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(54) **BIOMARKERS FOR DIAGNOSIS OF STROKE AND ITS CAUSES**

BIOMARKER ZUR DIAGNOSE VON SCHLAGANFÄLLEN UND IHREN URSACHEN

BIOMARQUEURS UTILISÉS POUR DIAGNOSTIQUER UN ACCIDENT VASCULAIRE CÉRÉBRAL ET SES CAUSES

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(73) Proprietor: **The Regents of the University of California**
Oakland, CA 94607 (US)

(72) Inventors:
- **SHARP, Frank**
  Davis
  California 95618 (US)
- **STAMOVA, Boryana**
  Davis
  California 95616 (US)
- **JICKLING, Glen C.**
  Sacramento
  California 95819 (US)

(74) Representative: **Walker, Ross Thomson**
Forresters
Skygarden
Erika-Mann-Strasse 11
80636 München (DE)

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Description

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/364,449, filed on July 15, 2010, the entire disclosure of which is hereby incorporated herein by reference for all purposes.

FIELD OF THE INVENTION

[0002] The present invention provides compositions and methods for diagnosing stroke and the risk of stroke, as well as the cause of stroke.

BACKGROUND OF THE INVENTION

[0003] Stroke is a leading cause of adult death and disability [Thom T et al., Circulation, 113:e85-151 (2006); WHO, The atlas of heart disease and stroke (2005)]. The diagnosis of ischemic stroke (IS) is made with clinical assessment in combination with brain imaging. However, the diagnosis is not always straightforward, particularly in the acute setting where an accurate, inexpensive and rapid diagnosis is critical to optimally treat patients.

[0004] Extensive efforts have been directed toward identifying blood based biomarkers for IS. More than 58 proteins and 7 panels of proteins have been described as biomarkers of IS [Whiteley W et al., Stroke, 39:2902-2909 (2008); Foerch C et al., Neurology, 73:393-399 (2009); Jensen MB et al., Expert Rev Cardiovasc Ther., 7:389-393 (2009)]. RNA expression profiles in the blood have also been described in IS [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006); Moore DF et al., Circulation, 111:212-221 2005]. We previously reported a 29-probe set expression profile predictive of IS [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)]. This profile required validation in a second cohort, which has been done in the current study. Herein is described a 97-probe set expression profile that differentiates IS from controls, e.g., individuals who are healthy, have vascular risk factors, or who have experienced myocardial infarction. These profiles represent further refinement of gene expression as a diagnostic tool in patients with acute IS.

[0005] Ischemic stroke is most commonly classified using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria, dividing patients into cardioembolic, large vessel, small vessel lacunar, other, and cryptogenic causes [Adams HP, Jr., et al., Stroke, 24:35-41 (1993)]. TOAST criteria improves rater reliability and guides treatment when a known cause can be clearly identified [Goldstein LB et al., Stroke, 32:1091-1098 (2001); Ay H et al., Stroke, 38:2979-2984 (2007)]. However, in many patients the cause of stroke remains unknown or cryptogenic in spite of extensive investigation. Given cryptogenic stroke accounts for approximately 30% of all ischemic strokes, better tools identify the cause of stroke are required [Ionita CC et al., Prev Cardiol., 8:41-46 (2005)].

[0006] Blood based biomarkers present a valuable tool to determine the cause of stroke. A number of protein biomarkers have been associated with stroke subtypes. For example, cardioembolic stroke is associated with brain natriuretic peptide and D-dimer; large vessel stroke is associated with C-reactive protein; and small vessel lacunar stroke is associated with homocysteine, ICAM-1, and thrombomodulin [Laskowitz DT et al., Stroke, 40:77-85 (2009); Shibazaki K et al., Intern Med., 48:259-264 (2009); Montaner J et al., Stroke, 39:2280-2287 (2008); Hassan A et al., Brain, 126:424-432 (2003)]. However, biomarkers of ischemic stroke subtype currently lack sufficient sensitivity and specificity to be used in clinical practice. Thus, a combination of biomarkers into a biomarker profile might be one method by which diagnostic specificity and sensitivity can be improved.

[0007] The present study determined that gene expression signatures in blood can be used to distinguish cardioembolic from large vessel ischemic stroke, and can be used to predict the cardioembolic and large vessel causes in patients with cryptogenic stroke. The rationale for why changes in blood cell RNA expression occur in ischemic stroke include inflammatory changes associated with acute cerebral ischemia, symptomatic atherosclerosis and thromboembolism [Xu H et al., J Cereb Blood Flow Metab., 28:1320-1328 (2008)9; Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006); Du X et al., Genomics, 87:693-703 (2006)]. Using whole genome microarrays, a 40 gene profile was identified to distinguish cardioembolic stroke from large vessel stroke, and a 37 gene profile was identified to distinguish cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes. These genes play roles in inflammation and represent a step toward better determining the cause of cryptogenic stroke.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention provides methods for diagnosing or predicting the occurrence of stroke and the cause of stroke by determining the overexpression and underexpression of biomarkers in blood.

[0009] Accordingly, in one aspect, the invention provides methods for diagnosing the occurrence and cause of ischemic

stroke or a predisposition for developing ischemic stroke, the method comprising:

a) determining a level of expression of at least 15 ischemic stroke-associated biomarkers in a biological sample from a patient, wherein the biomarkers are selected from the group consisting of a plurality of biomarkers selected from Table 7A, a plurality of biomarkers selected from Table 13A, a plurality of biomarkers selected from Table 14 and a plurality of biomarkers selected from Table 15;
b) comparing the level of expression of the ischemic stroke-associated biomarkers to the expression level of a plurality of stably expressed endogenous reference biomarkers,

wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 7A compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing ischemic stroke;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 13A compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing cardioembolic stroke;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 14 compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing carotid stenosis;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 15 compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing atrial fibrillation, thereby diagnosing the occurrence and cause of ischemic stroke or the predisposition for developing ischemic stroke. The levels of expression of the plurality of biomarkers can be concurrently or sequentially determined.

[0010] In a related aspect, the invention provides methods for diagnosing the occurrence and cause of ischemic stroke or a predisposition for developing ischemic stroke, the method comprising:

a) determining a level of expression of a plurality, e.g., at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more or all, ischemic stroke-associated biomarkers in a biological sample from a patient, wherein the biomarkers are selected from the group consisting of a plurality of biomarkers selected from Table 7A, a plurality of biomarkers selected from Table 13A, a plurality of biomarkers selected from Table 14 and a plurality of biomarkers selected from Table 15;
b) comparing the level of expression of the ischemic stroke-associated biomarkers to a control expression level,

wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 7A compared to the control expression level indicates that the patient suffers from or is at risk of developing ischemic stroke;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 13A compared to the control expression level indicates that the patient suffers from or is at risk of developing cardioembolic stroke;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 14 compared to the control expression level indicates that the patient suffers from or is at risk of developing carotid stenosis;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 15 compared to the control expression level indicates that the patient suffers from or is at risk of developing atrial fibrillation, thereby diagnosing the occurrence and cause of ischemic stroke or the predisposition for developing ischemic stroke. The levels of expression of the plurality of biomarkers can be concurrently or sequentially determined. The control expression level can be, *e.g.,* with respect to a plurality of stably expressed endogenous reference biomarkers, with respect to the expression level of the same ischemia-associated biomarker in an otherwise healthy individual (optionally normalized to the expression levels of a plurality of stably expressed endogenous reference biomarkers), or with respect to a threshold level representative of the expression level of the same ischemia-associated biomarker in an otherwise healthy individual (optionally normalized to the expression levels of a plurality of stably expressed endogenous reference biomarkers).

[0011] In various embodiments, the plurality of biomarkers determined are from Table 7A. In various embodiments, the plurality of biomarkers determined are from Table 13A. In various embodiments, the plurality of biomarkers determined are from Table 14. In various embodiments, the plurality of biomarkers determined are from Table 15. In various embodiments, the plurality of biomarkers determined are from two or more of Table 7A, Table 13A, Table 14 and Table 15.

[0012] In some embodiments, the plurality of stably expressed endogenous reference biomarkers are selected from the biomarkers listed in Table 16. In some embodiments, the ischemic stroke-associated biomarkers are overexpressed or underexpressed at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference

biomarkers, e.g., those listed in Table 16. In some embodiments, the expression levels of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or all, the endogenous reference biomarkers selected from the group consisting of USP7, MAPRE2, CSNK1G2, SAFB2, PRKAR2A, PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, CDC2L1 /// CDC2L2, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF1, KCMF1, PRKRIP1, CHMP4A, TMEM184C, TINF2, PODNL1, FBXO42, LOC441258, RRP1, C10orf104, ZDHHC5, C9orf23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16 are determined as a control.

[0013] In some embodiments, the level of expression of about 15-85, 20-70, 30-60 or 40-50 biomarkers are determined. In some embodiments, about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 biomarkers are determined. In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30 or more biomarkers from Table 7A are determined. In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30 or more biomarkers from Table 13A are determined. In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30 or more biomarkers from Table 14 are determined. In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30 or more biomarkers from Table 15 are determined. In some embodiments, the level of expression of all biomarkers listed in Table 7A are determined. In some embodiments, the level of expression of all biomarkers listed in Table 13A are determined. In some embodiments, the level of expression of all biomarkers listed in Table 14 are determined. In some embodiments, the level of expression of all biomarkers listed in Table 15 are determined. Stroke-associated biomarkers with increased and/or decreased expression levels, *e.g.,* in comparison to a control expression level, can be determined.

[0014] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined within 3 hours of a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of FAT3, GADL1, CXADR, RNF141, CLEC4E, TIMP2, ANKRD28, TIMM8A, PTPRD, CCRL1, FCRL4, DLX6, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, MCTP1 and SH3GL3 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, SNRPN, GLYATL1, DKRZP434L187, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, CCDC144A, ALDOAP2, LDB3, LOC729222 /// PPFIBP1, HNRNPUL2, ELAVL2, PRTG, FOXA2, SCD5, LOC283027, LOC344595, RPL22, LOC100129488 and RPL22 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0015] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined within 3 hours of a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more or all, ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of FGD4, F5, ABCA1, LOC100290882, LTB4R, UBXN2B, CKLF, CLEC4E, PHTF1, ENTPD1, OSBPL1A, RRAGD, CPEB2, CKLF, BST1 and CKLF indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0016] In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of CLEC4E, TIMP2, FGD4, CPEB2, LTB4R and VNN3 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CKLF, LOC100290882, UBXN2B, ENTPD1, BST1, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22, MCTP1 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0017] In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more or all, ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of THSD4, SNRPN, ASTN2, SNIP, FAT3, TIMM8A, CCDC144C /// LOC100134159, ANKRD28, TBX5, PGM5, SCD5, FCRL4, SHOX, CCRL1, LECT2, PTPRD, CCDC144A, LDB3, LOC729222 /// PPFIBP1, RBMS3, P704P, GYPA, PRTG, GABRB2, HNRNPUL2, ELAVL2, SPTLC3, FOXA2, DLX6, ALDOAP2, and FLJ35934 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting

with myocardial infarction within 3 hours of a suspected ischemic event, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, or more or all, ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of THSD4, SNRPN, ASTN2, SNIP, FAT3, TIMM8A, CCDC144C /// LOC100134159, ANKRD28, TBX5, PGM5 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0018] In various embodiments, in an individual presenting with one or more vascular risk factors (e.g., hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking) within 3 hours of a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors within 3 hours of a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0019] In various embodiments, in an individual presenting with one or more vascular risk factors (e.g., hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking) within 3 hours of a suspected ischemic event, an increased expression level of 1, 2, 3 or 4 ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, ELL2, TIMP2 and CLEC4E indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors within 3 hours of a suspected ischemic event, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or all, ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of SNIP, BXDC5, FAT3, LECT2, THSD4, CCDC144C /// LOC100134159, OVOL2, SPTLC3, GLYATL1, RBMS3, SPIB, DKFZP434L187, GADL1, SHOX, TBX5, UNC5B, PGM5 and CXADR indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0020] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined 3 or more hours after a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, CLEC4E, BXDC5, UNC5B, TIMP2, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488 and MCTP1 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of SPTLC3, DKRZP434L187, SPIB, HNRNPUL2, FOXA2, RPL22 and SH3GL3 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0021] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined at least 24 hours after a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of ZNF608, FCHO2, ST3GAL6, ABCA1, THBD, AMN1, QKI, KIAA0319, MCTP1, VNN3, UBR5, FAR2, RBM25, CHMP1B, LAMP2, VAPA, IFRD1, HNRNPH2, REM2 and GAB1 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, or more or all, ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of ZNF608, FCHO2, ST3GAL6, ABCA1, THBD, AMN1, QKI, KIAA0319, MCTP1 and VNN3 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0022] In various embodiments, in an individual presenting with myocardial infarction 3 or more hours after a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, HNRNPUL2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, UBXN2B, BST1, LTB4R, F5, IFRD1, KIAA0319, MCTP1, VNN3, AMN1, LAMP2, ZNF608, FAR2, GAB1, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with myocardial infarction 3 or more hours after a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from

the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC100290882, ENTPD1, CHMP1B, FCHO2, LOC283027, REM2, QKI, RBM25, ST3GAL6, HNRNPH2, UBR5, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0023] In various embodiments, in an individual presenting with myocardial infarction at least 24 hours after a suspected ischemic event, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, or more or all, ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RPL22, LOC100129488, LOC283027, LOC344595, THSD4, FAT3, P704P indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0024] In various embodiments, in an individual presenting with one or more vascular risk factors 3 or more hours after a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors 3 or more hours after a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0025] In various embodiments, in an individual presenting with one or more vascular risk factors at least 24 hours after a suspected ischemic event, an increased expression level of one or both ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of TIMP2 and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors at least 24 hours after a suspected ischemic event, a decreased expression level of 1, 2, 3, 4, 5, 6, or 7 ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RPL22, SNIP, SH3GL3, FAT3, SPTLC3, RBMS3 and SNRPN indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0026] With respect to the determination of the cause of stroke, in some embodiments an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A /// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP and LRRC37A3 indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of LOC284751, CD46, ENPP2, C19orf28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, C16orf68, TFDP1 and GSTK1 indicates that the patient has experienced or is at risk for cardioembolic stroke.

[0027] In some embodiments an increased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of EBF1, GRM5, AP3S2, LRRC37A3, IRF6, LHFP, BANK1, ARHGEF5, ZNF254, COL13A1, P2RX5, LHFP, PIK3C2B, EXT2, HLA-DOA, OOEP, ZNF185, TMEM 19, FCRL1 and PTPN20A///PTPN20B indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or 13 ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of TSKS, ENPP2, C16orf68, LOC284751, TFDP1, GSTK1, ADAMTSL4, CHURC 1, FLJ40125, ARHGEF12, CLEC18A, CD46 and C19orf28 indicates that the patient has experienced or is at risk for cardioembolic stroke.

[0028] In some embodiments an increased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more or all, ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of EBF1, GRM5, AP3S2, LRRC37A3, IRF6, LHFP, BANK1, ARHGEF5, ZNF254 and COL13A1 indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more or all, ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of TSKS, ENPP2, C16orf68, LOC284751, TFDP1, GSTK1, ADAMTSL4, CHURC1, FLJ40125 and ARHGEF12 indicates that the patient has experienced or is at risk for cardioembolic stroke.

[0029] With respect to the determination of the cause of stroke, in some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12orf42 and LOC100127980 indicates that the patient has experienced or is at risk for carotid stenosis. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 14 selected from

the group consisting of FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880, SLC20A1, SLC6A19, ARHGEF12, C16orf68, GIPC2 and LOC100144603 indicates that the patient has experienced or is at risk for carotid stenosis.

[0030] With respect to the determination of the cause of stroke, in some embodiments, an increased expression level of 2, 5, 10, 15, or more or all, ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of EBF1, COL13A1, LHFP, GRM5, ARHGEF5, RNF7, CLASP2, RANBP10, LOC100127980, CYTH3, PROCR, C12orf42, PRSS35, NT5E, and AKR1C3 indicates that the patient has experienced or is at risk for carotid stenosis. In some embodiments, a decreased expression level of 2, 5, 10, 15, or more or all ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of FLJ31945, C16orf68, SLC20A1, DOPEY2, LOC284751, LOC100144603, MTBP, SHOX2, GIPC2, CMBL, LOC146880, SLC6A19, ICAM4, ARHGEF12, and LOC10027183 indicates that the patient has experienced or is at risk for carotid stenosis.

[0031] With respect to the determination of the cause of stroke, in some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of SMC1A, SNORA68, GRLF 1, SDC4, HIPK2, LOC100129034, CMTM1 and TTC7A indicates that the patient has experienced or is at risk for atrial fibrillation. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6orf164 and MAP3K7IP1 indicates that the patient has experienced or is at risk for atrial fibrillation.

[0032] With respect to the determination of the cause of stroke, in some embodiments, an increased expression level of 1, 2, 3, 4, 5, 6, 7 or 8 ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of CMTM1, SDC4, SNORA68, HIPK2, TTC7A, GRLF1, LOC100129034, SMC1A indicates that the patient has experienced or is at risk for atrial fibrillation. In some embodiments, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of COL13A1, C6orf164, GPR176, BRUNOL6, MIF///SLC2A11, DUSP16, PPIE, MAP3K7IP1, PER3, LRRC43 indicates that the patient has experienced or is at risk for atrial fibrillation.

[0033] In a related aspect, the invention provides methods for determining whether a stroke has occurred or predicting whether a stroke will occur. Accordingly, the invention provides methods for diagnosing ischemic stroke or a predisposition for developing ischemic stroke, the method comprising: determining a level of expression of a plurality of ischemic stroke-associated biomarkers in a biological sample from a patient, wherein an increase or decrease of the level compared to a control indicates that the patient suffers from or is at risk of developing ischemic stroke, wherein the plurality of ischemic stroke-associated biomarkers is selected from the biomarkers set forth in Table 7A. In some embodiments, the methods for determining the occurrence of stroke comprise further determining the level of expression of one or biomarkers listed in Table 7B. In some embodiments, the ischemic stroke is a member selected from the group consisting of: embolic stroke, thrombotic stroke, transient ischemic attack, cardioembolic stroke and atherothrombotic stroke.

[0034] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined within 3 hours of a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of FAT3, GADL1, CXADR, RNF141, CLEC4E, TIMP2, ANKRD28, TIMM8A, PTPRD, CCRL1, FCRL4, DLX6, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, MCTP1 and SH3GL3 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, SNRPN, GLYATL1, DKRZP434L187, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, CCDC144A, ALDOAP2, LDB3, LOC729222 /// PPFIBP1, HNRNPUL2, ELAVL2, PRTG, FOXA2, SCD5, LOC283027, LOC344595, RPL22, LOC100129488 and RPL22 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0035] In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of CLEC4E, TIMP2, FGD4, CPEB2, LTB4R and VNN3 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CKLF, LOC100290882, UBXN2B, ENTPD1, BST1, F5, IFRD1, KIAA0319, CHMP1B,

MCTP1, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22, MCTP1 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0036] In various embodiments, in an individual presenting with one or more vascular risk factors (e.g., hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking) within 3 hours of a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors within 3 hours of a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0037] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined 3 or more hours after a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, CLEC4E, BXDC5, UNC5B, TIMP2, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488 and MCTP1 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of SPTLC3, DKRZP434L187, SPIB, HNRNPUL2, FOXA2, RPL22 and SH3GL3 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0038] In various embodiments, in an individual presenting with myocardial infarction 3 or more hours after a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, HNRNPUL2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, UBXN2B, BST1, LTB4R, F5, IFRD1, KIAA0319, MCTP1, VNN3, AMN1, LAMP2, ZNF608, FAR2, GAB1, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with myocardial infarction 3 or more hours after a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC100290882, ENTPD1, CHMP1B, FCHO2, LOC283027, REM2, QKI, RBM25, ST3GAL6, HNRNPH2, UBR5, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0039] In various embodiments, in an individual presenting with one or more vascular risk factors 3 or more hours after a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors 3 or more hours after a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0040] In a further aspect, the invention provides methods for determining the occurrence of or the predisposition of

a subject to experience cardioembolic stroke, the method comprising: determining a level of expression of a plurality of ischemic stroke-associated biomarkers in a biological sample from a patient, wherein an increase or decrease of the level compared to a control indicates that the patient has experienced cardioembolic stroke, wherein the plurality of ischemic stroke-associated biomarkers is selected from the biomarkers set forth in Table 13A. In some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers selected from the group consisting of IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A /// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP and LRRC37A3 indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers selected from the group consisting of LOC284751, CD46, ENPP2, C19orf28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, C16orf68, TFDP1 and GSTK1 indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a level of expression of a plurality of ischemic stroke-associated biomarkers listed in Table 13B is further determined, wherein an increase or decrease of the level compared to a control indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments an increased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more or all, ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of EBF1, GRM5, AP3S2, LRRC37A3, IRF6, LHFP, BANK1, ARHGEF5, ZNF254 and COL13A1 indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more or all, ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of TSKS, ENPP2, C16orf68, LOC284751, TFDP1, GSTK1, ADAMTSL4, CHURC1, FLJ40125 and ARHGEF12 indicates that the patient has experienced or is at risk for cardioembolic stroke.

[0041] In a further aspect, the invention provides methods for determining the occurrence of or the predisposition of a subject to experience carotid stenosis, the method comprising: determining a level of expression of a plurality of ischemic stroke-associated biomarkers in a biological sample from a patient who has suffered ischemic stroke, wherein an increase or decrease of the level compared to a control indicates that the patient has experienced carotid stenosis, wherein the plurality of ischemic stroke-associated biomarkers is selected from the biomarkers set forth in Table 14. In some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers selected from the group consisting of NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12orf42 and LOC100127980 indicates that the patient has experienced or is at risk for carotid stenosis. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers selected from the group consisting of FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880, SLC20A1, SLC6A19, ARHGEF12, C16orf68, GIPC2 and LOC100144603 indicates that the patient has experienced or is at risk for carotid stenosis. In some embodiments, an increased expression level of 2, 5, 10, 15, or more or all, ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of EBF1, COL13A1, LHFP, GRM5, ARHGEF5, RNF7, CLASP2, RANBP10, LOC100127980, CYTH3, PROCR, C12orf42, PRSS35, NT5E, and AKR1C3 indicates that the patient has experienced or is at risk for carotid stenosis. In some embodiments, a decreased expression level of 2, 5, 10, 15, or more or all ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of FLJ31945, C16orf68, SLC20A1, DOPEY2, LOC284751, LOC100144603, MTBP, SHOX2, GIPC2, CMBL, LOC146880, SLC6A19, ICAM4, ARHGEF12, and LOC10027183 indicates that the patient has experienced or is at risk for carotid stenosis.

[0042] In a further aspect, the invention provides methods for determining the occurrence of or the predisposition of a subject to experience atrial fibrillation in a patient, the method comprising: determining a level of expression of a plurality of ischemic stroke-associated biomarkers in a biological sample from the patient, wherein an increase or decrease of the level compared to a control indicates that the patient has experienced or is at risk for experiencing atrial fibrillation, wherein the plurality of ischemic stroke-associated biomarkers is selected from the biomarkers set forth in Table 15. In some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers selected from the group consisting of SMC1A, SNORA68, GRLF1, SDC4, HIPK2, LOC100129034, CMTM1 and TTC7A indicates that the patient has experienced or is at risk for atrial fibrillation. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers selected from the group consisting of LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6orf164 and MAP3K7IP1 indicates that the patient has experienced or is at risk for atrial fibrillation. In some embodiments, an increased expression level of 1, 2, 3, 4, 5, 6, 7 or 8 ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of CMTM1, SDC4, SNORA68, HIPK2, TTC7A, GRLF1, LOC100129034, SMC1A indicates that the patient has experienced or is at risk for atrial fibrillation. In some embodiments, a decreased expression level of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of COL13A1, C6orf164, GPR176, BRUNOL6, MIF///SLC2A11, DUSP16, PPIE, MAP3K7IP1, PER3, LRRC43 indicates that the patient has experienced or is at risk for atrial fibrillation.

[0043] With respect to embodiments of the methods for determination of occurrence and/or cause of stroke, in some embodiments, the level of expression of about 15-85, 20-70, 30-60 or 40-50 total biomarkers are determined. In some embodiments, about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 biomarkers are determined.

The levels of expression of the plurality of biomarkers can be concurrently or sequentially determined.

[0044] In some embodiments, the control level is the expression level of a plurality of stably expressed endogenous reference biomarkers. In some embodiments, the plurality of stably expressed endogenous reference biomarkers are selected from the biomarkers listed in Table 16. In some embodiments, the ischemic stroke-associated biomarkers are overexpressed or underexpressed at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference biomarkers, e.g., those listed in Table 16. In some embodiments, the expression levels of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or all, the endogenous reference biomarkers selected from the group consisting of USP7, MAPRE2, CSNK1G2, SAFB2, PRKAR2A, PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, CDC2L1 /// CDC2L2, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF 1, KCMF 1, PRKRIP 1, CHMP4A, TMEM184C, TINF2, PODNL 1, FBXO42, LOC441258, RRP1, C10orf104, ZDHHC5, C9orf23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16 are determined as a control.

[0045] In some embodiments, the control level is the expression level of the same biomarker in a healthy individual, e.g. an individual who has not experienced a vascular event and/or who is not at risk of experiencing a vascular event (e.g., TIA, ischemic stroke, myocardial infarction, peripheral vascular disease, or venous thromboembolism). In some embodiments, the control is a threshold level of expression, e.g., of the same ischemic stoke-associated biomarker, optionally normalized to the expression level of a stably expressed endogenous reference biomarker, representative of a population of healthy individuals.

[0046] Methods for determining the occurrence or predisposition of an ischemic event, may further comprise the step of determining whether the patient has suffered a myocardial infarction or whether the patient has vascular risk factors.

