40. The method of claim 1, further comprising administering to the subject a treatment to avoid or reduce ischemic injury if the presence of differential expression indicates that the subject has had an ischemic stroke.

41. The method of claim 40, wherein the selected treatment comprises administration of t-PA to the subject within three hours of onset of the ischemic stroke.

42. The method of claim 40, wherein the selected treatment comprises treating the subject with an anticoagulant agent, a thrombolytic agent, or combinations thereof.

43. The method of claim 38, wherein detecting differential expression of any combination of at least four ischemic stroke-related molecules comprises quantitatively or qualitatively analyzing a nucleic acid molecule or protein obtained from the subject.

44. The method of claim 1, wherein evaluating the ischemic stroke comprises predicting a likelihood of neurological recovery of the subject.

45. The method of claim 44, wherein differential expression is detected in a sample obtained from the subject within 24 hours of onset of clinical indications of stroke.

46. The method of claim 45, wherein differential expression is detected in at least 22 ischemic-stroke related molecules of the subject, wherein the at least 22 ischemic-stroke related molecules are represented by molecules listed in Table 5, and wherein the presence of differential expression of at least 22 ischemic-stroke related molecules indicates that the subject has a lower likelihood of neurological recovery.

47. The method of claim 1, wherein detecting differential expression comprises determining a t-statistic value that indicates upregulation or downregulation.

48. The method of claim 47, wherein a t-statistic value of at least 3.8 indicates upregulation and a t-statistic value of less than -3.7 indicates downregulation.

49. An array comprising oligonucleotides complementary to ischemic stroke-related gene sequences, wherein the ischemic stroke-related gene sequences comprise any combination of at least four of the genes listed in Tables 2-5.

50. The array of claim 49, wherein the ischemic stroke-related gene sequences comprise CD163.

51. The array of claim 50, wherein the ischemic stroke-related gene sequences further comprise one or more of hypothetical protein FLJ22662 laminin A motif; BST-1; FcγRI; baculoviral IAP repeat-containing protein 1; and KIAA0146.

52. The array of claim 49, wherein the ischemic stroke-related gene sequences comprise any combination of four or more ischemic stroke-related genes listed in Table 5.

53. The array of claim 52, wherein the ischemic stroke-related gene sequences comprise at least one gene from each class of genes listed in Table 5, wherein the class of genes comprise: sequences involved in white blood cell activation and differentiation, sequences involved in vascular repair, sequences involved in a response to hypoxia, and sequences involved in a response to altered cerebral microenvironment

54. The array of claim 49, wherein the ischemic stroke-related gene sequences consist of all genes listed in Table 5.

55. The array of claim 49, wherein the ischemic stroke-related gene sequences comprise all ischemic stroke-related genes listed in Table 4.

56. The array of claim 49, wherein the ischemic stroke-related gene sequences comprise any combination of 150 or more ischemic stroke-related genes listed in Table 3.

57. The array of claim 49, wherein the ischemic stroke-related gene sequences consist of all ischemic stroke-related genes listed in Table 3.

58. The array of claim 49, wherein the ischemic stroke-related gene sequences comprise any combination of 500 or more ischemic stroke-related genes listed in Table 2.

59. The array of claim 49, wherein the ischemic stroke-related gene sequences consist of all ischemic stroke-related genes listed in Table 2.

60. A kit for evaluating an ischemic stroke in a subject, comprising:

a solid phase nucleic acid molecule array comprising a plurality of oligonucleotides chemically linked to a solid polymeric support surface in a predetermined pattern, wherein the oligonucleotides are capable of hybridizing under stringent conditions to any combination of at least four ischemic stroke-related nucleic acid molecules listed in Tables 2-5.

61. The kit of claim 60, wherein the oligonucleotides are capable of hybridizing under stringent conditions to any combination of at least four ischemic stroke-related nucleic acid molecules listed in Table 5.