[0047] In some embodiments, the patient is asymptomatic. In some embodiments, the patient is exhibiting symptoms of ischemic stroke, *e.g.,* of having experienced an ischemic event, of experiencing an ischemic event, or of an imminent ischemic event. In some embodiments, the patient has suffered an ischemic event. In some embodiments, the determining step is performed at 3 or fewer hours after the ischemic event. In some embodiments, the determining step is performed 3 or more hours after the ischemic event.

[0048] In some embodiments, the methods further comprise the step of recommending or providing a regime of treatment to the patient appropriate to the determined cause of stroke. For example, in patients diagnosed as experiencing or having a predisposition for experiencing cardioembolic stroke, the methods further provide for recommending or providing a regime of treatment or prevention for cardioembolic stroke. In patients diagnosed as experiencing or having a predisposition for experiencing carotid stenosis, the methods further provide for recommending or providing a regime of treatment or prevention for carotid stenosis. In patients diagnosed as experiencing or having a predisposition for experiencing atrial fibrillation, the methods further provide for recommending or providing a regime of treatment or prevention for atrial fibrillation.

[0049] With respect to embodiments for determination of the level of expression of the biomarkers, in some embodiments, the level of expression of the biomarker is determined at the transcriptional level. For example, in some embodiments, the level of expression is determined by detecting hybridization of an ischemic stroke-associated gene probe to gene transcripts of the biomarkers in the biological sample. In some embodiments, the hybridization step is performed on a nucleic acid array chip. In some embodiments, the hybridization step is performed in a microfluidics assay plate. In some embodiments, the level of expression is determined by amplification of gene transcripts of the biomarkers. In some embodiments, the amplification reaction is a polymerase chain reaction (PCR).

[0050] In some embodiments, the level of expression of the biomarker is determined at the protein level.

[0051] In some embodiments, the methods further comprise obtaining a biological sample from the patient. In some embodiments, the biological sample is blood, serum or plasma.

[0052] In a further aspect, the invention provides a solid support comprising a plurality of nucleic acids that hybridize to a plurality of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15 (and optionally Table 16), wherein the plurality of nucleic acids are attached to the solid support. The solid support may optionally comprise a plurality of nucleic acids that hybridize to a plurality of the genes set forth in Table 16. In various embodiments, the solid support is a microarray. In various embodiments, the solid support is attached to at least about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 80, 85, 90, 95 or 100, or more or all, genes set forth in Tables 7A, 7B, 13A, 13B, 14, 15 and/or 16.

[0053] In one embodiment, the solid support comprises a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 7A (and 7B). For example, in one embodiment, the solid support comprises 2, 5, 10, 15, 20, or more or all, nucleic acids that hybridize to a plurality of stroke-associated biomarkers selected from SNIP, BXDC5, FAT3, LECT2, THSD4, CCDC144C /// LOC100134159, OVOL2, SPTLC3, CLEC4E, GLYATL1, RBMS3, SPIB, DKFZP434L187, GADL1, SHOX, TBX5, UNC5B, PGM5, TIMP2, ELL2, CXADR, and RNF141. In one embodiment, the solid support comprises 2, 3, 4, 5, 6, 7, 8, or 9, nucleic acids that hybridize to a plurality of stroke-associated biomarkers selected from RPL22, SNIP, SH3GL3, MCTP1, FAT3, SPTLC3, RBMS3, SNRPN, and TIMP2. In one embodiment, the solid support comprises 2, 5, 10, 15, or more or all, nucleic acids that hybridize to a plurality of stroke-associated

biomarkers selected from FGD4, F5, ABCA1, LOC100290882, LTB4R, UBXN2B, CKLF, CLEC4E, PHTF1, ENTPD1, OSBPL1A, RRAGD, CPEB2, CKLF, BST1, and CKLF. In one embodiment, the solid support comprises 2, 5, 10, 15, 20, or more or all, nucleic acids that hybridize to a plurality of stroke-associated biomarkers selected from ZNF608, FCHO2, ST3GAL6, ABCA1, THBD, AMN1, QKI, KIAA0319, MCTP1, VNN3, UBR5, FAR2, RBM25, CHMP1B, LAMP2, VAPA, IFRD1, HNRNPH2, REM2, and GAB1. In one embodiment, the solid support comprises 2, 5, 10, 15, 20, 25, 30, or more or all, nucleic acids that hybridize to a plurality of stroke-associated biomarkers selected from THSD4, SNRPN, ASTN2, SNIP, FAT3, TIMM8A, CCDC144C /// LOC100134159, ANKRD28, TBX5, PGM5, SCD5, FCRL4, SHOX, CCRL1, LECT2, PTPRD, CCDC144A, LDB3, LOC729222 /// PPFIBP1, RBMS3, P704P, GYPA, PRTG, GABRB2, HNRNPUL2, ELAVL2, SPTLC3, FOXA2, DLX6, ALDOAP2, and FLJ35934. In one embodiment, the solid support comprises 2, 5, 6, 7, or more or all, nucleic acids that hybridize to a plurality of stroke-associated biomarkers selected from RPL22, LOC100129488, LOC283027, LOC344595, THSD4, FAT3, and P704P. In one embodiment, the solid support comprises 15, 20, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more or all, nucleic acids that hybridize to a plurality of stroke-associated biomarkers selected from SNIP, BXDC5, FAT3, LECT2, THSD4, CCDC144C /// LOC100134159, OVOL2, SPTLC3, CLEC4E, GLYATL1, RBMS3, SPIB, DKFZP434L187, GADL1, SHOX, TBX5, UNC5B, PGM5, TIMP2, ELL2, CXADR, RNF141, RPL22, SH3GL3, MCTP1, SNRPN, FGD4, F5, ABCA1, LOC100290882, LTB4R, UBXN2B, CKLF, PHTF1, ENTPD1, OSBPL1A, RRAGD, CPEB2, CKLF, BST1, ZNF608, FCHO2, ST3GAL6, THBD, AMN1, QKI, KIAA0319, MCTP1, VNN3, UBR5, FAR2, RBM25, CHMP1B, LAMP2, VAPA, IFRD1, HNRNPH2, REM2, GAB1, ASTN2, TIMM8A, CCDC144C /// LOC100134159, ANKRD28, SCD5, FCRL4, CCRL1, LECT2, PTPRD, CCDC144A, LDB3, LOC729222 /// PPFIBP1, P704P, GYPA, PRTG, GABRB2, HNRNPUL2, ELAVL2, FOXA2, DLX6, ALDOAP2, FLJ35934, LOC100129488, LOC283027, and LOC344595.

[0054] In one embodiment, the solid support comprises a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 13A (and 13B). In one embodiment, the solid support comprises 2, 5, 10, 15, 20, 25, 30, 35, or more or all, nucleic acids that hybridize to a plurality of cardioembolic stroke-associated biomarkers selected from EBF1, GRM5, TSKS, ENPP2, AP3S2, LRRC37A3, C16orf68, LOC284751, IRF6, LHFP, BANK1, ARHGEF5, ZNF254, TFDP1, COL13A1, GSTK1, ADAMTSL4, P2RX5, LHFP, PIK3C2B, CHURC1, EXT2, HLA-DOA, OOEP, ZNF185, TMEM 19, FCRL 1, FLJ40125, ARHGEF12, CLEC18A, CD46, PTPN20A /// PTPN20B, and C19orf28.

[0055] In one embodiment, the solid support comprises a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 14. In one embodiment, the solid support comprises 2, 5, 10, 15, 20, 25, 30, 35, or more or all, nucleic acids that hybridize to a plurality of atrial fibrillation stroke-associated biomarkers selected from EBF1, FLJ31945, C16orf68, SLC20A1, DOPEY2, COL13A1, LHFP, LOC284751, GRM5, LOC100144603, MTBP, SHOX2, ARHGEF5, RNF7, CLASP2, GIPC2, RANBP10, CMBL, LOC100127980, CYTH3, PROCR, LOC146880, SLC6A19, ICAM4, C12orf42, ARHGEF12, PRSS35, NT5E, LOC100271832, LHFP, NT5E and AKR1C3.

[0056] In one embodiment, the solid support comprises a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 15. In one embodiment, the solid support comprises 2, 5, 10, 15, 18, or more or all, nucleic acids that hybridize to a plurality of atrial fibrillation stroke-associated biomarkers selected from CMTM1, COL13A1, SDC4, C6orf164, GPR176, BRUNOL6, SNORA68, MIF /// SLC2A11, DUSP16, HIPK2, TTC7A, PPIE, GRLF1, MAP3K7IP1, LOC100129034, PER3, SMC1A, and LRRC43.

[0057] In various embodiments, the solid support further comprises a plurality of nucleic acids that hybridize to a plurality of endogenous reference genes selected from the group consisting of USP7, MAPRE2, CSNK1G2, SAFB2, PRKAR2A, PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF1, KCMF1, PRKRIP1, CHMP4A, TMEM184C, TINF2, PODNL1, FBXO42, LOC441258, RRP1, C10orf104, ZDHHC5, C9orf23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16.

DEFINITIONS

[0058] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. Generally, enzymatic reactions and purification steps are performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (*see generally,* Sambrook et al. MOLECULAR CLONING: A LABORATORY MANUAL, 3rd ed. (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel, et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 1990-2008, Wiley Interscience), which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical chemistry, and organic synthetic described below are those well known and commonly employed in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

[0059] "Ischemia" or "ischemic event" as used herein refers to diseases and disorders characterized by inadequate blood supply (*i.e.,* circulation) to a local area due to blockage of the blood vessels to the area. Ischemia includes for example, strokes and transient ischemic attacks. Strokes include, e.g., ischemic stroke (including, but not limited to, cardioembolic strokes, atheroembolic or atherothrombotic strokes, *i.e.,* strokes caused by atherosclerosis in the carotid, aorta, heart, and brain, small vessel strokes (*i.e.,* lacunar strokes), strokes caused by diseases of the vessel wall, *i.e.,* vasculitis, strokes caused by infection, strokes caused by hematological disorders, strokes caused by migraines, and strokes caused by medications such as hormone therapy), hemorrhagic ischemic stroke, intracerebral hemorrhage, and subarachnoid hemorrhage.

[0060] "Ischemia reference expression profile" refers to the pattern of expression of a set of genes *(e.g.,* a plurality of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15) differentially expressed (*i.e.,* overexpressed or underexpressed) in ischemia relative to a control *(e.g.,* the expression level in an individual free of an ischemic event or the expression level of a stably expressed endogenous reference biomarker). A gene from Tables 7A, 7B, 13A, 13B, 14 and 15 that is expressed at a level that is at least about 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 3.1-, 3.2-, 3.3-, 3.4- or 3.5-fold higher than the level in a control is a gene overexpressed in ischemia and a gene from Tables 7A, 7B, 13A, 13B, 14 and 15 that is expressed at a level that is at least about 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 3.1-, 3.2-, 3.3-, 3.4- or 3.5-fold lower than the level in a control is a gene underexpressed in ischemia. Alternately, genes that are expressed at a level that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% higher than the level in a control is a gene overexpressed in ischemia and a gene that is expressed at a level that is at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% lower than the level in a control is a gene underexpressed in ischemia.

[0061] A "plurality" refers to two or more or all, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or more (e.g., genes). In some embodiments, a plurality refers to concurrent or sequential determination of about 15-85, 20-60 or 40-50 genes, for example, about 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100, or more or all, genes. In some embodiments, "plurality" refers to all genes listed in one or more tables, *e.g.,* all genes listed in Tables 7A, 7B, 13A, 13B, 14 and 15.

[0062] "Sample" or "biological sample" includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histologic purposes. Such samples include blood, sputum, tissue, lysed cells, brain biopsy, cultured cells, e.g., primary cultures, explants, and transformed cells, stool, urine, etc. A biological sample is typically obtained from a eukaryotic organism, most preferably a mammal such as a primate, e.g., chimpanzee or human; cow; dog; cat; a rodent, e.g., guinea pig, rat, mouse; rabbit; or a bird; reptile; or fish.

[0063] "Array" as used herein refers to a solid support comprising attached nucleic acid or peptide probes. Arrays typically comprise a plurality of different nucleic acid or peptide probes that are coupled to a surface of a substrate in different, known locations. These arrays, also described as "microarrays" or colloquially "chips" have been generally described in the art, for example, U.S. Patent Nos. 5,143,854, 5,445,934, 5,744,305, 5,677,195, 6,040,193, 5,424,186 and Fodor et al., Science, 251:767-777 (1991). These arrays may generally be produced using mechanical synthesis methods or light directed synthesis methods which incorporate a combination of photolithographic methods and solid phase synthesis methods. Techniques for the synthesis of these arrays using mechanical synthesis methods are described in, *e.g.,* U.S. Patent No. 5,384,261. Arrays may comprise a planar surface or may be nucleic acids or peptides on beads, gels, polymeric surfaces, fibers such as fiber optics, glass or any other appropriate substrate as described in, *e.g.,* U.S. Patent No. 5,770,358, 5,789,162, 5,708,153, 6,040,193 and 5,800,992. Arrays may be packaged in such a manner as to allow for diagnostics or other manipulation of an all inclusive device, as described in, *e.g.,* U.S. Patent Nos. 5,856,174 and 5,922,591.

[0064] The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region

[0065] (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

[0066] The terms "nucleic acid" and "polynucleotide" are used interchangeably herein to refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0067] Unless otherwise indicated, a particular nucleic acid sequence also encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, mRNA, oligonucleotide, and

polynucleotide.

[0068] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent hybridization conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent hybridization conditions are selected to be about 5-10°C lower than the thermal melting point for the specific sequence at a defined ionic strength Ph. The T_m is the temperature (under defined ionic strength, Ph, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent hybridization conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at Ph 7.0 to 8.3 and the temperature is at least about 30°C for short probes *(e.g.,* 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent hybridization conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42°C, or, 5x SSC, 1% SDS, incubating at 65°C, with wash in 0.2x SSC, and 0.1% SDS at 65°C.

[0069] Nucleic acids that do not hybridize to each other under stringent hybridization conditions are still substantially identical if the polypeptides which they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

[0070] The terms "isolated," "purified," or "biologically pure" refer to material that is substantially or essentially free from components that normally accompany it as found in its native state. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein that is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

[0071] The term "heterologous" when used with reference to portions of a nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.,* a promoter from one source and a coding region from another source. Similarly, a heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature *(e.g.,* a fusion protein).

[0072] An "expression vector" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a host cell. The expression vector can be part of a plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic acid to be transcribed operably linked to a promoter.

[0073] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer.

[0074] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, α-carboxyglutamate, and O-phosphoserine. "Amino acid analogs" refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.,* an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0075] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be re-

ferred to by their commonly accepted single-letter codes.

[0076] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0077] As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[0078] The following eight groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine I, Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M)

(*see, e.g.,* Creighton, Proteins (1984)).

[0079] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.,* 60% identity, preferably 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity over a specified region of an ischemia-associated gene *(e.g.,* a gene set forth in Tables 7A, 7B, 13A, 13B, 14 and 15), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Such sequences are then said to be "substantially identical." This definition also refers to the compliment of a test sequence. Preferably, the identity exists over a region that is at least about 25 amino acids or nucleotides in length, or more preferably over a region that is 50-100 amino acids or nucleotides in length.

[0080] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. For sequence comparison of nucleic acids and proteins to ischemia-associated nucleic acids and proteins, the BLAST and BLAST 2.0 algorithms and the default parameters discussed below are used.

[0081] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, *e.g.,* Current Protocols in Molecular Biology (Ausubel et al., eds. 1995 supplement)).

[0082] A preferred example of algorithm that is suitable for determining percent sequence identity and sequence

similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402 (1977) and Altschul et al., J. Mol. Biol. 215:403-410 (1990), respectively. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity for the nucleic acids and proteins of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al., supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands.

[0083] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.,* Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0084] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0085] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture *(e.g.,* total cellular or library DNA or RNA).

[0086] By "host cell" is meant a cell that contains an expression vector and supports the replication or expression of the expression vector. Host cells may be, for example, prokaryotic cells such as *E. coli* or eukaryotic cells such as yeast cells or mammalian cells such as CHO cells.

[0087] "Inhibitors," "activators," and "modulators" of expression or of activity are used to refer to inhibitory, activating, or modulating molecules, respectively, identified using *in vitro* and *in vivo* assays for expression or activity, e.g., ligands, agonists, antagonists, and their homologs and mimetics. The term "modulator" includes inhibitors and activators. Inhibitors are agents that, e.g., inhibit expression of a polypeptide or polynucleotide of the invention or bind to, partially or totally block stimulation or enzymatic activity, decrease, prevent, delay activation, inactivate, desensitize, or down regulate the activity of a polypeptide or polynucleotide of the invention, e.g., antagonists. Activators are agents that, e.g., induce or activate the expression of a polypeptide or polynucleotide of the invention or bind to, stimulate, increase, open, activate, facilitate, enhance activation or enzymatic activity, sensitize or up regulate the activity of a polypeptide or polynucleotide of the invention, e.g., agonists. Modulators include naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Assays to identify inhibitors and activators include, e.g., applying putative modulator compounds to cells, in the presence or absence of a polypeptide or polynucleotide of the invention and then determining the functional effects on a polypeptide or polynucleotide of the invention activity. Samples or assays comprising a polypeptide or polynucleotide of the invention that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative activity value of 100%. Inhibition is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is about 80%, optionally 50% or 25-1%. Activation is achieved when the activity value of a polypeptide or polynucleotide of the invention relative to the control is 110%, optionally 150%, optionally 200-500%, or 1000-3000% higher.

[0088] The term "test compound" or "drug candidate" or "modulator" or grammatical equivalents as used herein de-

scribes any molecule, either naturally occurring or synthetic, e.g., protein, oligopeptide (e.g., from about 5 to about 25 amino acids in length, preferably from about 10 to 20 or 12 to 18 amino acids in length, preferably 12, 15, or 18 amino acids in length), small organic molecule, polysaccharide, lipid, fatty acid, polynucleotide, RNAi, oligonucleotide, etc. The test compound can be in the form of a library of test compounds, such as a combinatorial or randomized library that provides a sufficient range of diversity. Test compounds are optionally linked to a fusion partner, e.g., targeting compounds, rescue compounds, dimerization compounds, stabilizing compounds, addressable compounds, and other functional moieties. Conventionally, new chemical entities with useful properties are generated by identifying a test compound (called a "lead compound") with some desirable property or activity, e.g., inhibiting activity, creating variants of the lead compound, and evaluating the property and activity of those variant compounds. Often, high throughput screening (HTS) methods are employed for such an analysis.

[0089] A "small organic molecule" refers to an organic molecule, either naturally occurring or synthetic, that has a molecular weight of more than about 50 Daltons and less than about 2500 Daltons, preferably less than about 2000 Daltons, preferably between about 100 to about 1000 Daltons, more preferably between about 200 to about 500 Daltons.

BRIEF DESCRIPTION OF THE DRAWINGS

[0090]

Figure 1. PAM prediction accuracy of IS and Healthy controls using the set of 29 gene predictors of IS from Tang et al, 2006. The Prediction Analysis of Microarrays (PAM) algorithm (K-NN, number of neighbors n=10) was trained on the expression values of a first random half of IS (n=35, 100 samples) and healthy (n=19) subjects from the current study using the 29 IS predictors from Tang et al, 2006. Then, these 29 IS predictors were used to predict the class of the second half of the samples (IS n=35, 99 samples; and healthy n=19, Test Set) and calculate the prediction accuracy. The X-axis represents the patient sample number and the Y-axis represents the Test Set probability of diagnosis. A sample is considered misclass¬ified if the predicted class does not match the known class with a probability greater than 0.5.

Figures 2A-C. PAM prediction accuracy of IS predictors in the current study. Prediction accuracy of the Test Set using PAM. Prediction Analysis of Microarrays (PAM) was used to perform the predictions (K-NN, neighbors n=10; threshold =0). For panels A, B and C the X-axis represents the patient sample number and the Y -axis represents Test Set probabilities. A sample is considered miss-classified if its correct class predicted probability is less than 0.5. The numbers of subjects in the Training Set were: 3h IS n=34; 24h IS n =33; SAVVY vascular controls n=26; and MI n=9. The numbers of subjects in the Test Set were: 3h IS n=33; 24h IS n=33; SAVVY n=26; and MI n=8. **A**. **3h IS predictors.** The 60-probe set predictors for 3h IS (combined from comparisons of 3h IS samples to healthy, MI and SAVVY samples from the Training Set) were put into PAM to predict the class of the Test Set subject samples by calculating the probability that they were in a given class. **B. 24h IS predictors.** The 46-probe set predictors for 24h IS (combined from comparisons of 24h IS samples to healthy, MI and SAVVY samples from the Training Set) were put into PAM to predict the class of the Test Set subject samples by calculating the probability that they were in a given class. **C. Combined 3h and 24h IS predictors.** The 97-probe set predictors for 3h IS and 24h IS (combined from comparisons of 3h IS and 24h IS samples to healthy, MI and SAVVY samples from the Training Set) were put into PAM to predict the class of the Test Set subject samples by calculating the probability that they were in a given class.

Figure 3. Diagram of the analysis work flow for the identification of IS predictors.

Figure 4. PAM prediction accuracy of IS and healthy using the 29 probe set predictors of IS from Tang et al, 2006. The internal gene normalized expression values of all IS (n=70, 199 samples) and healthy (n=38) for the 29 IS predictors from Tang et al, 2006 were used as input in PAM. K-NN (number of neighbors n=10) threshold =0 (including all 29 predictors, and a 10-fold cross-validation was used to estimate prediction accuracy. X-axis represents sample number and the Y-axis represents cross-validated probability of diagnosis. A sample is considered misclassified if the predicted class does not match the known class with a probability greater than 0.5.

Figure 5. PAM 3h vs. Healthy test set + test set confusion matrix

Figure 6. PAM 3h vs. MI CV + CV confusion matrix

Figure 7. PAM 3h vs. SAVVY test set + test set confusion matrix

Figure 8. PAM 24h vs. healthy test set + test set confusion matrix

Figure 9. PAM 24h vs. MI CV + CV confusion matrix

Figure 10. PAM 24h vs. SAVVY test set + test set confusion matrix

Figures 11A-C. PAM on Combined 3h, 24h and 3+24h IS predictors. CV Probabilities. **Figure 11A.** 3h IS predictors. Combined 60-probe set predictors from combined analysis on 3h IS vs all controls (healthy, MI and SAVVY) were input in PAM. **Figure 11B**. 24h IS predictors. Combined 46-probe set predictors from combined analysis on 24h IS vs all controls (healthy, MI and SAVVY) were input in PAM. **Figure 11C.** Combined 3h and 24h IS predictors. Combined 97-probe set predictors from combined analysis on 3h IS and 24h IS vs all controls (healthy, MI and SAVVY) were input in PAM.

Figures 12A-B A. Hierarchical cluster plot of the 40 genes found to differentiate cardioembolic stroke from large vessel stroke. Genes are shown on the y-axis and subjects are shown on the x-axis. Red indicates a high level of gene expression and blue indicates a low level of gene expression. Subjects can be observed to cluster by diagnosis. A group of genes have a high level of expression in cardioembolic stroke and a low level of expression in large vessel stroke. A separate group of genes have a low level of expression in cardioembolic stroke and a high level of expression in large vessel stroke. The cardioembolic group appears to cluster into two subgroups. **B**. Principal Component Analysis (PCA) of the 40 genes found to differentiate cardioembolic stroke from large vessel stroke. Each sphere represents a single subject. The ellipsoid surrounding the spheres represents two standard deviations from the group mean.

Figure 13. Leave one out cross-validation prediction analysis of the 40 total genes found to differentiate cardioembolic stroke from large vessel stroke. The probability of the predicted diagnosis in shown on the y-axis. The actual diagnosis of is shown on the x-axis. Subjects with cardioembolic stroke were predicted to have cardioembolic stroke for 69 out of 69 samples (100% correct prediction). Subjects with large vessel stroke were predicted to have large vessel stroke for 29 out of 30 samples (96.7% correct prediction). A sample is considered misclassified if the predicted class does not match the known class with a probability greater than 0.5.

Figure 14. Venn diagram of genes identified from the comparison of cardioembolic to controls, and large vessel stroke to control ($p<0.005$, FC> |1.2|). A total of 503 genes were found to be unique to cardioembolic stroke, 554 genes unique to large vessel stroke and 228 genes were common to stroke subtypes. These gene lists were used for functional analyses shown in Tables 9-11.

Figure 15A-B. A. Hierarchical cluster analysis of the 37 genes found to differentiate cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes. Genes are shown on the y-axis and subjects are shown on the x-axis. Red indicates a high level of gene expression and blue indicates a low level of gene expression. Subjects can be observed to cluster by diagnosis. A group of genes have a high level of expression in cardioembolic stroke due to atrial fibrillation and a low level of expression in non-atrial fibrillation causes. A group of genes have a low level of expression in cardioembolic stroke due to atrial fibrillation and a high level of expression in non-atrial fibrillation causes. **B.** Principal Component Analysis of the 37 genes found to differentiate cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes. Each sphere represents a single subject. The ellipsoid surrounding the spheres represents two standard deviations from the group mean.

Figure 16. Leave one out cross-validation prediction analysis of the 37 genes found to differentiate cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes. The probability of the predicted diagnosis in shown on the y-axis. The actual diagnosis of is shown on the x-axis. Subjects with cardioembolic stroke due to atrial fibrillation were predicted to have atrial fibrillation as a cause of stroke in 30 out of 30 samples (100% correct prediction). Subjects with cardioembolic stroke due to non-atrial fibrillation causes were correctly predicted in 22 out of 24 samples (91.7% correct prediction). A sample is considered misclassified if the predicted class does not match the known class with a probability greater than 0.5.

Figure 17. Hierarchical cluster plots and PCAs of the 40 genes found to differentiate cardioembolic stroke from large vessel stroke at 3 hours, 5 hours and 24 hours following stroke onset. The hierarchical clusters show that separation by the 40 genes of cardioembolic stroke from large vessel stroke is achieved at 3 hours, 5 hours and 24 hours following onset of ischemic stroke. This is confirmed by the PCAs which show that subjects with cardioembolic stroke are separated by greater than two standard deviations from large vessel stroke.

Figure 18. Hierarchical cluster plots and PCAs of the 37 genes found differentiate cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes at 3 hours, 5 hours and 24 hours following the stroke onset. The hierarchical clusters show the 37 genes can separate cardioembolic stroke due atrial fibrillation non-atrial fibrillation causes at 3 hours, 5 hours and 24 hours following onset of ischemic stroke. This is confirmed by the PCA analyses which show that subjects with cardioembolic stroke due to atrial fibrillation are separated by greater than two standard deviations from non-atrial fibrillation causes.

DETAILED DESCRIPTION

1. Introduction

[0091] The present invention provides biomarkers for diagnosing the occurrence and risk of stroke in a patient, and further biomarkers for determining the cause of stroke in an individual diagnosed as experiencing a stroke or with a predisposition for experiencing a stroke. Evaluation of the expression levels of combined biomarkers, *e.g.,* in a sample of blood, serum or plasma, allows the rapid diagnosis of the occurrence and cause of stroke in a patient who has experienced a suspected stroke event or who is experiencing symptoms indicative of a risk of stroke. By simultaneously determining whether a stroke has occurred, and the underlying cause of the stroke, appropriate medical treatment or intervention regimes are delivered to the patient as rapidly as possible. It is particularly desirable to be able to diagnose and treat a patient within 3 hours of a suspected stroke event. The present invention makes this possible, e.g., using available microarray technologies.

[0092] The biomarkers described herein for the diagnosis of the occurrence and risk of stroke can be used together, *e.g.,* on a single microarray or in a single assay procedure. The biomarkers also find use independently for the diagnosis of the occurrence of stroke, *e.g.,* in conjunction with alternative methods for determining the cause of stroke, and for determining the cause of stroke, *e.g.,* in conjunction with alternative methods for determining whether a stroke has occurred.

2. Patients Who Can Benefit from the Present Methods

[0093] Individuals who will benefit from the present methods may be exhibiting symptoms of ischemic stroke. In some embodiments, the subject has experienced an ischemic event (e.g., TIA, ischemic stroke, myocardial infarction, peripheral vascular disease, or venous thromboembolism). Alternatively, the subject may be suspected of having experienced an ischemic event. In some embodiments, the subject has not experienced and/or is not at risk of having an intracerebral hemorrhage or hemorrhagic stroke. In some embodiments, the subject has been diagnosed as having not experienced and/or not at risk of having an intracerebral hemorrhage or hemorrhagic stroke.

[0094] In some embodiments, the levels of expression of the panel of biomarkers are determined within 3 hours of a suspected ischemic event. In some embodiments, the levels of expression of the panel of biomarkers are determined at 3 or more hours after a suspected ischemic event. In some embodiments, the levels of expression of the panel of biomarkers are determined within 6, 12, 18, 24, 36, 48 hours of a suspected ischemic event.

[0095] In some cases, the subject is asymptomatic, but may have a risk or predisposition to experiencing ischemic stroke, e.g., based on genetics, a related disease condition, environment or lifestyle. In some embodiments, the patient has one or more vascular risk factors, e.g., hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking.

3. Biomarkers Useful for the Prediction or Diagnosis of Stroke

[0096] Biomarkers useful for the prediction, diagnosis or confirmation of the occurrence of ischemic stroke are listed in Tables 7A and 7B. Determination of the expression levels of a plurality of the biomarkers of Table 7A can be performed for the prediction, diagnosis or confirmation of the occurrence of stroke in conjunction with other biomarkers known in the art for the prediction, diagnosis or confirmation of the occurrence of stroke, in conjunction with other methods known in the art for the diagnosis of stroke, in conjunction with biomarkers described herein and known in the art useful for determining the cause of stroke *(e.g.,* as described herein) and/or in conjunction with methods known in the art for determining the cause of stroke.

[0097] Determination of the expression levels of a plurality of the biomarkers of Table 7A can be performed for the prediction, diagnosis or confirmation of the occurrence of stroke can also be performed independently, e.g., to diagnose that a stroke has occurred or determine the risk that a patient may suffer a stroke, independently of its cause.

[0098] In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30, 40, 50, 60 or more biomarkers from Table 7A (and Table 7B) are determined. In some embodiments, the expression levels of a plurality of biomarkers in Table 7A and a plurality of biomarkers in Table 7B are determined.

[0099] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined

within 3 hours of a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of FAT3, GADL1, CXADR, RNF141, CLEC4E, TIMP2, ANKRD28, TIMM8A, PTPRD, CCRL1, FCRL4, DLX6, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, MCTP1 and SH3GL3 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, SNRPN, GLYATL1, DKRZP434L187, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, CCDC144A, ALDOAP2, LDB3, LOC729222 /// PPFIBP1, HNRNPUL2, ELAVL2, PRTG, FOXA2, SCD5, LOC283027, LOC344595, RPL22, LOC100129488 and RPL22 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0100] In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of CLEC4E, TIMP2, FGD4, CPEB2, LTB4R and VNN3 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with myocardial infarction within 3 hours of a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CKLF, LOC100290882, UBXN2B, ENTPD1, BST1, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22, MCTP1 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0101] In various embodiments, in an individual presenting with one or more vascular risk factors (e.g., hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking) within 3 hours of a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors within 3 hours of a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0102] In some embodiments, the level of expression of biomarkers indicative of the occurrence of stroke is determined 3 or more hours after a suspected ischemic event. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, CLEC4E, BXDC5, UNC5B, TIMP2, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488 and MCTP1 indicates that the patient suffers from or is at risk of developing ischemic stroke. In an otherwise healthy individual (*i.e.,* no myocardial infarction, no vascular risk factors), a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of SPTLC3, DKRZP434L187, SPIB, HNRNPUL2, FOXA2, RPL22 and SH3GL3 indicates that the patient suffers from or is at risk of developing ischemic stroke.

[0103] In various embodiments, in an individual presenting with myocardial infarction 3 or more hours after a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, HNRNPUL2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, UBXN2B, BST1, LTB4R, F5, IFRD1, KIAA0319, MCTP1, VNN3, AMN1, LAMP2, ZNF608, FAR2,

GAB1, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with myocardial infarction 3 or more hours after a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC100290882, ENTPD1, CHMP1B, FCHO2, LOC283027, REM2, QKI, RBM25, ST3GAL6, HNRNPH2, UBR5, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0104] In various embodiments, in an individual presenting with one or more vascular risk factors 3 or more hours after a suspected ischemic event, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA and MCTP1 indicates that the individual suffers from or is at risk of developing ischemic stroke. In various embodiments, in an individual presenting with one or more vascular risk factors 3 or more hours after a suspected ischemic event, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 7A selected from the group consisting of PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, RPL22, LOC100129488, RPL22 and SH3GL3 indicates that the individual suffers from or is at risk of developing ischemic stroke.

[0105] Overexpression or underexpression of a plurality of biomarkers from Table 7A (and Table 7B) that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference biomarkers, e.g., those listed in Table 16 indicates that the subject has experienced or is at risk of experiencing an ischemic stroke. Overexpression or underexpression of a plurality of biomarkers from Table 7A (and Table 7B) that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression level of the same biomarker in an individual or a population of individuals who have not experienced a vascular event indicates that the subject has experienced or is at risk of experiencing an ischemic stroke.

4. Biomarkers Useful for the Diagnosis of Cause of Stroke

[0106] Biomarkers useful for the determination and diagnosis of the cause of stroke are listed in Tables 13A, 13B, 14 and 15. Determination of the expression levels of a plurality of the biomarkers of Tables 13A, 13B, 14 and 15 independently can be performed for the determination of the cause of stroke in conjunction with biomarkers described herein and known in the art for the prediction, diagnosis or confirmation of the occurrence of stroke, in conjunction with other methods known in the art for the diagnosis of stroke, in conjunction with other biomarkers known in the art useful for determining the cause of stroke (*e.g.,* as described herein) and/or in conjunction with methods known in the art for determining the cause of stroke. Classification of stroke subtypes is known in the art and reviewed in, *e.g.,* in Amarenco, et al., Cerebrovasc Dis (2009) 27:493-501.

[0107] Determination of the expression levels of a plurality of the biomarkers of Tables 13A, 14 and 15 can be performed for the determination of the cause of stroke can also be performed independently, e.g., to diagnose the cause of a stroke when it is already known that a stroke has occurred or that the patient has a predisposition to experience ischemic stroke.

[0108] In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30, 40, 50, 60 or more biomarkers from Tables 13A (and Table 13B) are independently determined. In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30, 40, 50, 60 or more biomarkers from Table 14 are independently determined. In some embodiments, the expression levels of at least about 3, 5, 10, 15, 20, 25, 30, 40, 50, 60 or more biomarkers from Table 15 are independently determined. In some embodiments, the expression levels of a plurality of biomarkers in Table 13A and a plurality of biomarkers in Table 13B are determined. In some embodiments, the expression levels of a plurality of biomarkers in Table 14 are determined. In some embodiments, the expression levels of a plurality of biomarkers in Table 15 are determined.

[0109] The biomarkers in Tables 13A and 13B find use in the determination of whether a patient has experienced or has a predisposition to experience cardioembolic stroke (*a.k.a,* cardiac embolism, cardioembolism emboligenic heart disease). A cardioembolic stroke occurs when a thrombus (clot) dislodges from the heart, travels through the cardiovascular system and lodges in the brain, first cutting off the blood supply and then often causing a hemorrhagic bleed.

In some embodiments an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A /// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP and LRRC37A3 indicates that the patient has experienced or is at risk for cardioembolic stroke. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of LOC284751, CD46, ENPP2, C19orf28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, C16orf68, TFDP1 and GSTK1 indicates that the patient has experienced or is at risk for cardioembolic stroke.

[0110] Overexpression or underexpression of a plurality of biomarkers from Table 13A (and Table 13B) that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference biomarkers, *e.g.*, those listed in Table 16 indicates that the subject has experienced or is at risk of experiencing cardioembolic stroke. Overexpression or underexpression of a plurality of biomarkers from Table 13A (and Table 13B) that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression level of the same biomarker in an individual or a population of individuals who have not experienced a vascular event indicates that the subject has experienced or is at risk of experiencing cardioembolic stroke.

[0111] The biomarkers in Table 14 find use in the determination of whether a patient has experienced or has a predisposition to experience carotid stenosis. Carotid stenosis is a narrowing or constriction of the inner surface (lumen) of the carotid artery, usually caused by atherosclerosis. An inflammatory buildup of plaque can narrow the carotid artery and can be a source of embolization. Emboli break off from the plaque and travel through the circulation to blood vessels in the brain, causing ischemia that can either be temporary (*e.g.,* a transient ischemic attack), or permanent resulting in a thromboembolic stroke (*a.k.a.,* atherothrombosis, large-artery atherosclerosis, atherosclerosis with stenosis). In some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12orf42 and LOC100127980 indicates that the patient has experienced or is at risk for carotid stenosis. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880, SLC20A1, SLC6A19, ARHGEF12, C16orf68, GIPC2 and LOC100144603 indicates that the patient has experienced or is at risk for carotid stenosis.

[0112] Overexpression or underexpression of a plurality of biomarkers from Table 14 that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference biomarkers, *e.g.,* those listed in Table 16 indicates that the subject has experienced or is at risk of experiencing carotid stenosis. Overexpression or underexpression of a plurality of biomarkers from Table 14 that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression level of the same biomarker in an individual or a population of individuals who have not experienced a vascular event indicates that the subject has experienced or is at risk of experiencing carotid stenosis.

[0113] The biomarkers in Table 15 find use in the determination of whether a patient has experienced or has a predisposition to experience atrial fibrillation. Atrial fibrillation (AF or A-fib) is the most common cardiac arrhythmia and involves the two upper chambers (atria) of the heart fibrillating (i.e., quivering) instead of a coordinated contraction. In some instances, cardioembolic stroke can occur as a result of atrial fibrillation. Cardioembolic stroke can be a downstream result of atrial fibrillation in that stagnant blood in the fibrillating atrium can form a thrombus that then embolises to the cerebral circulation, blocking arterial blood flow and causing ischaemic injury. In some embodiments, an increased expression level of one or more or all ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of SMC1A, SNORA68, GRLF1, SDC4, HIPK2, LOC100129034, CMTM1 and TTC7A indicates that the patient has experienced or is at risk for atrial fibrillation. In some embodiments, a decreased expression level of one or more or all ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6orf164 and MAP3K7IP1 indicates that the patient has experienced or is at risk for atrial fibrillation.

[0114] Overexpression or underexpression of a plurality of biomarkers from Table 15 that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference biomarkers, e.g., those listed in Table 16 indicates that the subject has experienced or is at risk of experiencing atrial fibrillation. Overexpression or underexpression of a plurality of biomarkers from Table 15 that is at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-

fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression level of the same biomarker in an individual or a population of individuals who have not experienced a vascular event indicates that the subject has experienced or is at risk of experiencing atrial fibrillation.

5. Comparison to a Control Level of Expression

[0115] The expression of the ischemic stroke-associated biomarkers are compared to a control ischemic stroke level of expression. As appropriate, the control level of expression can be the expression level of the same ischemic stroke-associated biomarker in an otherwise healthy individual (*e.g.,* in an individual who has not experienced and/or is not at risk of experiencing TIA). In some embodiments, the control level of expression is the expression level of a plurality of stably expressed endogenous reference biomarkers, as described herein or known in the art. In some embodiments, the control level of expression is a predetermined threshold level of expression of the same ischemic stroke-associated biomarker, *e.g.,* based on the expression level of the biomarker in a population of otherwise healthy individuals. In some embodiments, the expression level of the ischemic stroke-associated biomarker and the ischemic stroke-associated biomarker in an otherwise healthy individual are normalized to (*i.e.,* divided by), *e.g.,* the expression levels of a plurality of stably expressed endogenous reference biomarkers.

[0116] In some embodiments, the overexpression or underexpression of a ischemic stroke-associated biomarker is determined with reference to the expression of the same ischemic stroke associated biomarker in an otherwise healthy individual. For example, a healthy or normal control individual has not experienced and/or is not at risk of experiencing ischemic stroke. The healthy or normal control individual generally has not experienced a vascular event (*e.g.,* TIA, ischemic stroke, myocardial infarction, peripheral vascular disease, or venous thromboembolism). The healthy or normal control individual generally does not have one or more vascular risk factors (*e.g.,* hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking). As appropriate, the expression levels of the target ischemic stroke-associated biomarker in the healthy or normal control individual can be normalized (*i.e.,* divided by) the expression levels of a plurality of stably expressed endogenous reference biomarkers.

[0117] In some embodiments, the overexpression or underexpression of a ischemic stroke-associated biomarker is determined with reference to one or more stably expressed endogenous reference biomarkers. Internal control biomarkers or endogenous reference biomarkers are expressed at the same or nearly the same expression levels in the blood of patients with stroke or TIAs as compared to control patients. Target biomarkers are expressed at higher or lower levels in the blood of the stroke or TIA patients. The expression levels of the target biomarker to the reference biomarker are normalized by dividing the expression level of the target biomarker to the expression levels of a plurality of endogenous reference biomarkers. The normalized expression level of a target biomarker can be used to predict the occurrence or lack thereof of stroke or TIA, and/or the cause of stroke or TIA.

[0118] In some embodiments, the expression level of the ischemic stroke-associated biomarker from a patient suspected of having or experiencing ischemic stroke and from a control patient are normalized with respect to the expression levels of a plurality of stably expressed endogenous. The expression levels of the normalized expression of the ischemic stroke-associated biomarker is compared to the expression levels of the normalized expression of the same ischemic stroke-associated biomarker in a control patient. The determined fold change in expression = normalized expression of target biomarker in ischemic stroke patient/ normalized expression of target biomarker in control patient. Overexpression or underexpression of the normalized ischemic stroke-associated biomarker in the ischemic stroke patient by at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of the normalized ischemic stroke-associated biomarker in a healthy control patient indicates that the ischemic stroke patient has experienced or is at risk of experiencing ischemic stroke.

[0119] In some embodiments, the control level of expression is a predetermined threshold level. The threshold level can correspond to the level of expression of the same ischemic stroke-associated biomarker in an otherwise healthy individual or a population of otherwise healthy individuals, optionally normalized to the expression levels of a plurality of endogenous reference biomarkers. After expression levels and normalized expression levels of the ischemic stroke-associated biomarkers are determined in a representative number of otherwise healthy individuals and individuals predisposed to experiencing ischemic stroke, normal and ischemic stroke expression levels of the ischemic stroke-associated biomarkers can be maintained in a database, allowing for determination of threshold expression levels indicative of the presence or absence of risk to experience ischemic stroke or the occurrence of ischemic stroke. If the predetermined threshold level of expression is with respect to a population of normal control patients, then overexpression or underexpression of the ischemic stroke-associated biomarker (usually normalized) in the ischemic stroke patient by at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the threshold level indicates that the ischemic stroke patient has experienced or is at risk of experiencing

ischemic stroke. If the predetermined threshold level of expression is with respect to a population of patients known to have experienced ischemic stroke or known to be at risk for experiencing ischemic stroke, then an expression level in the patient suspected of experiencing ischemic stroke that is approximately equal to the threshold level (or overexpressed or underexpressed greater than the threshold level of expression), indicates that the ischemic stroke patient has experienced or is at risk of experiencing ischemic stroke.

[0120] With respect to the endogenous reference biomarkers used for comparison, preferably, Exemplary endogenous reference biomarkers that find use are listed in Table 16, below. Further suitable endogenous reference biomarkers are published, *e.g.,* in Stamova, et al., BMC Medical Genomics (2009) 2:49. In some embodiments, the expression levels of a plurality of endogenous reference biomarkers are determined as a control. In some embodiments, the expression levels of at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or more or all, endogenous reference biomarkers, *e.g.,* as listed in Table 16 or known in the art, are determined as a control.

[0121] In some embodiments, the expression levels of the endogenous reference biomarkers GAPDH, ACTB, B2M, HMBS and PPIB are determined as a control. In some embodiments, the expression levels of 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or more or all, endogenous reference biomarkers selected from the group consisting of USP7, MAPRE2, CSNK1G2, SAFB2, PRKAR2A, PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, CDC2L1 /// CDC2L2, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF1, KCMF1, PRKRIP1, CHMP4A, TMEM184C, TINF2, PODNL1, FBXO42, LOC441258, RRP1, C10orf104, ZDHHC5, C9orf23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16 are determined as a control.

[0122] Biomarkers indicative of stroke or a particular cause of stroke have levels of expression that are at least about 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, 2.0-fold, 2.1 fold, 2.2-fold, 2.3-fold, 2.4-fold, 2.5-fold, 2.6-fold, 2.7-fold, 2.8-fold, 2.9-fold, 3.0-fold, 3.1-fold, 3.2-fold, 3.3-fold, 3.4-fold or 3.5-fold, or more, in comparison to the expression levels of a plurality of stably expressed endogenous reference biomarkers, e.g., the geometric average expression level of the evaluated endogenous reference biomarkers, *e.g.,* 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, or more biomarkers listed in Table 16.

6. Methods of Detecting Biomarkers

[0123] Gene expression may be measured using any method known in the art. One of skill in the art will appreciate that the means of measuring gene expression is not a critical aspect of the invention. The expression levels of the biomarkers can be detected at the transcriptional or translational (*i.e.,* protein) level.

[0124] In some embodiments, the expression levels of the biomarkers are detected at the transcriptional level. A variety of methods of specific DNA and RNA measurement using nucleic acid hybridization techniques are known to those of skill in the art (*see,* Sambrook, *supra* and Ausubel, *supra*) and may be used to detect the expression of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15. Some methods involve an electrophoretic separation (e.g., Southern blot for detecting DNA, and Northern blot for detecting RNA), but measurement of DNA and RNA can also be carried out in the absence of electrophoretic separation (e.g., by dot blot). Southern blot of genomic DNA (e.g., from a human) can be used for screening for restriction fragment length polymorphism (RFLP) to detect the presence of a genetic disorder affecting a polypeptide of the invention. All forms of RNA can be detected, including, *e.g.,* message RNA (mRNA), microRNA (miRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA).

[0125] The selection of a nucleic acid hybridization format is not critical. A variety of nucleic acid hybridization formats are known to those skilled in the art. For example, common formats include sandwich assays and competition or displacement assays. Hybridization techniques are generally described in Hames and Higgins Nucleic Acid Hybridization, A Practical Approach, IRL Press (1985); Gall and Pardue, Proc. Natl. Acad. Sci. U.S.A., 63:378-383 (1969); and John et al. Nature, 223:582-587 (1969).

[0126] Detection of a hybridization complex may require the binding of a signal-generating complex to a duplex of target and probe polynucleotides or nucleic acids. Typically, such binding occurs through ligand and anti-ligand interactions as between a ligand-conjugated probe and an anti-ligand conjugated with a signal. The binding of the signal generation complex is also readily amenable to accelerations by exposure to ultrasonic energy.

[0127] The label may also allow indirect detection of the hybridization complex. For example, where the label is a hapten or antigen, the sample can be detected by using antibodies. In these systems, a signal is generated by attaching fluorescent or enzyme molecules to the antibodies or in some cases, by attachment to a radioactive label (*see, e.g.,* Tijssen, "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Burdon and van Knippenberg Eds., Elsevier (1985), pp. 9-20).

[0128] The probes are typically labeled either directly, as with isotopes, chromophores, lumiphores, chromogens, or indirectly, such as with biotin, to which a streptavidin complex may later bind. Thus, the detectable labels used in the assays of the present invention can be primary labels (where the label comprises an element that is detected directly or that produces a directly detectable element) or secondary labels (where the detected label binds to a primary label, e.g., as is common in immunological labeling). Typically, labeled signal nucleic acids are used to detect hybridization.

Complementary nucleic acids or signal nucleic acids may be labeled by any one of several methods typically used to detect the presence of hybridized polynucleotides. The most common method of detection is the use of autoradiography with ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P-labeled probes or the like.

[0129] Other labels include, e.g., ligands that bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden Introduction to Immunocytochemistry, 2nd ed., Springer Verlag, NY (1997); and in Haugland Handbook of Fluorescent Probes and Research Chemicals, a combined handbook and catalogue Published by Molecular Probes, Inc. (1996).

[0130] In general, a detector which monitors a particular probe or probe combination is used to detect the detection reagent label. Typical detectors include spectrophotometers, phototubes and photodiodes, microscopes, scintillation counters, cameras, film and the like, as well as combinations thereof. Examples of suitable detectors are widely available from a variety of commercial sources known to persons of skill in the art. Commonly, an optical image of a substrate comprising bound labeling moieties is digitized for subsequent computer analysis.

[0131] Most typically, the amount of RNA is measured by quantifying the amount of label fixed to the solid support by binding of the detection reagent. Typically, the presence of a modulator during incubation will increase or decrease the amount of label fixed to the solid support relative to a control incubation which does not comprise the modulator, or as compared to a baseline established for a particular reaction type. Means of detecting and quantifying labels are well known to those of skill in the art.

[0132] In preferred embodiments, the target nucleic acid or the probe is immobilized on a solid support. Solid supports suitable for use in the assays of the invention are known to those of skill in the art. As used herein, a solid support is a matrix of material in a substantially fixed arrangement.

[0133] For example, in one embodiment of the invention, microarrays are used to detect the pattern of gene expression. Microarrays provide one method for the simultaneous measurement of the expression levels of large numbers of genes. Each array consists of a reproducible pattern of a plurality of nucleic acids (e.g., a plurality of nucleic acids that hybridize to a plurality of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15) attached to a solid support. In one embodiment, the array contains a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 7A (and 7B). In one embodiment, the array contains a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 13A (and 13B). In one embodiment, the array contains a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 14. In one embodiment, the array contains a plurality of nucleic acids that hybridize to a plurality of the genes listed in Table 15. Labeled RNA or DNA is hybridized to complementary probes on the array and then detected by laser scanning. Hybridization intensities for each probe on the array are determined and converted to a quantitative read-out of relative gene expression levels in ischemia (e.g., stroke or transient ischemic attacks).

[0134] In some embodiments, a sample is obtained from a subject, total mRNA is isolated from the sample and is converted to labeled cRNA and then hybridized to an array. Relative transcript levels are calculated by reference to appropriate controls present on the array and in the sample. See Mahadevappa and Warrington, Nat. Biotechnol. 17, 1134-1136 (1999).

[0135] A variety of automated solid-phase assay techniques are also appropriate. For instance, very large scale immobilized polymer arrays (VLSIPS™), available from Affymetrix, Inc. (Santa Clara, CA) can be used to detect changes in expression levels of a plurality of genes involved in the same regulatory pathways simultaneously. *See,* Tijssen, *supra*., Fodor et al. (1991) Science, 251: 767- 777; Sheldon et al. (1993) Clinical Chemistry 39(4): 718-719, and Kozal et al. (1996) Nature Medicine 2(7): 753-759. Integrated microfluidic systems and other point-of-care diagnostic devices available in the art also find use. *See, e.g.,* Liu and Mathies, Trends Biotechnol. (2009) 27(10):572-81 and Tothill, Semin Cell Dev Biol (2009) 20(1):55-62. Microfluidics systems for use in detecting levels of expression of a plurality of nucleic acids are available, *e.g.,* from NanoString Technologies, on the internet at nanostring.com.