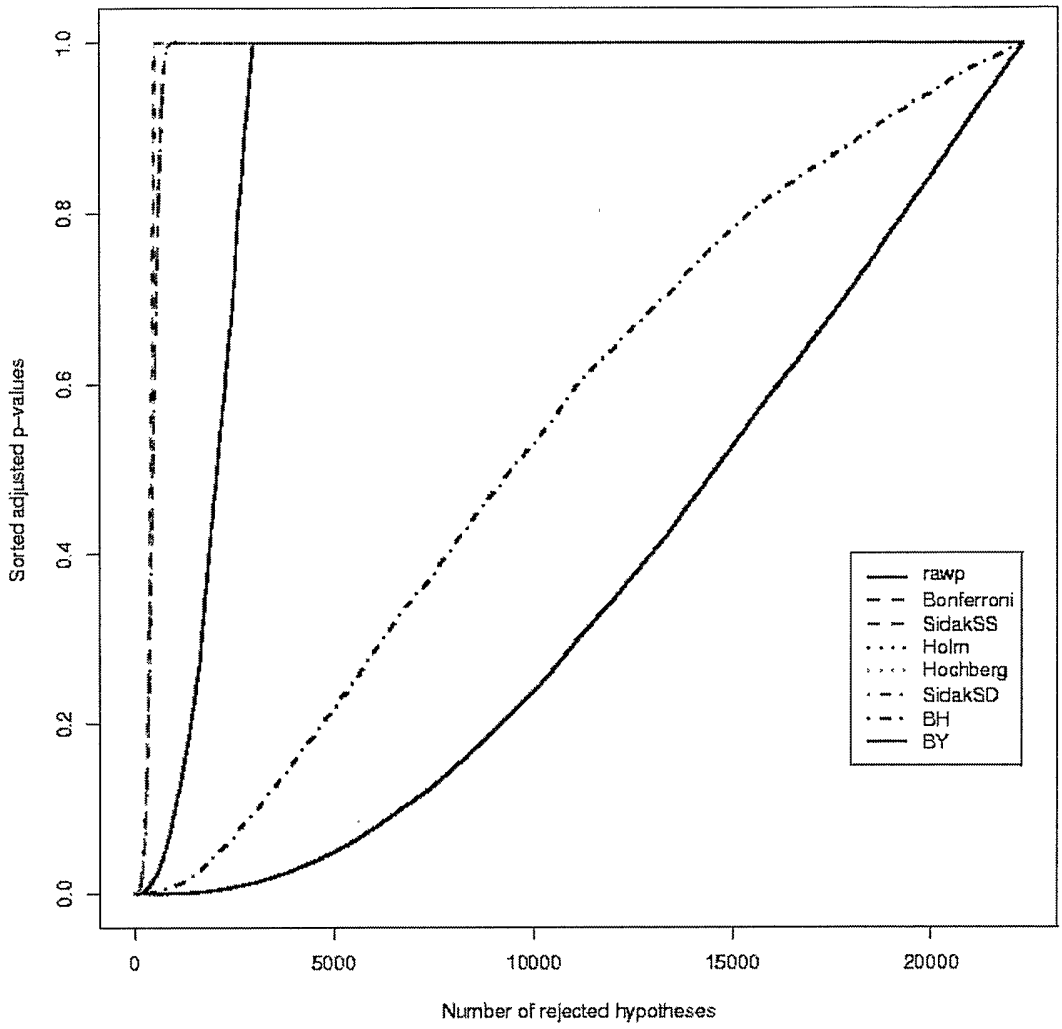
62. The kit of claim 60, wherein the oligonucleotides are capable of hybridizing under stringent conditions to any combination of at least 150 ischemic stroke-related nucleic acid molecules listed in Table 3.

63. The kit of claim 60, further comprising a buffer solution, in separate packaging.

64. The kit of claim 60, wherein the oligonucleotides are capable of hybridizing under stringent conditions to at least 5, at least 10, at least 15, at least 22, at least 50, at least 82, at least 100, at least 150, at least 190, at least 200, at least 250, at least 300, at least 500, or at least 600 ischemic stroke-related molecules listed in Tables 2-5.

65. The method of claim 36, wherein the subject had an ischemic stroke at least 72 hours prior to determining whether there is differential expression of at least four ischemic stroke-related molecules.

66. The method of claim 1, wherein detecting differential expression comprises quantitating expression of the at least four ischemic stroke-related molecules.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SEQUENCE LISTING

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<120> DIFFERENTIAL EXPRESSION OF MOLECULES ASSOCIATED WITH ACUTE STROKE

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