[0136] Detection can be accomplished, for example, by using a labeled detection moiety that binds specifically to duplex nucleic acids (e.g., an antibody that is specific for RNA-DNA duplexes). One preferred example uses an antibody that recognizes DNA-RNA heteroduplexes in which the antibody is linked to an enzyme (typically by recombinant or covalent chemical bonding). The antibody is detected when the enzyme reacts with its substrate, producing a detectable product. Coutlee et al. (1989) Analytical Biochemistry 181:153-162; Bogulavski (1986) et al. J. Immunol. Methods 89:123-130; Prooijen-Knegt (1982) Exp. Cell Res. 141:397-407; Rudkin (1976) Nature 265:472-473, Stollar (1970) Proc. Nat'l Acad. Sci. USA 65:993-1000; Ballard (1982) Mol. Immunol. 19:793-799; Pisetsky and Caster (1982) Mol. Immunol. 19:645-650; Viscidi et al. (1988) J. Clin. Microbial. 41:199-209; and Kiney et al. (1989) J. Clin. Microbiol. 27:6-12 describe antibodies to RNA duplexes, including homo and heteroduplexes. Kits comprising antibodies specific for DNA:RNA hybrids are available, e.g., from Digene Diagnostics, Inc. (Beltsville, MD).

[0137] In addition to available antibodies, one of skill in the art can easily make antibodies specific for nucleic acid duplexes using existing techniques, or modify those antibodies that are commercially or publicly available. In addition to the art referenced above, general methods for producing polyclonal and monoclonal antibodies are known to those of skill in the art (*see, e.g.,* Paul (3rd ed.) Fundamental Immunology Raven Press, Ltd., NY (1993); Coligan, et al., Current

Protocols in Immunology, Wiley Interscience (1991-2008); Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor Press, NY (1988); Harlow and Lane, Using Antibodies, Cold Spring Harbor Press, NY (1999); Stites et al. (eds.) Basic and Clinical Immunology (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Goding Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY, (1986); and Kohler and Milstein Nature 256: 495-497 (1975)). Other suitable techniques for antibody preparation include selection of libraries of recombinant antibodies in phage or similar vectors (*see,* Huse et al. Science 246:1275-1281 (1989); and Ward et al. Nature 341:544-546 (1989)). Specific monoclonal and polyclonal antibodies and antisera will usually bind with a K_D of at least about 0.1 μM, preferably at least about 0.01 μM or better, and most typically and preferably, 0.001 μM or better.

[0138] The nucleic acids used in this invention can be either positive or negative probes. Positive probes bind to their targets and the presence of duplex formation is evidence of the presence of the target. Negative probes fail to bind to the suspect target and the absence of duplex formation is evidence of the presence of the target. For example, the use of a wild type specific nucleic acid probe or PCR primers may serve as a negative probe in an assay sample where only the nucleotide sequence of interest is present.

[0139] The sensitivity of the hybridization assays may be enhanced through use of a nucleic acid amplification system that multiplies the target nucleic acid being detected. Examples of such systems include the polymerase chain reaction (PCR) system, in particular RT-PCR or real time PCR, and the ligase chain reaction (LCR) system. Other methods recently described in the art are the nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario) and Q Beta Replicase systems. These systems can be used to directly identify mutants where the PCR or LCR primers are designed to be extended or ligated only when a selected sequence is present. Alternatively, the selected sequences can be generally amplified using, for example, nonspecific PCR primers and the amplified target region later probed for a specific sequence indicative of a mutation. High throughput multiplex nucleic acid sequencing or "deep sequencing" to detect captured expressed biomarker genes also finds use. High throughput sequencing techniques are known in the art (*e.g.,* 454 Sequencing on the internet at 454.com).

[0140] An alternative means for determining the level of expression of the nucleic acids of the present invention is *in situ* hybridization. *In situ* hybridization assays are well known and are generally described in Angerer et al., Methods Enzymol. 152:649-660 (1987). In an *in situ* hybridization assay, cells, preferentially human cells, *e.g.,* blood cells, are fixed to a solid support, typically a glass slide. If DNA is to be probed, the cells are denatured with heat or alkali. The cells are then contacted with a hybridization solution at a moderate temperature to permit annealing of specific probes that are labeled. The probes are preferably labeled with radioisotopes or fluorescent reporters.

[0141] In other embodiments, quantitative RT-PCR is used to detect the expression of a plurality of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15. In one embodiment, quantitative RT-PCR is used to detect a plurality of the genes listed in Table 7A (and 7B). In one embodiment, quantitative RT-PCR is used to detect a plurality of the genes listed in Table 13A (and 13B). In one embodiment, quantitative RT-PCR is used to detect a plurality of the genes listed in Table 14. In one embodiment, quantitative RT-PCR is used to detect a plurality of the genes listed in Table 15. A general overview of the applicable technology can be found, for example, in A-Z of Quantitative PCR, Bustin, ed., 2004, International University Line; Quantitative PCR Protocols, Kochanowski and Reischl, eds., 1999, Humana Press; Clinical Applications of PCR, Lo, ed., 2006, Humana Press; PCR Protocols: A Guide to Methods and Applications (Innis et al. eds. (1990)) and PCR Technology: Principles and Applications for DNA Amplification (Erlich, ed. (1992)). In addition, amplification technology is described in U.S. Patent Nos. 4,683,195 and 4,683,202. Methods for multiplex PCR, known in the art, are applicable to the present invention.

[0142] Accordingly, in one embodiment of the invention provides a reaction mixture comprising a plurality of polynucleotides which specifically hybridize (*e.g.,* primers) to a plurality of nucleic acid sequences of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15. In some embodiments, the invention provides a reaction mixture comprising a plurality of polynucleotides which specifically hybridize (*e.g.,* primers) to a plurality of nucleic acid sequences of the genes set forth in Table 7A (and 7B). In some embodiments, the invention provides a reaction mixture comprising a plurality of polynucleotides which specifically hybridize (*e.g.,* primers) to a plurality of nucleic acid sequences of the genes set forth in Table 13A (and 13B). In some embodiments, the invention provides a reaction mixture comprising a plurality of polynucleotides which specifically hybridize (*e.g.,* primers) to a plurality of nucleic acid sequences of the genes set forth in Table 14. In some embodiments, the invention provides a reaction mixture comprising a plurality of polynucleotides which specifically hybridize (*e.g.,* primers) to a plurality of nucleic acid sequences of the genes set forth in Table 15. In some embodiments, the reaction mixture is a PCR mixture, for example, a multiplex PCR mixture.

[0143] This invention relies on routine techniques in the field of recombinant genetics. Generally, the nomenclature and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. Basic texts disclosing the general methods of use in this invention include Sambrook et al., Molecular Cloning, A Laboratory Manual (3rd ed. 2001); Kriegler, Gene Transfer and Expression:

A Laboratory Manual (1990); and Current Protocols in Molecular Biology (Ausubel et al., eds., 1994-2008, Wiley Interscience)).

[0144] For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Proteins sizes are estimated from gel electrophoresis, from sequenced proteins, from derived amino acid sequences, or from published protein sequences.

[0145] Oligonucleotides that are not commercially available can be chemically synthesized according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, Tetrahedron Letts. 22:1859-1862 (1981), using an automated synthesizer, as described in Van Devanter et. al., Nucleic Acids Res. 12:6159-6168 (1984). Purification of oligonucleotides is by either native acrylamide gel electrophoresis or by anion-exchange HPLC as described in Pearson & Reanier, J. Chrom. 255:137-149 (1983).

[0146] In some embodiments, the expression level of the biomarkers described herein are detected at the translational or protein level. Detection of proteins is well known in the art, and methods for protein detection known in the art find use. Exemplary assays for determining the expression levels of a plurality of proteins include, *e.g.,* ELISA, flow cytometry, mass spectrometry (*e.g.,* MALDI or SELDI), surface plasmon resonance (*e.g.,* BiaCore), microfluidics and other biosensor technologies. *See, e.g.,* Tothill, Semin Cell Dev Biol (2009) 20(1):55-62.

7. Ischemic Stroke Reference Profiles

[0147] The invention also provides ischemia reference profiles. The reference profiles comprise information correlating the expression levels of a plurality of ischemia-associated genes *(i.e.,* a plurality of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15) to particular types of ischemia. In one embodiment, the ischemia reference profile correlates the expression levels of a plurality of the genes listed in Tables 7A (and 7B) to the occurrence or risk of ischemia. In one embodiment, the ischemia reference profile correlates the expression levels of a plurality of the genes listed in Tables 13A (and 13B) to the occurrence or risk of cardioembolic stroke. In one embodiment, the ischemia reference profile correlates the expression levels of a plurality of the genes listed in Table 14 to the occurrence or risk of carotid stenosis. In one embodiment, the ischemia reference profile correlates the expression levels of a plurality of the genes listed in Table 15 to the occurrence or risk of atrial fibrillation. The profiles can conveniently be used to diagnose, monitor and prognose ischemia.

[0148] One embodiment of the invention provides an ischemia reference profile for subjects who have experienced or are at risk for experiencing stroke, regardless of cause. Accordingly, the ischemia reference profile correlates the expression levels of a plurality of the genes selected from Table 7A (and Table 7B). For example, an expression profile exhibiting at least about a 1.2-fold increase in expression of a plurality of the following genes: PGM5, CCDC144C /// LOC100134159, LECT2, SHOX, TBX5, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, RNF141, CLEC4E, BXDC5, UNC5B, TIMP2, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, FCRL4, ELAVL2, PRTG, DLX6, SCD5, GABRB2, GYPA, PHTF1, CKLF, CKLF, RRAGD, CLEC4E, CKLF, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, LOC283027, LOC344595, RPL22, LOC100129488 and MCTP1 when compared to the control level, and at least about a 1.2-fold decrease in expression of a plurality of the following genes: SPTLC3, DKRZP434L187, SPIB, HNRNPUL2, FOXA2, RPL22 and SH3GL3 when compared to the control level is a reference profile for a subject who has experienced or is at risk for stroke.

[0149] One embodiment of the invention provides an ischemia reference profile for subjects who have experienced or are at risk for experiencing cardioembolic stroke. Accordingly, the ischemia reference profile correlates the expression levels of a plurality of the genes selected from Table 13A (and Table 13B). For example, an expression profile exhibiting at least about a 1.2-fold increase in expression of a plurality of the following genes: IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A /// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP and LRRC37A3 when compared to the control level, and at least about a 1.2-fold decrease in expression of a plurality of the following genes: LOC284751, CD46, ENPP2, C19orf28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, C16orf68, TFDP1 and GSTK1 when compared to the control level is a reference profile for a subject who has experienced or is at risk for a cardioembolic stroke.

[0150] One embodiment of the invention provides an ischemia reference profile for subjects who have experienced or are at risk for experiencing carotid stenosis and atherosclerotic stroke. Accordingly, the ischemia reference profile correlates the expression levels of a plurality of the genes selected from Table 14. For example, an expression profile exhibiting at least about a 1.2-fold increase in expression of a plurality of the following genes: NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12orf42 and LOC100127980 when compared to the control level, and at least about a 1.2-fold decrease in expression of a plurality of the following genes: FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880,

SLC20A1, SLC6A19, ARHGEF12, C16orf68, GIPC2 when compared to the control level is a reference profile for a subject who has experienced or is at risk for carotid stenosis and atherothrombotic stroke.

[0151] One embodiment of the invention provides an ischemia reference profile for subjects who have experienced or are at risk for experiencing atrial fibrillation. Accordingly, the ischemia reference profile correlates the expression levels of a plurality of the genes selected from Table 15. For example, an expression profile exhibiting at least about a 1.2-fold increase in expression of a plurality of the following genes: SMC1A, SNORA68, GRLF1, SDC4, HIPK2, LOC100129034, CMTM1 and TTC7A when compared to the control level, and at least about a 1.2-fold decrease in expression of a plurality of the following genes: LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6orf164 and MAP3K7IP1 when compared to the control level is a reference profile for a subject who has experienced or is at risk for atrial fibrillation.

[0152] The reference profiles can be entered into a database, e.g., a relational database comprising data fitted into predefined categories. Each table, or relation, contains one or more data categories in columns. Each row contains a unique instance of data for the categories defined by the columns. For example, a typical database for the invention would include a table that describes a sample with columns for age, gender, reproductive status, expression profile and so forth. Another table would describe a disease: symptoms, level, sample identification, expression profile and so forth. In one embodiment, the invention matches the experimental sample to a database of reference samples. The database is assembled with a plurality of different samples to be used as reference samples. An individual reference sample in one embodiment will be obtained from a patient during a visit to a medical professional. Information about the physiological, disease and/or pharmacological status of the sample will also be obtained through any method available. This may include, but is not limited to, expression profile analysis, clinical analysis, medical history and/or patient interview. For example, the patient could be interviewed to determine age, sex, ethnic origin, symptoms or past diagnosis of disease, and the identity of any therapies the patient is currently undergoing. A plurality of these reference samples will be taken. A single individual may contribute a single reference sample or more than one sample over time. One skilled in the art will recognize that confidence levels in predictions based on comparison to a database increase as the number of reference samples in the database increases.

[0153] The database is organized into groups of reference samples. Each reference sample contains information about physiological, pharmacological and/or disease status. In one aspect the database is a relational database with data organized in three data tables, one where the samples are grouped primarily by physiological status, one where the samples are grouped primarily by disease status and one where the samples are grouped primarily by pharmacological status. Within each table the samples can be further grouped according to the two remaining categories. For example the physiological status table could be further categorized according to disease and pharmacological status.

[0154] As will be appreciated by one of skill in the art, the present invention may be embodied as a method, data processing system or program products. Accordingly, the present invention may take the form of data analysis systems, methods, analysis software, etc. Software written according to the present invention is to be stored in some form of computer readable medium, such as memory, hard-drive, DVD ROM or CD ROM, or transmitted over a network, and executed by a processor. The present invention also provides a computer system for analyzing physiological states, levels of disease states and/or therapeutic efficacy. The computer system comprises a processor, and memory coupled to said processor which encodes one or more programs. The programs encoded in memory cause the processor to perform the steps of the above methods wherein the expression profiles and information about physiological, pharmacological and disease states are received by the computer system as input. Computer systems may be used to execute the software of an embodiment of the invention (see, *e.g.,* U.S. Patent No. 5,733,729).

8. Providing Appropriate Treatment and Prevention Regimes to Patient

[0155] Upon a positive determination or confirmation that a patient has experienced a stroke, and a determination of the cause of stroke, *e.g.,* using the biomarkers provided herein, the methods further provide for the step of prescribing, providing or administering a regime for the prophylaxis or treatment of ischemic stroke. By diagnosing the occurrence and/or the cause of stroke using the biomarkers described herein, a patient can rapidly receive treatment that is tailored to and appropriate for the type of stroke that has been experienced, or that the patient is at risk of experiencing.

[0156] If the expression levels of the plurality of biomarkers evaluated from Table 7A (and 7B) indicate the occurrence or risk of stroke, a positive diagnosis of stroke can be confirmed using methods known in the art. For example, the patient can be subject to MRI imaging of brain and vessels, additional blood tests, EKG, and/or echocardiogram.

[0157] If the expression levels of the plurality of biomarkers evaluated from Table 13A (and 13B) indicate the occurrence or risk of cardioembolic stroke, the patient can be prescribed or administered a regime of an anticoagulant. Exemplary anticoagulants include aspirin, heparin, warfarin, and dabigatran.

[0158] If the expression levels of the plurality of biomarkers evaluated from Table 14 indicate the occurrence or risk of carotid stenosis, the patient can be prescribed or administered a regime of an anti-platelet drug. The most frequently used anti-platelet medication is aspirin. An alternative to aspirin is the anti-platelet drug clopidogrel (Plavix). Some studies

indicate that aspirin is most effective in combination with another anti-platelet drug. In some embodiments, the patient is prescribed a combination of low-dose aspirin and the anti-platelet drug dipyridamole (Aggrenox), to reduce blood clotting. Ticlopidine (Ticlid) is another anti-platelet medication that finds use. Patients having a moderately or severely narrowed neck (carotid) artery, may require or benefit from carotid endarterectomy. This preventive surgery clears carotid arteries of fatty deposits (atherosclerotic plaques) to prevent a first or subsequent strokes. In some embodiments, the patient may require or benefit from carotid angioplasty, or stenting. Carotid angioplasty involves using a balloon-like device to open a clogged artery and placing a small wire tube (stent) into the artery to keep it open.

[0159] If the expression levels of the plurality of biomarkers evaluated from Table 15 indicate the occurrence or risk of atrial fibrillation, the patient can be prescribed a regime of an anti-coagulant (to prevent stroke) and/or a pharmacological agent to achieve rate control. Exemplary anticoagulants include aspirin, heparin, warfarin, and dabigatran. Exemplary rate control drugs include beta blockers (*e.g.,* metoprolol, atenolol, bisoprolol), non-dihydropyridine calcium channel blockers (*e.g.,* diltiazem or verapamil), and cardiac glycosides (*e.g.,* digoxin).

9. Solid Supports and Kits

[0160] The invention further provides a solid supports comprising a plurality of nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes set forth in Tables 7A, 7B, 13A, 13B, 14, 15, and optionally 16. For example, the solid support can be a microarray attached to a plurality of nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes set forth in Table 7A, and optionally Table 16. For example, the solid support can be a microarray attached to a plurality of nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes set forth in Table 13A, and optionally Table 16. For example, the solid support can be a microarray attached to a plurality of nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes set forth in Table 14, and optionally Table 16. For example, the solid support can be a microarray attached to a plurality of nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes set forth in Table 15, and optionally Table 16.

[0161] In various embodiments, the solid supports are configured to exclude genes not associated with or useful to the diagnosis, prediction or confirmation of a stroke or the causes of stroke. For example, genes which are overexpressed or underexpressed less than 1.2-fold in subjects having or suspected of having stroke, regardless of cause, in comparison to a control level of expression can be excluded from the present solid supports. In some embodiments, genes that are overexpressed or underexpressed less than 1.2-fold in subjects with ischemic stroke, including cardioembolic stroke, atherothrombotic stroke, and stroke subsequent to atrial fibrillation, in comparison to a control level of expression can be excluded from the present solid supports. The solid support can comprise a plurality of nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes useful for the diagnosis of ischemic stroke, cardioembolic stroke, carotid stenosis, and/or atrial fibrillation, as described herein. As appropriate, nucleic acid probes that hybridize to a plurality (e.g., two or more, or all) of the genes useful for the diagnosis of ischemic stroke, cardioembolic stroke, carotid stenosis, and/or atrial fibrillation can be arranged in a predetermined array on the solid support. In various embodiments, nucleic acids not specifically identified and/or not relating to the diagnosis of and/or not associated with the diagnosis of ischemic stroke, cardioembolic stroke, carotid stenosis, and/or atrial fibrillation are not attached to the solid support. The solid support may be a component in a kit.

[0162] The invention also provides kits for diagnosing ischemia or a predisposition for developing ischemia. For example, the invention provides kits that include one or more reaction vessels that have aliquots of some or all of the reaction components of the invention in them. Aliquots can be in liquid or dried form. Reaction vessels can include sample processing cartridges or other vessels that allow for the containment, processing and/or amplification of samples in the same vessel. The kits may comprise a plurality of nucleic acid probes that hybridize to a plurality the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15. In one embodiment, the kits comprise a plurality of nucleic acid probes that hybridize to a plurality of the genes set forth in Table 7A (and 7B). In one embodiment, the kits comprise a plurality of nucleic acid probes that hybridize to a plurality of the genes set forth in Table 13A (and 13B). In one embodiment, the kits comprise a plurality of nucleic acid probes that hybridize to a plurality of the genes set forth in Table 14. In one embodiment, the kits comprise a plurality of nucleic acid probes that hybridize to a plurality of the genes set forth in Table15. The probes may be immobilized on an array as described herein.

[0163] In some embodiments, the kits comprise a solid support comprising a plurality of nucleic acid probes that hybridize to a plurality the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15, and optionally Table 16. For example, the solid support can be a microarray attached to a plurality of nucleic acid probes that hybridize to a plurality the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15, and optionally Table 16.

[0164] In addition, the kit can comprise appropriate buffers, salts and other reagents to facilitate amplification and/or detection reactions (*e.g.,* primers, labels) for determining the expression levels of a plurality of the genes set forth in Tables 7A, 7B, 13A, 13B, 14 and 15. In one embodiment, the kit comprises appropriate buffers, salts and other reagents to facilitate amplification and/or detection reactions (*e.g.,* primers, labels) for determining the expression levels of a plurality of the genes set forth in Table 7A (and 7B). In one embodiment, the kit comprises appropriate buffers, salts and

other reagents to facilitate amplification and/or detection reactions (*e.g.,* primers) for determining the expression levels of a plurality of the genes set forth in Table 13A (and 13B). In one embodiment, the kit comprises appropriate buffers, salts and other reagents to facilitate amplification and/or detection reactions (*e.g.,* primers) for determining the expression levels of a plurality of the genes set forth in Table 14. In one embodiment, the kit comprises appropriate buffers, salts and other reagents to facilitate amplification and/or detection reactions (*e.g.,* primers) for determining the expression levels of a plurality of the genes set forth in Table 15. The kits can also include written instructions for the use of the kit.

[0165] In one embodiment, the kits comprise a plurality of antibodies that bind to a plurality of the biomarkers set forth in Tables 7A, 7B, 13A, 13B, 14 and 15. The antibodies may or may not be immobilized on a solid support, *e.g.,* an ELISA plate.

EXAMPLES

[0166] The following examples are offered to illustrate, but not to limit the claimed invention.

Example 1: Biomarkers for the Diagnosis of the Occurrence and/or Risk of Ischemic Stroke

Materials and Methods

[0167] The study had two objectives: (1) Demonstrate that the previously identified 29 probes distinguish IS from healthy controls [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] in a new cohort; and (2) Identify additional genes that discriminate IS from vascular risk factor (SAVVY) controls and myocardial infarction (MI) controls. Whole blood was drawn from IS patients (n=70, 199 samples) at ≤ 3, 5 and 24 hours (3h IS, 5h IS, 24 IS) as part of the CLEAR trial [Pancioli AM et al., Stroke, 39:3268-3276 (2008)] (NCT00250991 at Clinical-Trials.gov). IS subjects were treated with r-tPA with or without eptifibatide after the 3h blood sample was obtained. Controls included healthy subjects (n=38), subjects with acute myocardial infarction (MI, n=17) and subjects with at least one cardiovascular risk factor (hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking) recruited from the SAVVY (Sex, Age and Variation in Vascular functionalitY) study (n=52). The institutional review board at each site approved the study, and each patient provided informed consent. Blood samples were collected in PAXgene tubes (PreAnlytix, Germany). Isolated RNA was processed using Ovation Whole Blood reagents (Nugen Technologies, San Carlos, CA) and hybridized onto Affymetrix Genome U133 Plus 2 GeneChips (Affymetrix Santa Clara, CA). Data was normalized using Robust Multichip Averaging (RMA) [Bolstad BM et al., Bioinformatics, 19:185-193 (2003)] and our internal-gene normalization approach. [Stamova BS et al., BMC Med Genomics, 2:49 (2009)]

Objective 1: The predictive ability of the 29 previously identified genes was determined using k-nearest neighbor in PAM (Prediction Analysis of Microarrays) [Tibshirani R et al., Proc Natl Acad Sci USA, 99:6567-6572 (2002)]. IS and healthy subjects were randomly split in half, stratified by Group and Time-Point (for the IS samples) into a Training Set to develop the prediction algorithm and an independent Test (Validation) Set for evaluating the accuracy of the prediction algorithm.

Objective 2: To identify genes able to discriminate between IS and all controls groups, an ANCOVA adjusted for age, gender and microarray batch effect was used. The numbers of predictive genes were minimized using the nearest-shrunken centroids algorithm (PAM). The ability of the identified genes to predict IS from controls was assessed using (1) 10-fold cross-validation (CV), and (2) assessed in a second (independent) Test (Validation) Set using several prediction algorithms (k-nearest neighbor (K-NN), support vector machine (SVM), linear discriminant analysis (LDA), and quadratic discriminant analysis (QDA)). Only the 3h IS (not treated) and 24h IS samples were analyzed for objective 2 since they were considered most clinically relevant. See supplementary materials and methods for details of the prediction and cross-validation analyses for Objectives 1 and 2.

Stady Participants

Ischemic Stroke (IS) Patients

[0168] Participants with acute IS (n=68) were recruited from the CLEAR trial, a multicenter, randomized double blind safety study of recombinant tissue-plasminogen activator (r-tPA) and eptifibatide as previously described [Pancioli AM et al., Stroke, 39:3268-3276 (2008)] (NCT00250991 at Clinical-Trials.gov). Blood samples were collected at ≤ 3 hours (3h IS), 5 hours (5hr IS) and 24 hours (24 IS) following ischemic stroke onset. r-tPA, with or without eptifibatide, was administered following the 3h blood draw. IS was diagnosed by a stroke neurologist with access to all clinical and diagnostic tests including neurovascular imaging data.

Control Groups

Vascular Risk Factor Subjects (SAVVY)

[0169] Subjects with at least one cardiovascular risk factor (hypertension, diabetes mellitus, hyperlipidemia, or tobacco smoking) were recruited from the SAVVY (Sex, Age and Variation in Vascular functionalitY) study (n=52). These subjects are referred to as vascular risk factor SAVVY Controls in the current study. Exclusion criteria were past history of cardiovascular disease (including stroke, coronary artery disease, peripheral artery disease or deep vein thrombosis), BMI > 46kg/m^2, history of cancer, chronic infection, autoimmune disease or blood dyscrasias.

Patients with Myocardial Infarction (MI)

[0170] Subjects with MI (n=16) were recruited from the University of California Davis Medical Center. The average time since the event was 58.0h (range 19.3-176.5). All were treated acutely with anti-platelet drugs and an anticoagulant prior to the blood draw. Angioplasty (n=8) or CABG (n=1) were performed in some of the patients prior to the blood draw. No MI patient received r-tPA.

Healthy Controls

[0171] Healthy controls were recruited from the University of Cincinnati (n=15), UC Davis (n=3) and Stanford (n=20). These subjects had never been hospitalized, were on no medications, and had no known major medical, surgical or psychiatric diseases.

[0172] Baseline demographic data were compared between the previous [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] and current study as well as between current IS and control subjects using Student's 2-tail t-test for continuous variables (age) and a χ^2 or Fisher Exact tests for categorical variables (gender, race).

Probe-level Data Analysis

[0173] Raw expression values of each probe from the Affymetrix U133 Plus 2.0 expression arrays were collapsed into probe set level data using Robust Multichip Averaging (RMA) normalization [Bolstad BM et al., Bioinformatics, 19:185-193 (2003)], as well as by modified internal-gene normalization (manuscript in preparation) to a subset of stably expressed internal genes [Stamova BS et al., BMC Med Genomics, 2:49 (2009)]. This involved Median Polishing summarization step, division of each individual gene expression value by the geometric mean of the reference genes, and $\log_2$-transformation. For the analysis in Objective 1, both RMA and Internal control gene normalized values were used. For all the analysis of Objective 2, the derivation of the discriminatory genes was performed using the internal control gene normalized values. The same values were used in developing the Classifiers.

Batch Correction

[0174] Due to the unbalanced nature of the batches, bias is introduced when batch is used as a factor in an ANCOVA model. However, it is still desirable to account for the existing technical variation. This was accomplished by selecting genes that were common to the ANCOVA output sets with and without batch as a factor. While this technique introduced strict criteria for the selection of discriminating genes, it was intended to improve the chance of validation of the results upon subsequent studies and to achieve greater generalization, which can be translated into IS predictive clinical test.

Identification of Discriminatory Genes

[0175] Analysis of each comparison (IS per time-point (3h and 24h) vs Healthy, MI and SAVVY, respectively) was performed individually. The samples were randomly split, stratified by Group, in order to perform a split-sample analysis, where the Prediction Algorithms are trained on half of the samples (Training Set), and the performance of the Classifiers is tested on the second half of the samples (Test Set). The Analysis Workflow Chart is shown in Figure 3. The feature selection for the derivation of the discriminatory genes between Healthy and IS at 3h and IS at 24h, respectively, involved finding common probe sets from four different ANCOVA analysis, referred to here as Models 1-4. All factors used in the analysis were common to all models (Group, Age, Gender) with the exception of Batch, which was only factored in Model 1 and 3. Models 1 and 2 were applied to a randomly selected one-half of the samples stratified by Group and time-point (for the IS samples) named here 1st random half, whereas Models 3 and 4 were applied to the complete data sets. Overlap of models with and without batch was performed due to the unbalanced nature of batches in an attempt to select more reliable probe sets. Overlap of complete-set and split-set models was performed to achieve greater generalization

compared to the split set model which can be translated into IS predictive clinical test.

[0176] Gene lists satisfying the following criteria were developed: FDR-corrected p-value (Group) <0.05 and fold-change ≤-1.5 or ≥1.5, as well as being not-significant for the rest of the factors (uncorrected p (Age)>0.5 and uncorrected p (Gender)>0.05 and, for the models including Batch, uncorrected p (Batch) >0.05). The goal is to find genes whose expressions are not affected by significant technical (batch), gender, or age effects.

[0177] Exception to Flow Chart Analysis for IS at 24h vs Healthy was at Model 1, where the uncorrected p (Group) <0.01 was used to generate a larger gene list. Analysis of SAVVY vs IS at 3h and IS at 24h, respectively, included only Models 2 and 4, since Batch could not be factored in, due to the complete confounding of the batches. Analysis of MI vs IS at 3h and IS at 24h, respectively, included only Models 3 and 4, since the sample size of the MI patients was very small (n=17). In this case a 10-fold cross-validation procedure was used to determine the performance of the Classification Algorithms. If the number of the probe sets at the feature selection step was large, we proceeded with excluding probe sets not annotated, annotated as chromosomal segments, annotated as hypothetical proteins, probe sets which per Affimetrix annotation may potentially detect more than one unique gene (* _x_at, *_a_at, *_s_at), and exclusion of duplicates.

Predictions/Classification

[0178] Different prediction algorithms were used. Prediction Analysis of Microarrays (PAM) uses the K-nearest neighbor as a classification engine (default k=10) as well as nearest shrunken centroid as a feature-selection method [Tibshirani R et al., Proc Natl Acad Sci USA, 99:6567-6572 (2002)]. The differentially expressed genes that passed the criteria outlined above were input into PAM and the minimum numbers of genes with the optimal classification accuracy were selected. In addition, multiple other classification methods were evaluated in the analysis of the combined 3h IS predictors, 24h IS predictors and 3h plus 24h IS predictors in order to find an optimal model and to produce an unbiased estimate of prediction accuracy (analysis performed in Partek Genomics Suite, Partek Inc., St. Louis, MI, USA). A combination of the ANCOVA models and nearest-shrunken centroids for our feature reduction step was used. In addition to PAM, the classification models used in this study were K-Nearest Neighbor (K-NN) with k = 1, 3, 5, 7, and 9 number of neighbors with Euclidian Distance similarity measure; Nearest-Centroid (NC) with equal and proportional prior probabilities; Quadratic Discriminant Analysis (QDA) with equal and proportional prior probabilities, Linear Discriminant Analysis (LDA) with equal and proportional prior probabilities, and Support Vector Machine, constituting a 121-model space. For overview of these methods, see [Asyali MH et al., Current Bioinformatics, 1:55-73 (2006); Jain AK et al., Statistical pattern recognition: A review, IEEE Transactions On Pattern Analysis and Machine Intelligence., 22:4-37 (2000)]. 2-level nested cross-validation (CV) was performed to generate a less biased estimate of classification success (reported as accuracy (normalized) estimate). In this approach, an "outer" cross-validation is performed in order to produce an unbiased estimate of prediction error (by holding out samples as an independent test set). To select the optimal model to be applied to the held out test sample, additional "inner" cross-validation is performed on the training data (which is the data not held out as test data by the "outer" cross-validation). Full leave-one-out cross validation (CV) was used in cases where the complete set was used to train and CV the prediction accuracy.

[0179] For Table 4 in the Results section, the following parameters were used: *Accuracy (normalized) estimate of 121-Model Space=91.2% (80.3/88). Best Model: SVM (shrink=yes, cost=101, nu=0.5, tol=0.001, kern rbf deg = 3, radial basis function (gamma) = 0.01, coef=0.0). Kappa =0.83. †Accuracy (normalized) estimate of 121-Model Space=87.9% (76.4/87). Best Model: SVM (shrink=yes, cost=101, nu=0.5, tol=0.001, kern rbf deg = 3, radial basis function (gamma) = 0.0001, coef=0.0). Kappa=0.83. ‡accuracy (normalized) estimate of 121-Model Space=91.2% (110/121). Best Model: SVM (shrink=yes, cost=701, nu=0.5, tol=0.001, kern rbf deg = 3, radial basis function (gamma) = 0.00001, coef=0.0). ‖ Correct classification at 3h=76%, at 24h=97%. #Correct classification at 3h=94%, at 24h=97%.

Gene Enrichment Analysis of Discriminatory Genes to Identify Biological Themes in the Combined 3h and 24h IS Predictors

[0180] Ingenuity Pathway Analysis (IPA 8.0, Ingenuity® Systems) was used for identifying over-represented biological functions in the combined 97 probe set list of 3h and 24h predictors. A Fisher's exact test (p<0.1) was used to determine whether there was over representation of the 97 probe sets/genes in any given biological function. Gene ontology of the stroke predictors was extracted from Affymetrix NetAffix website (on the internet at affymetrix.com/user/login.jsp?toURL=-/analysis/netaffx/index.affx).

Results

Subject Demographic

[0181] Demographic information is presented in Table 1 (Objective 1) and Table 2 (Objective 2). Age was significantly different between IS and control groups ($p<0.05$) (Tables 1 and 2). Gender was significantly different ($p<0.05$) between IS and healthy subjects in the Tang et al, 2006 study [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] and the current study (Table 1), as well as between IS and Vascular Risk Factor (SAVVY) Control subjects from the current study (Table 2). Race was significantly different between IS compared to Healthy and MI controls (Table 2). Hypertension and diabetes were not significantly different between the groups.

Table 1. Demographic Summary of Subjects from our previous Tang et al. 2006 Study [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] and our Current Study of Ischemic Stroke (IS) and Healthy Controls. N= number of subjects.

	IS Tang *et. al,* 2006	Healthy Tang *et. al,* 2006	IS Current Study	Healthy Controls Current Study
N	15	15	70†	38
Mean Age, years (SD)	64± 14	49 ±11	66.8±12.7	45.0±19.8
Gender, %				
Male	73.3	87.5*	57.1	47.4*
Female	26.7	12.5*	42.9	52.6*
Race, %				
Caucasian	80.0	75.0	80.0	55.3
African American	20.0	0.0	20.0	15.8
Other	0.0	25.0	0.0	28.9
NIH Stroke Scale				
1st Blood Draw (3h)	15±7	N/A	14 ± 7	N/A
2nd Blood Draw (5h)	12±8		11 ± 8	
3rd Blood Draw (24h)	9±7		10 ± 8	

†N = 67 at 3h, 66 at 5h, 66 at 24h. 61 subjects had all three time points.

*Gender distribution significantly different ($p<0.05$) between healthy subjects in the current study compared to the Tang et al. 20061 study and marginally different between IS subjects in the current study compared to the Tang et al. 2006 [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] study.

Table 2. Demographic Summary of Current Study Participants.

	IS	Healthy Controls	MI Controls	Vascular SAVVY Controls
N	70†	38	17	52
Mean Age, years (SD)	66.8±12.7	45.0±19.8	59.6±12.2	56.2±5.4
Gender, %				
Male	57.1	47.4	70.6	32.7
Female	42.9	52.6	29.4	67.3*
Race, %				
Caucasian	80.0	55.3	47.1	86.5
African American	20.0	15.8	17.6	11.5
Other	0.0	28.9**	35.3**	2.0
NIH Stroke Scale				
1st Blood Draw (3h)	14 ± 7			
2nd Blood Draw (5h)	11 ± 8	N/A	N/A	N/A

(continued)

	IS	Healthy Controls	MI Controls	Vascular SAVVY Controls
3rd Blood Draw (24h)	10 ± 8			

†N = 67 at 3h, 66 at 5h, 66 at 24h. 61 subjects had all three time points;
*Gender distribution significantly different ($p<0.05$) between Ischemic Stroke (IS) and Vascular Risk Factor (SAVVY) controls. MI=myocardial infarction. N= number of subjects.
**Race significantly different ($p<0.05$) between IS compared to healthy and MI.

1) Replication of Tang et al, 2006 [Tang Y et al., *J Cereb Blood Flow Metab*., 26:1089-1102 (2006)] IS predictors in a larger cohort

[0182] Due to the different array processing protocols in the study by Tang et al, 2006 [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] and the current studies, the following analyses were performed: (1) the prediction algorithm was retrained on the first random half of the new samples (Training Set) and the performance of the 29 probe sets evaluated in the second half (Test/Validation Set); and (2) the samples used in the Tang et al, 2006 study [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)] and the current study were internal gene normalized. Overall, 92.9% sensitivity for IS and 94.7% specificity for healthy controls with high Test Set probabilities were achieved (Figure 1, Table 3). The results are similar to the ability of these predictors to classify the previously published patients [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)], with 88.9% sensitivity for IS and 100% specificity for healthy controls (Table 3). In addition, for comparison purposes to the previous study [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)], RMA normalization and Cross-Validation (used in the previous study [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)]) on our complete set of IS and healthy samples was performed. Similar results were obtained (Table 5 and Figure 4).

Table 3. Validation of the of the 29 probe sets from the Tang et al. 2006 study [Tang Y et al., *J Cereb Blood Flow Metab.,* 26:1089-1102 (2006)]. These probe sets were trained on the first half (Training Set) of the subjects in this study (n=35 IS, n=19 Healthy), and then used predict the Test Set probabilities on a second half of the ischemic stroke (IS) subjects (Test Set) (n =35, 99 samples) and Healthy subjects (n=19, 19 samples) in the Current Study. In addition, the same probe sets were used to predict the Test set probabilities on the original subjects in the Tang et al. 2006 study.

Class Prediction	Study	3h	5h	24h	All Time Points
IS, Sensitivity, %	Tang *et al,* 2006	73.3	93.3	100	88.9
	Current Study	84.8	97.0	97.0	92.9
Healthy, Specificity, %	Tang *et al,* 2006	N/A	N/A	N/A	100
	Current Study	N/A	N/A	N/A	94.7

Sensitivity = % correct classification of IS samples
Specificity = % correct classification of healthy samples

Table 4. Classification Accuracy (%) of 3h and 24h Ischemic Stroke (IS) Predictors. Half of the subjects (training set) were used to derive the IS Predictors. For the Test Set prediction accuracy estimate on the second half of the subjects, there were 3h IS (n=33), 24h IS (n=33), healthy (n=19), Vascular Risk Factor (SAVVY) (n=26) and MI (n=8). The 60-probe set 3h IS predictors represented the sum of the 3h IS comparison to the three control groups: Healthy(17), SAVVY(22) and MI(31), of which 10 were common to the 3h IS vs MI and 3h IS vs SAVVY predictors, yielding 60 probe sets. The 46-probe set 24h IS predictors represented the sum of the 24h IS comparison to the three control groups: Healthy(20), SAVVY(9) and MI(17). The 3h and 24h IS Combined predictors represent the sum of the 3h IS predictors (60) and 24h IS predictors(46) of which 9 were common, yielding 97 probe sets.

	60 probe sets 3h IS vs Controls (Healthy, MI, SAVVY)		46 probe sets 24h IS vs Controls (Healthy, MI, SAVVY)		97 probe sets 3h and 24h IS Combined vs Controls (Healthy, MI, SAVVY)	
Group	**PAM**	**SVM***	**PAM**	**SVM†**	**PAM**	**SVM‡**
IS	85	94	91	94	86‖	95#

(continued)

	60 probe sets 3h IS vs Controls (Healthy, MI, SAVVY)		46 probe sets 24h IS vs Controls (Healthy, MI, SAVVY)		97 probe sets 3h and 24h IS Combined vs Controls (Healthy, MI, SAVVY)	
SAVVY	92	96	92	96	96	96
MI	88	88	63	50	75	75
Healthy	84	68	89	84	84	68

‖ Correct classification at 3h=76%, at 24h=97%. #Correct classification at 3h=94%, at 24h=97%.

Table 5. Validation of the of the 29 probe sets from the Tang et al, 2006 study [Tang Y et al., *J Cereb Blood Flow Metab.,* 26:1089-1102 (2006)]. Cross-validated Probabilities. Trained and cross- validated on current study samples (IS: n =70, 199 samples) and Healthy (n=38, 38 samples).

Normalization Method	Class Prediction	Study	3h	5h	24h	All Time Points
RMA	IS, Sensitivity, %	Tang *et al,* 2006	66.7	86.7	100	84.4
		Current Study	86.6	98.5	89.4	91.5
	Healthy, Specificity, %	Tang *et al,* 2006	N/A	N/A	N/A	100
		Current Study	N/A	N/A	N/A	84.2
Internal Genes	IS, Sensitivity, %	Tang *et al,* 2006	73.3	93.3	100	88.9
		Current Study	86.6	98.5	95.5	93.5
	Healthy, Specificity, %	Tang *et al,* 2006	N/A	N/A	N/A	100
		Current Study	N/A	N/A	N/A	89.5

Sensitivity = % correct classification of IS samples
Specificity = % correct classification of healthy samples

2) Refinement of Prediction of IS Against Several Different Control Groups

Differentiation of IS Patients from Controls

[0183] Predictive gene expression signatures were derived individually for each comparison. To discriminate the 3h IS group from the healthy (training set), MI (Cross Validation set, due to small sample size for MI), and SAVVY (training set) control groups, the PAM classification algorithm derived 17, 31, and 22 predictor probesets/genes, respectively. Putting these genes into PAM to predict the class of the subjects in the test groups yielded 87.9/94.7%, 98.5/82.4%, and 100/96.2% sensitivity/specificity for 3h IS compared to healthy, MI and SAVVY control samples, respectively (Figures 5, 6 and 7, respectively).

[0184] To discriminate the 24h IS group from the healthy (training set), MI (CV set, due to small sample size for MI), and SAVVY (training set) control groups, the PAM classification algorithm derived 20, 19, and 9 predictor probesets/genes, respectively. Putting these genes into PAM to predict the class of the subjects in the test groups yielded 90.9/94.7%, 93.9/88.2%, and 97/100% sensitivity/specificity for 24h IS compared to healthy, MI and SAVVY control samples, respectively (Figures 8, 9, and 10, respectively).

Prediction Accuracy of 3h IS Predictors on 3h IS, Healthy, MI and SAVVY Subjects

[0185] Combining the lists of the 3h predictors from the individual comparison analyses yielded 60 unique probe sets representing 56 annotated genes. Their prediction probability using PAM on the Test Set is presented in Figure 2A. The percent correctly predicted samples from PAM as well as the best performing prediction model (SVM) are presented in Table 4. Overall (normalized) accuracy was 91.2%. With SVM the sensitivity was 94% and specificities were 96% for SAVVY, 88% for MI, and 68% for healthy. Analysis in PAM produced lower sensitivity for IS but higher specificity for healthy subjects compared to SVM (Table 4). In addition to the split sample analysis, a 10-fold Cross Validation was performed which is a preferred method for developing and evaluating prediction algorithms for small sample sizes. This produced the expected better prediction results (Table 6 and Figure 11A).

Table 6. Classification Accuracy (% correct classification) of 3h and 24h Ischemic Stroke (IS) Predictors. Sample sizes used for Cross-Validation were n=67 at 3h IS, n=66 at 24h IS, n=52 for SAVVY, n=17 for MI. Sample sizes used for split-sample prediction performance estimate on the test set were n=33 at 3h IS, n=33 at 24h IS, n=26 for SAVVY, n=8 for MI. The 60-probe set 3h IS predictors represented the sum of the 3h IS comparison to the three control groups: Healthy (17 probe sets), SAVVY controls (22 probe sets) and MI (31 probe sets). The 46-probe set 24h IS predictors represented the sum of the 24h IS comparison to the three control groups: Healthy (20 probe sets), SAVVY controls (9 probe sets) and MI (17 probe sets). The 3h and 24h IS Combined predictors represent the sum of the 3h IS predictors (60 probe sets) and 24h IS predictors (n=46) of which 9 were common, thus yielding 97 probe sets.

	60 probe sets 3h IS vs Controls (Healthy, MI, SAVVY)		46 probe sets 24h IS vs Controls (Healthy, MI, SAVVY)		97 probe sets 3h and 24h IS Combined vs Controls (Healthy, MI, SAVVY)	
Group	**PAM**	**SVM***	**PAM**	**svm†**	**PAM**	**svm‡**
IS	90	91	88	91	90‖	96
SAVVY	94	98	98	98	94	98
MI	71	88	65	82	71	82
Healthy	82	84	79	84	79	76

*Accuracy (normalized) estimate of 121-Model Space=86.4% (150/174). Best Model: SVM (shrink=yes, cost=201, nu=0.5, tol=0.001, kern rbf deg = 3, radial basis function (gamma) = 0.001, coef=0.0). †Accuracy (normalized) estimate of 121-Model Space=89.2% (154/173). Best Model: SVM (shrink=yes, cost=201, nu=0.5, tol=0.001, kern rbf deg = 3, radial basis function (gamma) = 0.0001, coef=0.0). ‡Accuracy (normalized) estimate of 121-Model Space=88.2% (212/240). Best Model: SVM (shrink=yes, cost=101, nu=0.5, tol=0.001, kern rbf deg = 3, radial basis function (gamma) = 0.01, coef=0.0). ‖ Correct classification at 3h=87%, at 24h=96%

Prediction Accuracy of 24h IS Predictors on 24h IS, Healthy, MI and SAVVY Subjects

[0186] Combining the lists of the 24h predictors from the individual comparison analyses yielded 46 unique probe sets representing 32 annotated genes. Their prediction probability using PAM on the Test Set is presented in Figure 2B. The percent correctly predicted samples from PAM as well as SVM (best performing prediction model) are presented in Table 4. Overall (normalized) accuracy was 89.2%. With SVM the sensitivity was 94% and specificities were 96% for SAVVY, 50% for MI and 84% for healthy. Better results were again obtained using a 10-fold cross validation (Table 6 and Figure 11B).

Prediction Accuracy of combined 3h and 24 IS Predictors on 3h and 24h IS, Healthy, MI and SAVVY Subjects

[0187] Combining the lists of the 3h and 24h predictors from the individual comparison analyses yielded 97 unique probe sets representing 79 annotated genes. Their prediction probability using PAM on the Test Set is presented in Figure 2C. The percent correctly predicted samples from PAM and SVM (best performing prediction model) are presented in Table 4. Overall (normalized) accuracy was 91.2%. With SVM the sensitivity was 95% and specificities were 96% for SAVVY, 75% for MI, and 68% for healthy. Analysis in PAM produced lower sensitivity for IS but higher specificity for healthy subjects compared to SVM (Table 4). Similarly, due to the small sample numbers of MI subjects, 10-fold cross-validation was performed which yielded somewhat better results (Table 6 and Figure 11 C).

IV. Main Biological Function of Biomarkers Described

[0188] Using Ingenuity Pathway analysis software (see Supplementary Materials) the coagulation system was the only statistically over-represented bio-function in the combined 97-probe set list of 3h and 24h IS predictors. The coagulation genes included coagulation factor V (proaccelerin, labile factor) (F5) and thrombomodulin (THBD). GO annotations and the complete list of predictors are presented in Tables 7A-C. Less stringent criteria yielded large numbers of genes with many more regulated pathways.

Table 7A. Combined 3h and 24h IS predictors - Identification of Genes

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
1554560_at	PGM5	phosphoglucomutase 5	BC033073.1	Hs.307835	NM_021965	NP_068800
1561271_at	CCDC144C /// LOC100134159	coiled-coil domain containing 144C /// similar to Coiled-coil domain containing 144B	BC036241.1	Hs.652797	NR_023380 /// XM_001718261	XP_001718313
207409_at	LECT2	leukocyte cell-derived chemotaxin	NM_002302.1	Hs.512580	NM_002302	NP_002293
207570_at	SHOX	short stature homeobox	NM_000451.2	Hs.105932	NM_000451 /// NM_006883	NP_000442 /// NP_006874
240715_at	TBX5	T-box 5	AW269421	Hs.381715	NM_000192 /// NM_080717 /// NM_080718 /// NM_181486	NP_000183 /// NP_542448 /// NP_542449 /// NP_852259
220456_at	SPTLC3	serine palmitoyltransferase, long chain base subunit 3	NM_018327.1	Hs.425023	NM_018327	NP_060797
232547_at	SNIP	SNAP25-interacting protein	BF062187	Hs.448872	NM_025248	NP_079524
238447_at	RBMS3	RNA binding motif, single stranded interacting protein	AA428240	Hs.696468	NM_001003792 /// NM_001003793 /// NM_014483	NP_001003792 /// NP_001003793 /// NP_055298
242912_at	P704P	prostate-specific P704P	A1041215	Hs.654289	NM_001145442	NP_001138914
222835_at	THSD4	thrombospondin, type I, containing	BG163478	Hs.387057	NM_024817	NP_079093
236029_at	FAT3	FAT tumor suppressor homolog 3 (Drosophila)	AI283093	Hs.98523	NM_001008781	NP_001008781
1559545_at	SNRPN	small nuclear ribonucleoprotein polypeptide N	AI371649	Hs.632166	NM_003097 /// NM_022805 /// NM_022806 /// NM_022807 /// NM_022808	NP_003088 /// NP_073716 /// NP_073717 /// NP_073718 /// NP_073719
1562089_at	GLYATL1	glycine-N-acyltransferase-like 1	BC013929.1	Hs.616909	NM_080661	NP_542392
1563533_at	GADL1	glutamate decarboxylase-like 1	AL832766.1	Hs.657052	NM_207359	NP_997242
203917_at	CXADR	coxsackie virus and adenovirus receptor	NM_001338.1	Hs.634837	NM_001338	NP_001329
206048_at	OVOL2	ovo-like 2 (Drosophila)	NM_021220.1	Hs.661013	NM_021220	NP_067043

(continued)

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
219104_at	RNF141	ring finger protein 141	NM_016422.1	Hs.44685	NM_016422	NP_057506
219859_at	CLEC4E	C-type lectin domain family 4, member E	NM_014358.1	Hs.236516	NM_014358	NP_055173
232739_at	SPIB	Spi-B transcription factor (Spi-1/PU.1 related)	AK025419.1	Hs.437905	NM_003121	NP_003112
234243_at	BXDC5	brix domain containing 5	AL359584.1	Hs.481202	NM_025065	NP_079341
226899_at	UNC5B	unc-5 homolog B (C.	AK022859.1	Hs.522997	NM_170744	NP_734465
203167_at	TIMP2	TIMP metallopeptidase inhibitor 2	NM_003255.2	Hs.633514	NM_003255	NP_003246
1554816_at	ASTN2	astrotactin 2	BC010680.1	Hs.601562	NM_014010 /// NM_198186 /// NM_198187 /// NM_198188	NP_054729 /// NP_937829 /// NP_937830 /// NP_937831
1557895_at	FLJ35934	FLJ35934 protein	BC033201.1	Hs.375092	XR_041166	-
1561079_at	ANKRD28	ankyrin repeat domain 28	BC035170.1	Hs.335239	NM_015199	NP_056014
1561477_at	CCDC144A	coiled-coil domain containing 144A	BC034617.1	---	NM_014695	Nip_055510
210800_at	TIMM8A	translocase of inner mitochondrial membrane 8 homolog A (yeast)	BC005236.1	Hs.447877	NM_001145951 /// NM_004085	NP_001139423 /// NP_004076
211617 at	ALDOAP2	aldolase A, fructose-bisphosphate pseudogene 2	M21191.1	Hs.652473	---	---
213371_at	LDB3	LIM domain binding 3	AI803302	Hs.657271	NM_001080114 /// NM_001080115 /// NM_001080116 /// NM_007078	NP_001073583 /// NP_001073584 /// NP_001073585 /// NP_009009
214043_at	PTPRD	protein tyrosine phosphatase, receptor type, D	BF062299	Hs.446083	NM_001040712 /// NM_002839 /// NM_130391 /// NM_130392 /// NM_130393	NP_001035802 /// NP_002830 /// NP_569075 /// NP_569076 /// NP_569077

(continued)

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
214375_at	LOC729222 /// PPFIBP1	similar to PTPRF interacting protein binding protein 1 /// PTPRF interacting protein, binding protein 1 (liprin beta 1)	AI962377	Hs.172445	NM_003622 /// NM_177444 /// XR_015484 /// XR_037707 /// XR_037871	NP_003613 /// NP_803193
220351_at	CCRL1	chemokine (C-C motif) receptor-like 1	NM_016557.1	Hs.310512	NM_016557 /// NM_178445	NP_057641 /// NP_848540
222264_at	HNRNPUL2	heterogeneous nuclear ribonucleoprotein U-like 2	BG167570	Hs.714969	NM_001079559	NP_001073027
224403_at	FCRL4	Fc receptor-like 4	AF343661.1	Hs.120260	NM_031282	NP_112572
228260_at	ELAVL2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	AL161628	Hs.166109	NM_004432	NP_004423
229073_at	PRTG	protogenin homolog (Gallus gallus)	AA912476	Hs.130957	NM_173814	NP_776175
239309_at	DLX6	distal-less homeobox 6	T65128	Hs.249196	NM_005222	NP_005213
40284_at	FOXA2	forkhead box A2	AB028021	Hs.155651	NM_021784 /// NM_153675 /// XM_002345401	NP_068556 /// NP_710141 /// XP_002345442
220232_at	SCD5	stearoyl-CoA desaturase 5	NM_024906.1	Hs.379191	NM_001037582 /// NM_024906	NP_001032671 /// NP_079182
242344_at	GABRB2	gamma-aminobutyric acid A receptor, beta 2	AA772920	Hs.303527	NM_000813 /// NM_021911	NP_000804/// NP_068711
1559520_at	GYPA	Glycophorin A (MNS blood group)	AL833104.1	Hs.434973	NM_002099	NP_002090
215285_s_at	PHTF1	putative homeodomain transcription factor 1	AA927671	Hs.655824	NM_006608	NP_006599
219161_s_at	CKLF	chemokine-like factor	NM_016951.2	Hs.15159	NM_001040138 /// NM_016326 /// NM_016951 /// NM_181640 /// NM_181641	NP_001035228 /// NP_057410 /// NP_058647 /// NP_857591 /// NP_857592

(continued)

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
221058_s_at	CKLF	chemokine-like factor	NM_016326.2	Hs.15159	NM_001040138 /// NM_016326 /// NM_016951 /// NM_181640 /// NM_181641	NP_001035228 /// NP_057410 /// NP_058647 /// NP_857591 /// NP_857592
221524_s_at	RRAGD	Ras-related GTP binding D	AF272036.1	Hs.31712	NM_021244	NP_067067
222934_s_at	CLEC4E	C-type lectin domain family 4, member E	BC000715.1	Hs.236516	NM_014358	NP_055173
223451_s_at	CKLF	chemokine-like factor	AF096895.2	Hs.15159	NM_001040138 /// NM_016326 /// NM_016951 /// NM_181640 /// NM_181641	NP_001035228 /// NP_057410 /// NP_058647 /// NP_857591 /// NP_857592
227948_at	FGD4	FYVE, RhoGEF and PH domain containing 4	AI949549	Hs.117835	NM_139241	NP_640334
235479_at	CPEB2	cytoplasmic polyadenylation element binding protein 2	AI948598	Hs.656937	NM_182485 /// NM_182646	NP_872291 /// NP_872587
236297_at	---	---	AI420817	Hs.585479	---	---
236898_at	LOC100290882	similar to hCG1994130	AW242604	---	XM_002347794	XP_002347835
238903_at	UBXN2B	UBX domain protein 2B	AI636090	Hs.155572	NM_001077619	NP_001071087
207691_x_at	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1	NM_001776.1	Hs.719076	NM_001098175 /// NM_001164178 /// NM_001164179 /// NM 001164181 /// NM_001164182 /// NM_001164183 /// NM_001776	NP_001091645 /// NP_001157650 /// NP_001157651 /// NP_001157653 /// NP_001157654 /// NP_001157655 /// NP_001767
205715_at	BST1	bone marrow stromal cell antigen 1	NM_004334.1	---	NM_004334	NP_004325
236172_at	LTB4R	leukotriene B4 receptor	AW206817	Hs.567248	NM_001143919 /// NM_181657	NP_001137391 /// NP_858043

(continued)

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
231029_at	F5	coagulation factor V (proaccelerin, labile factor)	AI740541	Hs.30054	NM_000130	NP_000121
202146_at	IFRD1	interferon-related developmental regulator 1	AA747426	Hs.7879	NM_001007245 /// NM_001550	NP_001007246 /// NP_001541
206017_at	KIAA0319	KIAA0319	NM_014809.1	Hs.26441	NM_014809	NP_055624
218177_at	CHMP1B	chromatin modifying protein 1B	AA293502	Hs.656244	NM_020412	NP_065145
220122_at	MCTP1	multiple C2 domains, transmembrane 1	NM_024717.1	Hs.655087	NM_001002796 /// NM_024717	NP_001002796 /// NP_078993
220528_at	VNN3	vanin 3	NM_018399.1	Hs.183656	NM_001024460 /// NM_018399 /// NM_078625 /// NR_028290 /// NR_028291	NP_001019631 /// NP_060869 /// NP_523239
226258_at	AMN1	antagonist of mitotic exit network 1 homolog (S. cerevisiae)	BG031897	Hs.591146	NM_001113402 /// NR_004854	NP_001106873
226671_at	LAMP2	lysosomal-associated membrane protein 2	AI150000	Hs.496684	NM_001122606 /// NM_002294 /// NM_013995	NP_001116078 /// NP_002285 /// NP_054701
228220_at	FCHO2	FCH domain only 2	AI627666	Hs.719247	NM_001146032 /// NM_138782	NP_001139504 /// NP_620137
229817_at	ZNF608	zinc finger protein 608	AI452715	Hs.266616	NM_020747	NP_065798
235699_at	REM2	RAS (RAD and GEM)-like GTP binding 2	H19232	Hs.444911	NM_173527	NP_775798
236154_at	QKI	Quaking homolog, KH domain RNA binding (mouse)	R41907	Hs.593520	NM_006775 /// NM_206853 /// NM_206854 /// NM_206855	NP_006766 /// NP_996735 /// NP_996736 /// NP_996737
236613_at	RBM25	RNA binding motif protein 25	BE466195	Hs.531106	NM_021239	NP_067062
239108_at	FAR2	Fatty acyl CoA reductase 2	H16791	Hs.719237	NM_018099	NP_060569
213355_at	ST3GAL6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6	AI989567	Hs.148716	NM_006100	NP_006091

(continued)

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
243201_at	HNRNPH2	Heterogeneous nuclear ribonucleoprotein H2 (H')	BF061744	Hs.632828	NM_001032393 /// NM_019597	NP_001027565 /// NP_062543
214987_at	GAB1	GRB2-associated binding protein 1	AL049449.1	Hs.80720	NM_002039 /// NM_207123	NP_002030 /// NP_997006
208883_at	UBR5	ubiquitin protein ligase E3 component n-recognin 5	BF515424	Hs.591856	NM_015902	NP_056986
228480_at	VAPA	VAMP (vesicle-associated membrane protein)-associated protein A, 33kDa	AW296039	Hs.699980	NM_003574 /// NM_194434	NP_003565 /// NP_919415
1556834_at	---	---	BC042986.1	Hs.562766	---	---
1561754_at	---	---	AF086134.1	Hs.671185	---	---
1561856_at	---	---	BC030088.1	Hs.398148	---	---
1562084_at	---	---	BC042866.1	Hs.571857	---	---
1562527_at	LOC283027	hypothetical protein LOC283027	AF519622.1	Hs.710809	---	---
1569539_at	---	---	BC037935.1	Hs.650514	---	---
1569664_at	---	---	BC035915.1	Hs.622886	---	---
230959_at	---	---	AW072078	Hs.656184	---	---
231597_x_at	---	---	AI371550	---	---	---
231598_x_at	---	---	AI379823	---	---	---
235606_at	LOC344595	hypothetical LOC344595	AA417117	Hs.655735	NR_028301 /// /// XM_001128525 /// XM_002345686 /// XM_943541	XP_001128525 /// XP_002345727 /// XP_948634
238370_x_at	RPL22	Ribosomal protein L22	AI252081	Hs.554762	NM_000983	NP_000974
243489_at	---	---	BF514098	Hs.678608	---	---
244723_at	LOC100129488	hypothetical protein LOC100129488	BF510430	Hs.656497	XM_001724110 /// XM_001724617	XP_001724162 /// XP_001724669

(continued)

Table 7A. Biomarkers Useful to Predict the Occurrence of Stroke						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
240331_at	---	---	AI820961	Hs.658892	---	---
238375_at	RPL22	Ribosomal protein L22	AI820887	Hs.554762	NM_000983	NP_000974
1554730_at	MCTP1	multiple C2 domains, transmembrane 1	BC030005.1	Hs.655087	NM_001002796 /// NM_024717	NP_001002796 /// NP_078993
211565_at	SH3GL3	SH3-domain GRB2-like 3	AF036272.1	Hs.666365	NM_003027 /// NR_026799	NP_003018

Table 7B. Combined 3h and 24h IS predictors - Identification of Additional Genes

Probe Set ID	Gene Symbol	Gene Title	GenBankID	UniGene ID	RefSeq Transcript ID	RefSeq Protein ID
203505_at	ABCA1	ATP-binding cassette, subfamily A (ABC1), member 1	AF285167.1	Hs.719214	NM_005502	NP_005493
1569476_at	DKFZP434L187	hypothetical LOC26082	BC033224.1	Hs.652128	NR_026771	---
226982_at	ELL2	elongation factor, RNA polymerase II, 2	AI745624	Hs.192221	NM_012081	NP_036213
208158_s_at	OSBPL1A	oxysterol binding protein-like 1A	NM_018030.1	Hs.370725	NM_018030 /// NM_080597	NP_060500 /// NP_542164
237252_at	THBD	thrombomodulin	AW119113	Hs.2030	NM_000361	NP_000352

Table 7C. Combined 3h and 24h IS predictors - Fold Change in Expression

Gene Symbol	GenBankID	Fold Change (Stroke_3h vs Healthy)	Fold Change (Stroke_24 h vs Healthy)	Fold Change (Stroke_3h vs MI)	Fold Change (Stroke_24 h vs MI)	Fold Change (Stroke_3h vs Vascular RF)	Fold Change (Stroke 24 h vs Vascular RF)
ABCA1	AF285167.1	2.07119	2.2176	1.09826	1.31812	2.53649	2.82053
PGM5	BC033073.1	-1.02257	1.0524	-2.95407	-1.70705	-2.71273	-2.09609
CCDC144C /// LOC100134159	BC036241.1	-1.10901	1.33794	-3.34373	-1.89213	-3.74796	-2.67466
LECT2	NM_002302.1	-1.02083	1.37036	-2.59881	-1.71369	-4.29623	-2.98873
SHOX	NM_000451.2	-1.14389	1.11225	-2.767	-1.72507	-3.06805	-2.39484
TBX5	AW269421	-1.03714	1.19129	-3.0574	-2.27761	-3.06348	-2.47297
SPTLC3	NM_018327.1	-1.14707	-1.03685	-2.19854	-1.83648	-3.30052	-2.75386
SNIP	BF062187	-1.17632	1.08103	-3.82871	-3.06017	-5.6754	-4.47024
RBMS3	AA428240	-1.12009	1.02386	-2.45005	-1.70022	-3.16789	-2.60285
P704P	AI041215	-1.05604	1.00723	-2.43469	-2.22274	-2.01936	-1.86268
THSD4	BG163478	-1.08685	1.12498	-4.69322	-2.63617	-3.79344	-3.11379
FAT3	AI283093	1.01071	1.24955	-3.79842	-2.32779	-4.30382	-3.5885
SNRPN	AI371649	-1.08724	1.34813	-4.11683	-2.74804	-3.4562	-2.56379
GLYATL1	BC013929.1	-1.02659	1.15432	-2.02763	-1.33612	-3.17767	-2.50921
GADL1	AL832766.1	1.08821	1.07407	-2.11355	-1.52718	-3.08374	-2.61596
DKFZP434L18 7	BC033224.1	-1.51544	-1.14093	-2.31467	-1.58952	-3.11353	-2.53751
CXADR	NM_001338.1	1.0038	1.24866	-2.17672	-1.37969	-2.59518	-1.96103
OVOL2	NM_021220.1	-1.00245	1.2244	-2.6229	-1.90303	-3.73623	-3.11322
RNF141	NM_016422.1	1.41652	1.76732	-1.2503	1.0572	2.50809	2.88426
CLEC4E	NM_014358.1	2.20581	1.74528	1.48355	1.23558	3.20009	2.54377
ELL2	AI745624	1.55833	1.55667	-1.1485	1.01227	2.61216	2.75015
SPIB	AK025419.1	-1.49579	-1.1927	-1.30814	-1.21513	-3.13558	-2.57627
BXDC5	AL359584.1	-1.12459	1.0218	-3.37543	-2.16061	-4.34359	-3.47716

(continued)

Gene Symbol	GenBankID	Fold Change (Stroke_3h vs Healthy)	Fold Change (Stroke_24 h vs Healthy)	Fold Change (Stroke_3h vs MI)	Fold Change (Stroke_24 h vs MI)	Fold Change (Stroke_3h vs Vascular RF)	Fold Change (Stroke 24 h vs Vascular RF)
UNC5B	AK022859.1	-1.06322	1.08493	-1.82774	-1.59757	-2.97215	-2.38636
TIMP2	NM_003255.2	1.28723	1.29348	1.17202	1.27056	2.65311	2.63656
ASTN2	BC010680.1	-1.03518	1.32302	-3.91463	-2.04726	-2.20417	-1.67438
FLJ35934	BC033201.1	-1.06005	1.08555	-1.7712	-1.53152	-2.90353	-2.69988
ANKRD28	BC035170.1	1.02142	1.52112	-3.23102	-1.72253	-3.75956	-2.19095
CCDC144A	BC034617.1	-1.04089	1.40116	-2.58087	-1.9595	-2.94944	-2.15249
TIMM8A	BC005236.1	1.05795	1.1857	-3.61689	-2.51479	-3.9091	-3.38927
ALDOAP2	M21191.1	-1.00693	1.18023	-2.05999	-1.4251	-2.14058	-1.77344
LDB3	AI803302	-1.02467	1.31867	-2.4936	-1.58633	-2.80843	-2.24309
PTPRD	BF062299	1.13646	1.22444	-2.58721	-2.91802	-2.00587	-1.70565
LOC729222 /// PPFIBP1	AI962377	-1.01017	1.07748	-2.45679	-2.23759	-3.62343	-2.8743
CCRL1	NM_016557.1	1.06414	1.45982	-2.599	-1.47982	-2.2814	-1.54199
HNRNPUL2	BG167570	-1.1163	-1.0024	2.22518	2.22789	-1.33616	-1.19626
FCRL4	AF343661.1	1.07525	1.21448	-2.78886	-1.89115	-2.49879	-2.24799
ELAVL2	AL161628	-1.01813	1.21927	-2.20416	-1.49881	-1.86331	-1.56998
PRTG	AA912476	-1.04494	1.0727	-2.3894	-1.62453	-2.99348	-2.50635
DLX6	T65128	1.00215	1.1532	-2.11674	-1.46511	-1.95595	-1.70623
FOXA2	AB028021	-1.0925	-1.03101	-2.1882	-1.81159	-1.99843	-1.7749
SCD5	NM 024906.1	-1.03966	1.02948	-2.87609	-1.91564	-2.53071	-2.01041
GABRB2	AA772920	1.04696	1.32248	-2.28193	-1.33566	-1.78517	-1.39109
GYPA	AL833104.1	1.04745	1.30732	-2.41685	-1.59859	-1.67937	-1.39989
OSBPL1A	NM_018030.1	1.69237	2.0161	1.00357	1.22861	1.80349	2.07533
PHTF1	AA927671	1.72132	1.82978	-1.11534	1.20764	1.90827	2.12932

(continued)

Gene Symbol	GenBankID	Fold Change (Stroke_3h vs Healthy)	Fold Change (Stroke_24 h vs Healthy)	Fold Change (Stroke_3h vs MI)	Fold Change (Stroke_24 h vs MI)	Fold Change (Stroke_3h vs Vascular RF)	Fold Change (Stroke 24 h vs Vascular RF)
CKLF	NM_016951.2	1.49288	1.67299	-1.083	1.05107	1.77407	1.85927
CKLF	NM_016326.2	1.74019	1.92981	-1.09886	1.052	1.87206	1.89616
RRAGD	AF272036.1	1.64361	1.82266	-1.00147	1.30328	1.59064	1.74087
CLEC4E	BC000715.1	1.72855	1.61958	1.25076	1.17072	1.52696	1.35715
CKLF	AF096895.2	1.59532	1.72012	-1.10928	1.00131	1.64747	1.65053
FGD4	AI949549	2.18122	1.77306	1.22089	1.20979	2.35983	1.917
CPEB2	AI948598	1.60268	1.66275	1.15005	1.40965	1.7109	1.90031
---	AI420817	1.55624	1.60124	-1.28503	1.01626	1.77952	2.0373
LOC100290882	AW242604	1.92911	2.33974	-1.89614	-1.13965	2.98199	3.56684
UBXN2B	AI636090	1.74169	1.9441	-1.11509	1.21234	1.70517	1.89673
ENTPD1	NM_001776.1	1.71167	1.76733	-1.21194	-1.07299	1.73969	1.87582
BST1	NM_004334.1	1.53532	1.62794	-1.06879	1.06228	1.26326	1.34039
LTB4R	AW206817	1.80645	1.74035	1.15737	1.21072	1.71929	1.59047
F5	AI740541	2.14346	2.2038	-1.11679	1.43859	2.20136	2.347
IFRD1	AA747426	1.47432	1.73008	-1.34744	1.06181	1.31407	1.4635
KIAA0319	NM_014809.1	1.63362	1.97771	-1.05306	1.43649	1.46648	1.73293
CHMP1B	AA293502	1.40135	1.80505	-1.3705	-1.09633	1.18349	1.53651
MCTP1	NM_024717.1	1.58307	1.95238	-1.1366	1.10386	1.53504	1.96499
VNN3	NM_018399.1	1.99343	1.93901	1.07486	1.19707	1.96766	1.8831
AMN1	BG031897	1.7461	2.0847	-1.39916	1.07802	1.95345	2.38444
LAMP2	AI150000	1.55435	1.79845	-1.13336	1.11498	1.66826	1.84881
FCHO2	AI627666	1.63852	2.28796	-2.06562	-1.09689	1.6281	2.30861
ZNF608	AI452715	1.83637	4.23691	-1.64571	1.60438	1.757	4.09293
REM2	H19232	1.51731	1.67729	-1.41824	-1.10004	1.24838	1.35338

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Gene Symbol	GenBankID	Fold Change (Stroke_3h vs Healthy)	Fold Change (Stroke_24 h vs Healthy)	Fold Change (Stroke_3h vs MI)	Fold Change (Stroke_24 h vs MI)	Fold Change (Stroke_3h vs Vascular RF)	Fold Change (Stroke 24 h vs Vascular RF)
QKI	R41907	1.71321	2.0559	-1.66896	-1.18819	1.49767	1.98251
RBM25	BE466195	1.61161	1.82166	-1.39912	-1.08457	1.43618	1.81597
FAR2	H16791	1.31592	1.85091	-1.43408	1.12536	1.15805	1.86642
ST3GAL6	AI989567	1.38604	2.22775	-1.83273	-1.10403	1.27982	2.05433
HNRNPH2	BF061744	1.5212	1.69734	-1.34191	-1.10041	1.42575	1.58788
GAB1	AL049449.1	1.20963	1.46575	-1.09271	1.28122	1.60125	1.95743
UBR5	BF515424	1.48189	1.85612	-1.61547	-1.08548	1.34557	1.71885
VAPA	AW296039	1.63693	1.7318	-1.19576	1.07236	1.6695	1.83796
THBD	AW119113	1.59427	2.17248	-1.25924	1.19969	2.00552	2.50204
---	BC042986.1	1.01757	1.24549	-3.49838	-2.38168	-3.50237	-2.86054
---	AF086134.1	-1.11298	1.08518	-4.34061	-3.27948	-7.60167	-5.23956
---	BC030088.1	-1.05299	1.36405	-5.35944	-2.82975	-5.70757	-4.37802
---	BC042866.1	1.02169	1.23725	-6.2228	-3.34786	-5.66994	-4.21957
LOC283027	AF519622.1	-1.02208	1.17339	-4.87073	-3.15597	-3.09193	-2.23434
---	BC037935.1	-1.18515	-1.10026	-4.36438	-3.29567	-6.29072	-4.67834
---	BC035915.1	-1.16002	-1.00454	-3.4789	-2.32404	-5.06879	-3.9396
---	AW072078	-1.13183	-1.03534	-2.5817	-2.45432	-2.97149	-2.42805
---	AI371550	-1.2797	-1.00935	-5.39252	-3.15227	-6.53552	-4.91151
---	AI379823	-1.43222	-1.11788	-7.45042	-4.9032	-8.79656	-6.41832
LOC344595	AA417117	-1.12772	-1.04434	-3.36106	-2.70588	-4.60892	-3.38362
RPL22	AI252081	-1.29183	1.00176	-10.6787	-5.52024	-13.1426	-9.07862
---	BF514098	-1.3354	-1.14637	-7.15023	-4.63994	-7.02866	-5.73683
LOC100129488	BF510430	-1.29144	1.03103	-5.98942	-3.38109	-10.5201	-8.12763
---	AI820961	-1.03836	1.07016	-4.96218	-3.43895	-4.54929	-3.90039

(continued)

Gene Symbol	GenBankID	Fold Change (Stroke_3h vs Healthy)	Fold Change (Stroke_24 h vs Healthy)	Fold Change (Stroke_3h vs MI)	Fold Change (Stroke_24 h vs MI)	Fold Change (Stroke_3h vs Vascular RF)	Fold Change (Stroke 24 h vs Vascular RF)
RPL22	AI820887	-1.41083	-1.1041	-10.3524	-5.84457	-7.0436	-5.09597
MCTP1	BC030005.1	1.65623	2.02722	-1.04349	1.29446	2.71543	3.28561
SH3GL3	AF036272.1	1.01474	-1.0085	-5.88871	-3.48898	-6.30725	-4.45099

Discussion

[0189] Diagnosis of ischemic stroke is based on clinical impression combined with brain imaging. However, in the acute setting, brain imaging is not always readily accessible, and clinical evaluation by persons experienced in stroke is not always readily available. In such patients, a blood test could be of use to diagnose ischemic stroke (IS). Several protein biomarkers have been associated with IS, but in the acute setting these have not yet shown sufficient sensitivity nor specificity to be clinically useful [Whiteley W et al., Stroke, 39:2902-2909 (2008); Foerch C et al., Neurology, 73:393-399 (2009); Jensen MB et al., Expert Rev Cardiovasc Ther., 7:389-393 (2009)]. In this study we show that gene expression profiles can be used as biomarkers of IS, replicated our previous findings, and refined the gene expression signature of IS by including more relevant control groups.

[0190] A 29-probe set profile was previously reported that distinguished IS from healthy controls [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006)]. When this profile was used to predict a larger cohort of patients in this study, it distinguished IS from healthy subjects with a sensitivity of 92.9% and specificity of 94.7%. This is important in that it represents a validation of the concept that gene expression profiles can identify patients with stroke. Replication of gene expression profiles has been a challenge in the field, in large part due to false discovery associated with performing multiple comparisons. Robust biological responses and careful analyses made it possible to validate this 29-probe set profile in this study.

[0191] To obtain more biologically useful predictors of IS, gene profiles that distinguish IS from patients with vascular risk factors (RF) and myocardial infarction (MI) were identified. Using the individual group comparisons, the diagnosis of IS compared to the vascular risk factor group with over 95% sensitivity and specificity was predicted. Using the individual group comparisons, patients with IS from MI with over 90% sensitivity and over 80% specificity were differentiated. Biologically, this suggests at least some differences in the immune responses to infarction in brain and heart.

[0192] The 3 hour time point was a focus of most comparisons because this represents the critical time when decisions are made regarding acute therapy such as thrombolysis. Thus, for the development of a point-of care test, this time period is when gene expression profiles could be of greatest use. With the 60-probe set signature, at the 3 hour time point, correct classification rates of 85-94%, 92-96%, 88% and 68-84% for IS, vascular risk factor, MI and healthy controls, respectively, was achieved. These are approaching clinical useful ranges.

[0193] Though RNA profiles were the focus in this study, the identified genes could be used as a guide in the evaluation of protein biomarkers for ischemic stroke. Genes for Factor 5 and throbomodulin were both identified as differentially expressed in IS compared to controls. Both of these molecules have also been identified as proteins associated with IS [Tang Y et al., J Cereb Blood Flow Metab., 26:1089-1102 (2006); Moore DF et al., Circulation, 111:212-221 2005; Kozuka K et al., Atherosclerosis, 161:161-168 (2002)].

[0194] The goal of this study was not to identify all differentially expressed genes between IS and controls, but rather identify sets of genes whose patterns of expression may be useful for stroke prediction. As a result, these analyses have excluded large numbers of differentially expressed genes that are biologically relevant in IS. These will be the subject of future studies. Limitations of this study include (1) lack of stroke "mimics" in the control groups (2) lack of validation by qRT-PCR which would likely be used for clinical applications (3) the confounding treatment effects in the 5h and 24h blood samples from IS patients (4) race was not factored in due to different distributions with zero subjects in some of the race categories and (5) age is a confounder that was addressed by factoring it in ANCOVA models and by selecting control groups with close age distribution to the IS patients.

Example 2: Biomarkers for the Diagnosis of the Cause of Ischemic Stroke

1. Study Patients

[0195] Patients with acute ischemic stroke were enrolled from the CLEAR trial, a multicenter, randomized double-blind safety study of recombinant tissue-plasminogen activator (rt-PA) and eptifibatide as previously described [Pancioli AM et al., Stroke, 39:3268-3276 (2008)] (NCT00250991 at Clinical-Trials.gov). The institutional review board of each site approved the study protocol and written informed consent was obtained from each patient prior to study entry. Eligible patients had a diagnosis of acute ischemic stroke, therapy initiated within 3 hours of stroke onset, a National Institutes of Health Stroke Scale (NIHSS) >5, and were 18-80 years of age. All patients had standardized clinical evaluations, including NIHSS, and brain imaging. Blood samples were drawn into PAXgene tubes (PreAnalytiX, Hilden, Germany) at $\leq$3 hours, 5 hours, and 24 hours after stroke onset for use in gene expression analysis. A total of 194 samples were obtained from 76 patients.

[0196] Etiology of ischemic stroke was classified according to TOAST [Adams HP, Jr., et al., Stroke, 24:35-41 (1993)]. Patients with cardioembolic stroke, large vessel stroke and cryptogenic stroke (undetermined etiology) were included for study. Cardioembolic stroke required at least one source of cardiac embolus to be identified and the exclusion of large vessel or small vessel causes of stroke. Large vessel stroke required stenosis greater than 50% of ipsilateral

extracranial or intracranial artery and the exclusion of cardioembolic and small vessel causes of stroke. Cause of stroke was determined using medical history, blood tests, brain imaging, Doppler and vascular angiography, and cardiac investigations. Patients with atrial fibrillation were identified using electrocardiogram, echocardiogram and 24-48 hour cardiac monitoring. Control blood samples were drawn from 23 control subjects similar in age, gender and race to stroke subjects. These subjects had no history of ischemic stroke or cardiovascular disease, no recent infection and no hematological disease.

2. Sample Processing

[0197] Whole blood was collected from the antecubital vein into PAXgene tubes (PreAnalytiX, Germany). PAXgene tubes were frozen at -80°C after 2 hours at room temperature. All samples were processed in the same laboratory. Total RNA was isolated according to the manufacturer's protocol (PAXgene blood RNA kit; Pre-AnalytiX). RNA was analyzed using Agilent 2100 Bioanalyzer for quality and Nano-Drop (Thermo Fisher) for concentration. Samples required A260/A280 absorbance ratios of purified RNA ≥2.0 and 28S/18S rRNA ratios ≥1.8. Reverse transcription, amplification, and sample labeling were carried out using Nugen's Ovation Whole Blood Solution (Nugen Technologies, San Carlos, CA). Each RNA sample was hybridized according to manufacturer's protocol onto Affymetrix Human U133 Plus 2.0 GeneChips (Affymetrix Santa Clara, CA), which contain 54,697 probe sets. The arrays were washed and processed on a Fluidics Station 450 and then scanned on a Genechip Scanner 3000. Samples were randomly assigned to microarray batch stratified by cause of stroke.

3. Gene Expression Profile Analyses

[0198] Raw expression values (probe level data) were imported into Partek software (Partek Inc., St. Louis, MO). They were log transformed and normalized using RMA (Robust Multichip Average) and our previously reported internal gene normalization method [Stamova BS et al., BMC Med Genomics, 2:49 (2009)]. Statistical analysis, principal components analysis, and hierarchical unsupervised clustering analysis were performed with Partek Genomics Suite 6.04. The fidelity of genetic biomarker subsets as class prediction tools was established using k-nearest neighbor and 10-fold leave-one-out cross-validation in PAM (Prediction Analysis of Microarrays) [Tibshirani RJ and Efron B., Stat Appl Genet Mol Biol., 1:Article 1 (2002)]. Leave-one-out cross-validation provides a relatively unbiased estimate of the generalization ability of the genetic classifier. A model is generated on 90% of the samples and used to predict the remaining 10% of samples. The procedure is repeated 10 times to compute the overall error in the model. Ingenuity Pathway Analysis (IPA, Ingenuity Systems®, www.ingenuity.com) was used to determine whether the numbers of genes regulated within given pathways or cell functions were greater than expected by chance (Fisher's exact test).

4. Statistical Analyses

[0199] Differences in demographic data between groups were analyzed using Fisher's exact test and a two-tailed t-test where appropriate. All data are presented as mean ± standard error. To identify the gene expression profiles that distinguish cardioembolic stroke from large vessel stroke, repeated measures analysis of variance (ANOVA) was used including stroke etiology, time, stroke etiology & time interaction, and the within subject variance in the model. Unsupervised hierarchical clustering and principal components analysis (PCA) were used to evaluate relationships between cardioembolic stroke and large vessel stroke. Gene probes with a p value ≤0.005 and a fold change ≥ |1.2| were considered significant.

[0200] A similar analysis was used to identify the gene expression profiles that distinguish cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes. A repeated measures ANOVA was used including cardioembolic stroke etiology, time, and within subject variance in the model. Unsupervised hierarchical clustering and PCA were used to evaluate relationships between cardioembolic stroke caused by atrial fibrillation and non-atrial fibrillation. Gene probes with a p value ≤0.005 and a fold change ≥ |1.2| were considered significant.

[0201] Functional analysis was performed by comparing subjects with cardioembolic stroke and large vessel stroke to control subjects. A one-way ANCOVA was used adjusting for age and gender. Gene probes with a p value ≤0.005 and a fold change ≥ |1.2| were considered significant and analyzed in IPA.

RESULTS

Cardioembolic versus Large Vessel Ischemic Stoke

[0202] Demographic and clinical characteristics of subjects used for the comparison of cardioembolic stroke to large vessel stroke are shown in Table 8. Atrial fibrillation was the only variable significantly different between groups ($p<0.05$).

There were 69 samples with cardioembolic stroke and 30 samples with large vessel stroke.

[0203] Initially the ability of the previously published 77 gene list to distinguish cardioembolic stroke from large vessel stroke was evaluated [Xu H et al., J Cereb Blood Flow Metab., 28:1320-1328 (2008)]. This gene list was based on the first 11 patients enrolled in the CLEAR trial, 7 with cardioembolic stroke and 4 with large vessel stroke. Using a k-nearest neighbor prediction model, the preliminary 77 gene list was used to predict the completed CLEAR trial patient population. Cardioembolic stroke was correctly predicted in 82.6% of samples, and large vessel stroke was correctly predicted in 80.0% of samples. However, on 10-fold leave one out cross-validation, 56.5% were correctly predicted as cardioembolic stroke and 60% were correctly predicted as large vessel stroke, with the probability of predicted diagnosis being below 90% in most samples. These results suggests that gene expression profiles in blood can distinguish cause of stroke, though further refinement is required to better generalize genomic predictors to a larger patient population.

[0204] Analysis of the complete CLEAR trial patients was thus performed. A repeated measures ANOVA identified 40 genes significantly different between cardioembolic stroke and large vessel stroke at all three time points (Table 13). A hierarchical cluster plot of the 40 genes is shown in Figure 12a, and a Principal Component Analysis (PCA) in Figure 12b. The 40 genes separate cardioembolic stroke from large vessel stroke by at least 2 standard deviations (Figure 12b). The hierarchical cluster plot demonstrates a group of genes that are up-regulated in cardioembolic stroke and down-regulated in large vessel stroke. There is also a group of genes that are down-regulated in cardioembolic stroke and up-regulated in large vessel stroke. The 40 genes separate cardioembolic from large vessel stroke at $\leq$ 3 hours, 5 hours and 24 hours following ischemic stroke as shown in Figure 17.

Prediction of Cardioembolic and Large Vessel Stroke

[0205] The ability of the 40 genes to predict cardioembolic stroke from large vessel stroke was evaluated using 10-fold leave one out cross-validation model in PAM. Of the 99 samples, 100% of the 69 samples with cardioembolic stroke were correctly predicted, and 96.7% of the 30 samples with large vessel stroke were correctly predicted (Figure 13). The probability of predicted diagnosis was >90% for the majority of samples (Figure 13). To further evaluate the 40 gene list, it was applied to a separate group of patients with known cardioembolic stroke. Of the 10 samples, 90% (9/10) were correctly predicted as cardioembolic stroke.

[0206] The 40 gene list was subsequently used to predict the cause of stroke in patients with cryptogenic stroke. There were 36 patients (85 samples) with cryptogenic stroke. To be considered classified by the prediction model, all samples from each patient were required to have a >90% probability of the same predicted diagnosis. A total of 15 patients (41 %) were predicted to have a profile similar to cardioembolic stroke with a probability >90%, and a total of 6 patients (17%) were predicted to have a profile similar to large vessel stroke with a probability >90%. This represents a potential reclassification of 58% of cryptogenic stroke to either cardioembolic or large vessel stroke.

Functional Analysis

[0207] To determine the functional pathways associated with cardioembolic and large vessel stroke, the subjects with cardioembolic and large vessel stroke were compared to controls. There were 731 genes significantly different between cardioembolic stroke subjects and controls, and 782 genes that were significantly different between large vessel stroke and controls ($p < 0.005$, fold change $\geq |1.2|$). These two gene lists are shown in a Venn diagram in Figure 14. There were 503 genes unique to cardioembolic stroke, 554 genes unique to large vessel stroke and 228 genes common to cardioembolic stroke and large vessel stroke. The top Canonical and molecular functions of these respective gene lists are shown in Tables 9-11.

[0208] Of the 503 cardioembolic stroke genes, specific genes that have been previously associated with three of the main cardiac diseases include: atrial fibrillation genes - CREM, SLC8A1, KNCH7, KCNE1; myocardial infarction genes - PDE4B, TLR2; and heart failure genes - MAPK1, HTT, GNAQ, CD52, PDE4B, RAF1, CFLAR, and MDM2 (Table 9). Cardioembolic stroke was associated with development of lymphocytes, inflammatory disorder, cardiomyocyte cell death, and phosphatidylinositiol 4-phosphate modification. Top canonical pathways included renin-angiotensin signaling, thrombopoietin signaling, NF-κB activation, cardiac hypertrophy, and B cell receptor signaling (Table 9).

[0209] Of the 554 large vessel stroke genes, specific genes that have been previously associated with atherosclerotic lesion and atherosclerotic plaque include MMP9, FASLG, CX3R1, RAG1, TNF, IRAG1, CX3CR, and THBS1 (Table 10). Large vessel stroke was associated with T cell and leukocyte development, inflammation, and invasion. Top canonical pathways include T cell activation and regulation, CCR5 signaling in macrophages, relaxin signaling, and corticotropin releasing hormone signaling (Table 10).

[0210] A total of 228 genes were common to cardioembolic stroke and large vessel stroke, representing ischemic stroke (Figure 14). They were associated with leukocyte and phagocyte development and movement, cardiovascular processes, NF-κB response element expression, and oxidative stress (Table 11). Top canonical pathways include p38 MAPK signaling, toll-like receptor signaling, IL-6 and IL-10 signaling, NK-κB signaling, B-cell receptor signaling, and

NRF-mediated oxidative stress (Table 11).

Atrial fibrillation versus Non-Atrial fibrillation Cardioembolic Stroke

[0211] There were 23 subjects with cardioembolic stroke, 10 with atrial fibrillation and 13 with no atrial fibrillation identified on routine investigation. Subjects in the non-atrial fibrillation group who are more likely to have paroxysmal atrial fibrillation were excluded. To do this, the 10 patients with stroke due to atrial fibrillation were initially compared to the 10 patients with large vessel stroke. Repeated measures ANOVA identified a 39 gene profile of atrial fibrillation. This profile was then used to predict which of the 13 cardioembolic stroke subjects without atrial fibrillation identified on routine investigation had the highest probability of being similar to atrial fibrillation. There were 5 subjects who fell within 4 standard deviations of the mean predicted probability of patients with known atrial fibrillation. These patients were considered more likely to have paroxysmal atrial fibrillation and thus were excluded from further analysis, as a conservative method to reduce the possibility of paroxysmal atrial fibrillation being present in the non-atrial fibrillation group. The remaining 8 non-atrial fibrillation patients were compared to the 10 patients with atrial fibrillation. The demographic and clinical characteristics of are shown in Table 12. Atrial fibrillation was the only variable significantly different between the two groups ($p<0.05$). A repeated measures ANOVA identified 37 genes that were significantly different between atrial fibrillation and non-atrial fibrillation causes of cardioembolic stroke (Table 14). A hierarchical cluster plot of the 37 genes is shown in Figure 15a, and a PCA in Figure 15b. The 37 genes clearly separate atrial fibrillation from non-atrial fibrillation (Figure 15). The 37 genes can separate atrial fibrillation from non-atrial fibrillation cardioembolic stroke at $\leq$ 3 hours, 5 hours and 24 hours following ischemic stroke (Figure 18). The 37 genes were applied to the 5 subjects excluded from analysis, with 2 being predicted to be atrial fibrillation, 2 being indeterminate, and 1 being predicted to be non-atrial fibrillation cardioembolic stroke.

Prediction of Atrial fibrillation and Non-Atrial Fibrillation Cardioembolic Stroke

[0212] The ability of the 37 genes to predict atrial fibrillation from non-atrial fibrillation causes of cardioembolic stroke was evaluated using a 10-fold leave one out cross-validation model in PAM. In the 60 samples, 100% of the 30 samples with atrial fibrillation cardioembolic stroke were correctly predicted, and 91.7% of the 30 samples with non-atrial fibrillation cardioembolic stroke were correctly predicted (Figure 16). Additionally, the probability of predicted diagnosis was >90% for most samples.

[0213] The 37 gene list was used to predict a test set of 10 samples with cardioembolic stroke who did not have atrial fibrillation identified on routine testing. Of these 10 samples, 3 (30%) were predicted to have paroxysmal atrial fibrillation with >90% probability when compared to the gene expression profile of subjects with known symptomatic atrial fibrillation. The 37 gene list was also used to predict the cause of stroke in patients with cryptogenic stroke. There were 11 patients with cryptogenic stroke who were predicted to have cardioembolic stroke based on the 40 gene profile. Of these 11 patients, 3 patients (27%) were predicted to have paroxysmal atrial fibrillation with a probability >90% based on a gene expression profile that was similar to subjects with known atrial fibrillation stroke.

DISCUSSION

[0214] Determining the cause of ischemic stroke is of paramount importance as it guides management decisions such as whether to initiate antiplatelet or anticoagulation treatment. However, identifying the cause of stroke remains a challenge in many patients as exemplified by cryptogenic stroke. Given that cryptogenic stroke accounts for approximately 30% of ischemic strokes, better classification tools are required. The use of gene expression profiles in blood to distinguish cardioembolic stroke from large vessel stroke on a molecular level are described herein. A 40 gene expression profile can distinguish cardioembolic stroke from large vessel stroke, and a 37 gene expression profile can distinguish cardioembolic stroke due to atrial fibrillation from non-atrial fibrillation causes. When applied to cryptogenic stroke, 58% of subjects can be reclassified with a probability >90% as being either cardioembolic or large vessel stroke.

[0215] Limitations of large-scale gene expression profiling have been well described [Schulze A and Downward J., Nat Cell Biol., 3:E190-195 (2001)]. However, comparable approaches have applied in patients with human malignancies and that have translated to PCR based arrays for diagnostic purposes [Hedenfalk I et al., N Engl J Med., 344:539-548 (2001); Valk PJ et al., N Engl J Med., 350:1617-1628 (2004)]. Unlike human malignancy with distinct histological criteria, ischemic stroke subtypes are heterogeneous and rely on a combination of clinical and investigational criteria. With strict patient selection, molecular classification of ischemic stroke subtypes into clinically relevant subgroups with biomarkers appears to be feasible. Indeed, several prothrombotic and inflammatory biomarkers in the blood are different in each subtype of ischemic stroke [Laskowitz DT et al., Stroke, 40:77-85 (2009); Shibazaki K et al., Inter Med., 48:259-264 (2009); Montaner J et al., Stroke, 39:2280-2287 (2008); Hassan A et al., Brain, 126:424-432 (2003); Xu H et al., J Cereb Blood Flow Metab., 28:1320-1328 (2008)].

Cardioembolic and Large Vessel Atherosclerotic Stroke

[0216] A gene expression profile able to differentiate cardioembolic stroke from large vessel stroke was identified. This distinction is clinically important as treatment and diagnostic testing are different between the two subtypes. In general, cardioembolic stroke benefit from anticoagulation, whereas large vessel stroke benefit from antiplatelet therapy and vascular surgery. Determining the etiology of stroke and thus the preventative treatments to be initiated relies on diagnostic tests. In fact, the TOAST criteria require that other causes of stroke be ruled out to make a probable diagnosis of cause [Adams HP, Jr., et al., Stroke, 24:35-41 (1993)]. As a result, patients with ischemic stroke frequently undergo extensive testing to image the vasculature and evaluate cardiac function. Diagnostic testing to determine the cause of stroke can be better focused by using gene expression profiles, particularly in cryptogenic stroke. In this manner, costly resources can be targeted to subjects where they will be of highest yield.

Cardioembolic Stoke

[0217] Currently, the selection of which patients with ischemic stroke require cardiac investigations such as Holter monitor and echocardiogram is based on clinical judgment combined with brain imaging. However, determining which ischemic stroke patients should be screened by transthoracic and transesophageal echocardiography is challenging. Though age <50 years is associated with higher diagnostic yield, many stroke patients are older than 50 years. Gene expression profiles in combination with clinical impression serve as a guide to direct echocardiography.

[0218] Cardiac monitoring for arrhythmias is also commonly performed following ischemic stroke. Identifying atrial fibrillation is important, as anticoagulation reduces recurrent embolic events. However, cardiac monitoring for 24 to 48 hours often misses paroxysmal atrial fibrillation [Tayal AH et al., Neurology, 71:1696-1701 (2008); Ziegler PD et al., Stroke, 41:256-260]. A gene expression profile suggesting a patient has a high probability of atrial fibrillation may be an additional tool to aid in preventing such missed treatment opportunities. In a group of 10 cardioembolic strokes who did not have atrial fibrillation identified on routine investigation, it is shown that a gene expression profile can predict 3 subjects (30%) to have paroxysmal atrial fibrillation with greater than 90% probability. This is consistent with previous studies of cardioembolic stroke without atrial fibrillation on routine investigation, where an additional 23-28% cases of paroxysmal atrial fibrillation can be identified using long term cardiac monitoring [Tayal AH et al., Neurology, 71:1696-1701 (2008), Ziegler PD et al., Stroke, 41:256-260]. Subjects who appear to have atrial fibrillation by gene expression profiles could be a target group for such prolonged cardiac recording.

Large Vessel Stroke

[0219] Gene expression profiles may also aid in the diagnosis of large vessel stroke. Evaluation of large vessel atherosclerotic disease includes imaging of extracranial and intracranial vessels using magnetic resonance angiography (MRA), computed tomography angiography (CTA), ultrasound and conventional angiography. Inconsistencies in the results of vascular imaging do occur. For example, the degree of carotid stenosis by ultrasound may not agree with the degree of stenosis by MRA or CTA. Supplementing imaging with a gene expression profile suggestive of symptomatic atherosclerotic disease could add confidence to the diagnosis of large vessel atherosclerotic disease. The presence of large vessel disease is large based on a single factor, the degree of vascular stenosis. In the TOAST criteria, a stenosis less than 50% is considered negative [Adams HP, Jr., et al., Stroke, 24:35-41 (1993)]. Gene expression profiles provide an additional measure of factors associated with symptomatic atherosclerotic disease, particularly inflammation. This is similar in concept to MRI methods to determine atheroma inflammation [Tang TY et al., Arterioscler Thromb Vasc Biol., 29:1001-1008 (2009)]. These proposed applications of gene expression profiles require further investigation. However, they show promise as methods to better target investigations and treatments to patients with ischemic stroke.

Cryptogenic Stroke

[0220] Cryptogenic stroke is a heterogeneous group of patients where better diagnostic tools are required. The gene expression profiles described herein were applied to the cryptogenic stroke group and predicted 41% to have cardioembolic stroke. Of these patients, 27% were suggested to have atrial fibrillation. Cryptogenic stroke patients with a molecular signature similar to cardioembolic stroke may represent a group where long term cardiac monitoring can be focused, and potentially a subgroup where a trial of anticoagulation could be performed [Tayal AH et al., Neurology, 71:1696-1701 (2008); Harloff A et al., Stroke, 37:859-864 (2006); Sacco RL et al., Cerebrovasc Dis., 22:4-12 (2006); Mohr JP et al., N Engl J Med., 345:1444-1451 (2001)]. 17% of the cryptogenic group were also predicted to have large vessel stroke. This finding may represent a symptomatic stenosis <50%, though further study with thorough vascular imaging is required.

Functional Analysis

[0221] The rationale for changes in gene expression in blood of patients with ischemic stroke rests largely in differences in patterns of inflammation. The major source of RNA in the blood is immune cells including leukocytes, neutrophils, and monocytes [Du X et al., Genomics, 87:693-703 (2006)]. Immune cells provide an indirect reflection of a patient's disease state and subsequent response, such as the immune response to ischemic brain tissue and immune response to disease mediated by vascular risk factors. The majority of these responses remain unclear, though it appears there are differences in the ways these responses are orchestrated between subjects with cardioembolic stroke and large vessel stroke. This is evidenced by the 40 gene profile for cardioembolic stroke and large vessel stroke, and the 37 gene profile for cardioembolic stroke due to atrial fibrillation and non-atrial fibrillation. The fact that different genes are associated with stroke of large vessel, cardioembolic, and atrial fibrillation origin suggests specific immune responses in each condition. The precise cause for these differences, including immune cell-endothelial interactions, remain unknown and should become clearer as each condition and cause is studied.

[0222] In conclusion, the present invention provides gene expression signatures can distinguish between cardioembolic and large vessel subtypes of ischemic stroke. Gene expression profiles find use for the development of blood tests to aid in the classification of ischemic stroke, target stroke investigation and treatment, and determine the causes of cryptogenic stroke.

TABLES

[0223]

Table 8: Demographic variables for subjects with cardioembolic stroke and large vessel stroke. p-values represent comparisons of subjects with cardioembolic to large vessel stroke using Fisher's exact test or two-tailed t-test where appropriate. (BP, blood pressure; CABG, coronary artery bypass graft)

Variables	**Cardioembolic** (n=23)	**Large Vessel** (n=10)	**p value**
Mean Age (years)	71.7±1.6	66.9±2.9	0.14
Sex, male (%)	12 (52.2%)	8 (80%)	0.25
Race, Caucasian (%)	15 (65.2%)	8 (80%)	0.68
Hypertension (%)	16 (69.6%)	8 (80%)	0.55
Mean Systolic BP	158.3±6.1	163.5±8.0	0.63
Mean Diastolic BP	80.6±3.6	88±6.7	0.30
Diabetes (%)	4 (17.4%)	4 (40%)	0.21
Hyperlipidemia (%)	6 (26.1%)	3 (30%)	1.00
Mean Weight (kg)	81.9±4.5	89.6±6.2	0.34
Atrial Fibrillation (%)	10 (43.4%)	0 (0%)	0.03
Myocardial Infarction (%)	4 (17.3%)	2 (20%)	1.00
Congestive Heart Failure	8 (34.8%)	2 (20%)	0.68
Coronary Artery Bypass	5 (21.7%)	1 (10%)	0.64
Carotid Endarterectomy	0 (0%)	2 (20%)	0.08
Femoral Popliteal Bypass	0 (0%)	1 (10%)	0.30
Prior Stroke	7 (30.4%)	1 (10%)	0.38
Mean NIHSS 3 hours	11.9±1.7	12.7±1.0	0.69
Mean NIHSS 24 hours	11.2±1.8	13.9±3.0	0.44
Mean NIHSS 5 days	10.3±2.1	12.1±4.7	0.69

Table 9: Functional analysis of 503 genes found to be unique to Cardioembolic strokes when compared to controls (p <0.005, FC > |1.2|)

TABLE 9			
	CE	**Genes**	**p-value**
Canonical Pathways	Renin-angiotensin signaling	ADCY4, GNAQ, PAPK1, MAPK14, PIK3C3, PIK3C2B, PRKARIA, PRKCZ, RAF1	2.6 x 10^{-4}
	Thrombopoietin signaling	MAPK1, PIK3C3, PIK3C2B, PRKCZ, RAF1, STAT5B* CXCR5, ITGAL, MAPK1, PIK3C3, PIK3C2B, PRKCZ, RAF1	5.8 x 10^{-4}
	NF-kB activation	MAPK1, MDM2, PIK3C3, PIK3C2B, PRKCZ, PTEN*, RAF1, TFDP1	6.0 x 10^{-4}
	Cardiac Hypertrophy role of NFAT	ADCY4, GNAQ, MAPK1, MAPK14, MEF2A, PIK3C3, PIK3C2B, PRKARIA, PRKCZ, RAF1, SLC8A1	9.9 x 10^{-4}
	B cell receptor Signaling	BCL6, FCGR2C, MAP3K2, MAPK1, MAPK14, PIK3C3, PIK3C2B, PTEN*, RAF1	2.4 x 10^{-3}
Molecular Functions	Lymphocyte development	APC, BCL6, CARD11, CD55, CFLAR, CXCR5, DTX1, GATA3, HIST1H1C, HLA-DOA, IFNGR1, IL13RA1, IL27RA, ITGAL, KLF13, MAP3K2, MAPK1, MAPK14, MBP, MDM2, PRKCZ, PTEN, RAF1, RBPJ, SEMA4A, SMARCA4, SRGN, STAT5B, STK17B, TXN, XRCC5	1.4 x 10^{-3}
	Cardiomyocytes cell death	CREM, GHRL, GNAQ, MAPK1, MAPK14, MDM2, NAMPT, PTEN, RAF1, SLC8A1, SOD2	1.8 x 10^{-4} 2.8 x 10^{-4}
	T lymphocyte development	APC, BCL6, CARD11, CD55, CFLAR, DTX1, GATA3, HIST1H1C, HLA-DOA, IFNGR1, IL27RA, KLF13, MAP3K2, MAPK1, MAPK14, MBP, MDM2, PRKCZ, PTEN, SEMA4A, SMARCA4, SRGN, STAT5B, STK17B, XRCC5	4.1 x 10^{-4}
	Inflammatory disorder	ABCB4, AHNAK, AKAP13, ANXA3, AQP9, ARF1, ASPH, B4GALT1, BCL6, CARD11, CASC4, CD55, CDH26, CFLAR, CLEC4D, CMIP, CR1, CREM, CXCR5, DHX37, DIS3L2, DYNC1LI1, ENG, ENTPD1, ETV5, EXOC6, FBXL13, FGGY, GATA3, GEMIN5, GLG1, H3F3B, HCG27, HDGFRP3, HLA-DOA, HTT, IFNGR1, IL18RAP, IL27RA, ITGAL, KALRN, KIF13A, KLF13, LHX2, LYST, MAP4, MAPK14, MBP, MDM2, MED24, MEF2A, MTHFS, NAMPT, NAT10, NBAS, NCOA2, NUMB, NUP62, OSBPL1A, PDE4B, PHF15, PHRF1, PRKCZ, PTEN, RAF1, S100A9, S100A12, SBF2, SLC22A4, SLC7A11, SLC8A1, SOD2, SOX6, STAT5B, STK17B, TPST1, TRRAP, TSPAN2, TTYH2, TXN, USP11, USP15, VIM, VSIG4, YEATS2, ZFHX3, ZNF230, ZNF831	4.4 x 10^{-4}
	Phosphatidylinositol4-phosphate modification	PI4KA, PIK3C3, PIK3C2B, PTEN	

Table 10: Functional analysis of the 554 genes unique to large vessel atherosclerotic stroke when compared to controls (p <0.005, FC > |1.2|).

TABLE 10			
	LV	**Genes**	**p-value**
Canonical Pathways	Cytotoxic T lymphocyte target cell apoptosis	APAF1, CD247, FADD, FASLG, TRA@, TRD@	1.3 x 10^{-5}
	CCR5 signaling in macrophages	CD247, FASLG, GNAI1, GNG*, MAPK13*, PRKCZ, TRA@, TRD@	1.3 x 10^{-4}
	Relaxin signaling	GNAI1, GNG2, GUCY2D, MAPK1, MMP9, NF-κB IE, NPR2, PDE2A, PIK3R3, PRKACA,	1.5 x 10^{-4}
	Corticotropin releasing hormone signaling	PRKCZ FASLG, GNAI1, GUCY2D, MAPK1, MAPK13, NPR2, NR4A1	1.0 x 10^{-3}
	T lymphocyte regulation	CD247, NR4A1, PRKCZ, TRA@, TRD@, ZAP70*	1.3 x 10^{-3}
Molecular Functions	T lymphocyte differentiation	ADA, TRA@, ZAP70	1.4 x 10^{-3}
	Leukocyte development, morphology	ADA, APAF1, CCR8, CD83, CD247, CSF1, CSF2RA, CXCL12, EZH2, F5, FADD, FASLG, HIVEP2, IL12RB1, IL21R, ITGB7, MAPK1, NR4A1, PDIA3, PRKCZ, RAG1, RNASEL, SMAD7, THBS1, TRA@, XIAP, ZAP70	1.5 x 10^{-3}
	Invasion of cells	MMP9, PLAUR	1.7 x 10^{-3}
	Inflammatory disorder	ADA, ADORA3, ADRB2, ALOX5AP, APAF1, ARHGDIB, ARHGEF 17, ASPH, ATP4B, C20ORF43, CA4, CA13, CAPN10, CD83, CD247, CDK6, CLCN6, COL9A3, COLQ, CORIN, CORO2A, CSF1, CSF2RA, CX3CR1, CXCL12, EGFL8, F5, FAM101B, FASLG, FBF1, GNG2, GRB10, HIC1, HIVEP1, HP, KIAA1908, LIMD1, LTB4R, MAPK13, MDC1, MMP9, MPHOSPH9, MSRA, MYH3, NFIA, NR4A1, NUMA1, OLAH, PACSIN2, PADI4, PCNX, PDE2A, PDIA3, PER1, PFTK1, PGLYRP1, PGM1, PHF19, PIK3R3, PITPNA, PLAUR, PMF1, PPARGC1B, PRKCZ, PRR5L, PTGDR, PXK, RAB7A, RAG1, RAPH1, RARG, ROPN1L, SAMSN1, SERPINE2, SEZ6L, SLC25A15, SLC26A8, SLC8A1, SLCO4C1, SPRED1, SPTLC2, SRPK2, STK36, TAF7L, TBC1D1, TGFBR3, THBS1, TKT, TNFSF8, TNIK, TRA@, TTC7A, TUBA4, TUBA4A, VARS2, ZEB1	2.8 x 10^{-3}
	T cell development	ADA, APAF1, CCR8, CD83, CD247, CXCL12, F5, FADD, FASLG, HIVEP2, IL12RB1, IL21R, ITGB7, MAPK1, NR4A1, PDIA3, PRKCZ, RAG1, RNASEL, SMAD7, THBS1, TRA@, XIAP, ZAP70	5.2 x 10^{-3}

Table 11: Functional analysis of 228 genes common to cardioembolic and large vessel atherosclerotic stroke when compared to controls (p <0.005, FC > |1.2|).

	LV-CE Common	**Genes**	**p-value**
Canonical Pathways	p38 MAPK Signaling	DUSP1*, IL1R2, IRAK3*, MAP2K6, MAPK14, MAX, MKNK1, TNF	3.6 x 10^{-6}
	Toll-like receptor signaling	IRAK3*, MAP2K6, MAPK14, TLR2, TOLLIP	1.2 x 10^{-4}
	IL-6 signaling	ABCB1, IL1R2*, MAP2K6, MAPK14, SOS2, TNF	2.4 x 10^{-4}
	NF-κB Signaling	GSK3B, IL1R2, IRAK3, MAP2K6, MAP3K3, TLR2, TNF	4.5 x 10^{-4}

(continued)

	LV-CE Common	Genes	p-value
	B Cell Receptor Signaling	GSK3B, MAP2K6, MAP3K3, MAPK14, NFATC2, PTEN, SOS2	5.6×10^{-3}
	Role of Macrophages, fibroblasts and endothelial cells in RA	CEBPD*, GSK3B, IL1R2*, IRAK3*, MAP2K6, MAPK14, NFATC2, TLR2, TNF	2.8×10^{-3}
	IL-10 Signaling	IL1R2, MAP2K6, MAPK14, TNF	3.1×10^{-3}
	NRF2-mediated Oxidative Stress	DNAJC3, FKBP5, GSK3B, MAP2K6, MAPK14, TXN	6.6×10^{-3}
Molecular Functions	Neutrophil / Phagocyte / Leukocyte movement	CAMP, CD55, CSF2RA, DUSP1, FCAR*, LILRA6, MAPK14, PTEN, SLPI, TLR2, TNF	1.7×10^{-5}
	Leukocyte development, activation	BST1, CAMP, CD55, CD59, CEBPD, CFLAR, CSF2RA, F5, GATA3, GSK3B, IL2RB, LILRA6, MAPK14, MLL, NFATC2, PRKDC, PTEN, RGL4, TLR2, TNF, TXN	5.4×10^{-5}
		BMX, GSK3B, IL18BP, MAP3K3, MAPK14, TLR2	1.2×10^{-4}
	Cardiovascular process	GSK3B, MAPK14, TLR2, TNF	2.5×10^{-4}
	NF-kappa B response element expression Leukocyte proliferation	CAMP, CD59, CFLAR, CSF2RA, GATA3, IL2RB, IRS2, MAPK14, MLL, NFATC2, PCYT1A, PTEN, SLPI, TLR2, TNF, TXN	2.9×10^{-4}
	Oxidative stress	TNF, TXN	3.0×10^{-4}

Table 12: Demographic variables for subjects with cardioembolic stroke due to atrial fibrillation and non-atrial fibrillation causes. p-values represent comparisons of subjects with atrial fibrillation to those with non-atrial fibrillation using Fisher's exact test or two-tailed t-test where appropriate. (BP, blood pressure; CABG, coronary artery bypass graft)

Variables	**Atrial Fibrillation** (n=10)	**Non-Atrial Fibrillation** (n=8)	**p value**
Mean Age (years)	72.9±2.3	68.5±3.1	0.26
Sex, male (%)	4 (40%)	6 (75%)	0.19
Race, Caucasian (%)	6 (60%)	5 (62%)	0.65
Hypertension (%)	8 (80%)	6 (75%)	1.00
Mean Systolic BP	158.3±9.3	160.4±10.7	0.88
Mean Diastolic BP	80.8±4.7	86.1±8.0	0.56
Diabetes (%)	1 (10%)	2 (25%)	0.56
Hyperlipidemia (%)	3 (30%)	3 (30%)	1.00
Mean Weight (kg)	86.9±8.2	84.9±5.7	0.85
Myocardial Infarction (%)	1 (10%)	3 (37%)	0.28
Congestive Heart Failure	5 (50%)	3 (37%)	0.28
Coronary Artery Bypass	1 (10%)	1 (10%)	1.00
Carotid Endarterectomy	0 (0%)	0 (0%)	-
Femoral Popliteal Bypass	0 (0%)	0 (0%)	-
Prior Stroke	3 (30%)	3 (37%)	1.00
Mean NIHSS 3 hours	17.3±3.1	13.6±2.4	0.39
Mean NIHSS 24 hours	11.6±3.5	10.6±3.0	0.83

(continued)

Variables	Atrial Fibrillation (n=10)	Non-Atrial Fibrillation (n=8)	p value
Mean NIHSS 5 days	9.9±3.5	10.0±4.0	0.98

Table 13A. A list of 40 genes that differentiate cardioembolic stroke from large vessel stroke ($p < 0.005$, fold change >|1.2|).

Table 13A. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
1552477_a_at	1.26832	IRF6	interferon regulatory factor 6	BC014852.1	3664	Hs.719361	NM_006147	NP_006138
1556896_at	-2.02233	LOC284751	hypothetical LOC284751	AK090605.1	284751	Hs.282325	NM_001025463	NP_001020634
1557542_at	-1.33949	---	---	AW069144	-	Hs.353829	---	---
1559449_a_at	1.34719	ZN F254	Zinc finger protein 254	BF679633	9534	Hs.434406	NM_203282	NP_975011
1565389_s_at	1.45568	GRM5	glutamate receptor, metabotropic 5	S64316.1	2915	Hs.147361	NM_000842 /// NM_001143831	NP_000833 /// NP_001137303
202012_s_at	1.19814	EXT2	exostoses (multiple) 2	AA196245	2132	Hs.368404	NM_000401 /// NM_207122	NP_000392 /// NP_997005
202399_s_at	1.20871	AP3S2	adaptor-related protein complex 3, sigma 2 subunit	NM_005829.1	10239	Hs.632161	NM_005829 /// NR_023361	NP_005820
204484_at	1.32829	PIK3C2B	phosphoinositide-3-kinase, class 2, beta polypeptide	NM_002646.1	5287	Hs.497487	NM_002646	NP_002637
204765_at	1.22689	ARHGEF5	Rho guanine nucleotide exchange factor (GEF) 5	NM_005435.1	7984	Hs.334	NM_005435	NP_005426

(continued)

Table 13A. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
207549_x_at	-1.23817	CD46	CD46 molecule, complement regulatory protein	NM_002389.1	4179	Hs.510402	NM_002389 /// NM_153826 /// NM_172350 /// NM_172351 /// NM_172352 /// NM_172353 /// NM_172354 /// NM_172355 /// NM_172356 /// NM_172357 /// NM_172358 /// NM_172359 /// NM_172360 /// NM_172361	NP_002380 /// NP_722548 /// NP_758860 /// NP_758861 /// NP_758862 /// NP_758863 /// NP_758864 /// NP_758865 /// NP_758866 /// NP_758867 /// NP_758868 /// NP_758869 /// NP_758870 /// NP_758871
210839_s_at	-1.35639	ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	D45421.1	5168	Hs.190977	NM_001040092 /// N_001130863 /// NM_006209	NP_001035181 /// NP_001124335 /// NP_006200

(continued)

Table 13A. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
211343_s_at	1.84752	COL13A1	collagen, type XIII, alpha 1	M33653.1	1305	Hs.695934	N_001130103 /// NM_005203 /// NM_080798 /// NM_080799 /// NM_080800 /// NM_080801 /// NM_080802 /// NM_080803 /// NM_080804 /// NM_080805 /// NM_080806 /// NM_080807 /// NM_080808 /// NM_080809 /// NM_080810 /// NM_080811 /// NM_080812 /// NM_080813 /// NM_080814 /// NM_080815	NP_001123575 /// NP_005194 /// NP_542988 /// NP_542989 /// NP_542990 /// NP_542991 /// NP_542992 /// NP_542993 /// NP_542994 /// NP_542995 /// NP_542996 /// NP_542997 /// NP_542998 /// NP_542999 /// NP_543000 /// NP_543001 /// NP_543002 /// NP_543003 /// NP_543004 /// NP_543005

(continued)

Table 13A. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
215172_at	1.46054	PTPN20A /// PTPN20B	protein tyrosine phosphatase, non-receptor type 20A /// protein tyrosine phosphatase, non-receptor type 20B	AL050040.1	26095 /// 653129	Hs.440733	NM_001042357 /// NM_001042358 /// NM_001042359 /// NM_001042360 /// NM_001042361 /// NM_001042362 /// NM_001042363 /// NM_001042364 /// NM_001042365 /// NM_001042387 /// NM_001042389 /// NM_001042390 /// NM_001042391 /// NM_001042392 /// NM_001042393 /// NM_001042394 /// NM_001042395 /// NM_001042396 /// NM_001042397 /// NM_015605	NP_001035816 /// NP_001035817 /// NP_001035818 /// NP_001035819 /// NP_001035820 /// NP_001035821 /// NP_001035822 /// NP_001035823 /// NP_001035824 /// NP_001035846 /// NP_001035848 /// NP_001035849 /// NP_001035850 /// *NP_001035851* /// NP_001035852 /// NP_001035853 /// NP_001035854 /// NP_001035855 /// NP_001035856 /// NP_056420
218656_s_at	1.51991	LHFP	lipoma HMGIC fusion partner	NM_005780.1	10186	Hs.507798	NM_005780	NP_005771
220178_at	-1.22676	C19orf28	chromosome 19 open reading frame 28	NM_021731.1	126321	Hs.656901	NM_001042680 /// NM_021731 /// NM_174983	NP_1036145 /// NP_068377 /// NP_778148
220545_s_ at	-1.41349	TSKS	testis-specific serine kinase substrate	NM_021733.1	60385	Hs.515858	NM_021733	NP_068379
222915_s_ at	1.52098	BANK1	B-cell scaffold protein with ankyrin repeats 1	AA811540	55024	Hs.480400	NM_001083907 /// N_001127507 /// NM_017935	NP_001077376 /// NP_001120979 /// NP_060405

(continued)

Table 13A. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
223210_at	-1.30772	CHURC1	churchill domain containing 1	AF060510.1	91612	Hs.325531	NM_145165	NP_660148
226071_at	-1.30444	ADAMTSL4	ADAMTS-like 4	AF217974.1	54507	Hs.516243	NM_019032 /// NM_025008	NP_61905 /// NP_079284
226878_at	1.47827	HLA-DOA	major histocompatibility complex, class II, DO alpha	AL581873	3111	Hs.631991	NM_002119	NP_002110
229487_art	1.81657	EBF1	early B-cell factor 1	W73890	1879	Hs.573143	NM_024007	NP_076870
229559_at	-1.29088	FLJ40125	protein phosphatase 1B-like	BE732320	147699	Hs.532872	NM_001080401	NP_001073870
230022_at	-1.5369	CLEC18A	C-type lectin domain family 18, member A	BF057185	348174	Hs.592064	N_001136214 /// NM_182619	NP_001129686 /// NP_872425
230676_s_at	1.23715	TMEM19	transmembrane protein 19	AW663887	55266	Hs.688627	NM_018279	NP_060749
231411_at	1.51995	LHFP	Lipoma HMGIC fusion partner	BE674089	10186	Hs.507798	NM_005780	NP_005771
233016_at	-1.2455	---	---	AK022893.1	---	Hs.288478	---	---
233621_s_at	-1.72591	ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	AL137456.1	23365	Hs.24598	NM_015313	NP_056128
233742_at	-1.30295	C16orf68	Chromosome 16 open reading frame 68	AK000114.1	79091	Hs.306380	NM_024109	NP_077014
235982_at	1.53186	FCRL1	Fc receptor-like 1	AA677057	115350	Hs.656112	*NM_001159397 ///* NM_052938	NP_001152869 /// NP_001152870 /// NP_443170
236592_at	-1.27563	-	-	AI791859	-	Hs.658362	-	-

(continued)

Table 13A. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
238218_at	1.3701	OOEP	oocyte expressed protein homolog (dog)	AW206656	441161	Hs.671212	NM_001080507	NP_001073976
239591_at	3.94309	LRRC37A3	leucine rich repeat containing 37, member A3	BF433269	374819	Hs.551962	NM_199340	NP_955372
239724_at	-1.32678	-	-	AI653368	-	Hs.658979	-	---
242939_at	-1.37839	TFDP1	transcription factor Dp-1	AI950069	7027	Hs.79353	*NM_007111* /// NR_026580	NP_009042
243185_at	1.89601	-	-	AA804267	---	Hs.438315	-	-
243325_at	-1.21991	GSTK1	Glutathione S-transferase kappa 1	AV722006	373156	Hs.390667	N_001143679 /// N_001143680 /// *NM_001143681* /// NM_015917	NP_001137151 /// NP_001137152 /// NP_001137153 /// NP_057001
243467_art	1.37052	-	-	AW406163	-	Hs.435736	---	-
244181_at	-1.81372	-	-	AA018968	-	-	-	-

Table 13B. Additional genes that differentiate cardioembolic stroke from large vessel stroke ($p < 0.005$, fold change >|1.2|).

Table 13B. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
203585-at	1.31961	ZNF185	zinc finger protein 185 domain)	NM_007150.1	7739	Hs.16622	NM_007150	NP-009081
210448_s_ at	1.51809	P2RX5	purinergic receptor P2X, ligand-gated ion channel, 5	U49396.1	5026	Hs.408615	NM_002561 /// NM_175080 /// NM_175081	NP_002552 /// NP_78255 /// NP_78256
221211_s_at		C21orf7	chromosome 21 open reading frame 7		56911			
226085_at		CBX5	chromobox homolog 5 (HP1 alpha homolog, Drosophila)		23468			
207979_s_ at		CD8B	CD8b molecule		926			
201280_s_ at		DAB2	disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)		1601			
219863_at		HERC5	hect domain and RLD 5		51191			
205821_at		KLRK1	killer cell lectin-like receptor subfamily K, member 1		22914			
1558882_at		LOC401233	similar to HIV TAT specific factor 1; cofactor required for Tat activation of HI		401233			
236930_at		NUMB	(clone S171)		8650			
215175-at		PCNX	pecanex homolog (Drosophila)		22990			
214146_s_at		PPBP	pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)		5473			
204507_s_at		PPP3R1 /// WDR92	protein phosphatase 3 (formerly 2B), regulatory subunit B, alpha isoform /// WD		5534			

(continued)

Table 13B. Biomarkers that differentiate cardioembolic stroke from large vessel stroke								
Probe Set ID	**Fold-Change (Large Vessel vs. Cardioembolic)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
232078_at		PVRL2	poliovirus receptor-related 2 (herpesvirus entry mediator B)		5819			
232079_s_ at		PVRL2	poliovirus receptor-related 2 (herpesvirus entry mediator B)		5819			
225418_at		PVRL2	poliovirus receptor-related 2 (herpesvirus entry mediator B)		5819			
228996_at		RC3H1	ring finger and CCCH-type zinc finger domains 1		149041			
202131_s_ at		RIOK3	RIO kinase 3 (yeast)		8780			
212589_at		RRAS2	related RAS viral (r-ras) oncogene homolog 2		22800			
206108_s_ at		SFRS6	splicing factor, arginine/serine-rich 6		6431			
239084_at		SNAP29	synaptosomal-associated protein, 29kDa		9342			
217104_at		ST20	suppressor of tumorigenicity 20		400410			
206366_x_at		XCL1	chemokine (C motif) ligand 1		6375			
214567_s_at		XCL1 /// XCL2	chemokine (C motif) ligand 1 /// chemokine (C motif) ligand 2		6375 /// 6846			

Table 14. Genes that differentiate carotid stenosis from atrial fibrillation ($p < 0.005$, fold change >|1.2|).

Table 14. Biomarkers that differentiate carotid stenosis from atrial fibrillation								
Probe Set ID	**Fold-Change (Carotid vs. Afib)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
1553994_at	1.42712	NT5E	5'-nucleotidase, ecto (CD73)	BC015940.1	4907	Hs.153952	NM_002526	NP_002517
1555469_a_at	1.20537	CLASP2	cytoplasmic linker associated protein 2	BC029035.1	23122	Hs.108614	NM_015097	NP_055912
1556578_a_at	-1.20603	FLJ31945	hypothetical protein LOC440137	AI911996	440137	Hs.183953	XM_001714983 /// XM_001716811 XM_001718431	XP_001715035 /// XP_001716863 /// XP_001718483
1556896_ at	-2.01983	LOC284751	hypothetical LOC284751	AK090605.1	284751	Hs.282325	NM_001025463	NP_001020634
1556999_ at	-1.29018	LOC100271832	hypothetical LOC100271832	BC035107.1	100271832	-	NR_027097	-
1557542_ at	-1.36504	-	-	AW069144	-	Hs.353829	---	-
1563614_ at	-1.34743	MTBP	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse) binding protein, 104kDa	AL832671.1	27085	Hs.657656	NM_022045	NP_071328
1565389_s at	1.64476	GRM5	glutamate receptor, metabotropic 5	S64316.1	2915	Hs.147361	*NM_000842* /// NM_001143831	NP_000833 /// NP_001137303
1565862_a_at	-1.28258	-	-	H65800	-	Hs.658642	-	---
203650_at	1.4381	PROCR	protein C receptor, endothelial (EPCR)	NM_006404.1	10544	Hs.647450	NM_006404	NP_006395
203939_at	1.73974	NT5E	5'-nucleotidase, ecto (CD73)	NM_002526.1	4907	Hs.153952	NM_002526	NP_002517

(continued)

Table 14. Biomarkers that differentiate carotid stenosis from atrial fibrillation								
204765_at	1.27552	ARHGEF5	Rho guanine nucleotide exchange factor (GEF) 5	NM_005435.1	7984	Hs.334	NM_005435	NP_005426
207194_s_at	-1.77347	ICAM4	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	NM_001544.2	3386	Hs.706750	NM_001039132 III NM_001544 /// NM_022377	NP_001034221 /// NP_001535 /// NP_071772
208443_x_at	-1.20918	SHOX2	short stature homeobox 2	NM_006884.1	6474	Hs.55967	NM_001163678 /// NM_003030 /// NM_006884	NP_001157150 /// NP_003021 /// NP_006875
209160_at	1.59131	AKR1C3	aldo-keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	AB018580.1	8644	Hs.78183	NM_003739	NP_003730
211343_s_at	2.22751	COL13A1	collagen, type XIII, alpha 1	M33653.1	1305	Hs.695934	NM_001130103 /// NM_005203 /// NM_080798 /// NM_080799 /// NM_080800 /// NM_080801 /// NM_080802 /// NM_080803 /// NM_080804 ///	NP_001123575 /// NP_005194 /// NP_542988 /// NP_542989 /// NP_542990 /// NP_542991 /// NP_542992 /// h in Tables 7A and and 7B selected

(continued)

Table 14. Biomarkers that differentiate carotid stenosis from atrial fibrillation								
							NM_080805 /// NM_080806 /// NM_080807 /// NM_080808 /// NM_080809 /// NM_080810 /// NM_080811 /// NM_080812 /// NM_080813 /// NM_080814 /// NM_080815	NP_542995 /// NP_542996 /// NP_542997 /// NP_542998 /// NP_542999 /// NP_543000 /// NP_543001 /// NP_543002 /// NP_543003 /// NP_543004 /// NP_543005
215219è_at	-1.46668	DOPEY2	dopey family member 2	AK025095.1	9980	Hs.204575	NM_005128	NP_005119
218656_s_at	1.57558	LHFP	lipoma HMGIC fusion partner	NM_005780.1	10186	Hs.507798	NM_005780	NP_005771
224394_at	1.20322	RNF7	ring finger protein 7	AF312226.1	9616	Hs.134623	NM_014245 /// NM_183237	NP_055060 /// NP_899060
225147_at	1.29667	CYTH3	cytohesin 3	AL521959	9265	Hs.487479	NM_004227	NP_004218
227522_at	-3.08723	CMBL	carboxymethylenebutenolidase homolog (Pseudomonas)	AA209487	134147	Hs.192586	NM_138809	NP_620164
228779_at	-1.24601	LOC146880	hypothetical LOC146880	AA524743	146880	Hs.117853	NR_026899 /// NR_027487	---
228818_at	1.4946	---	---	BF110792	---	Hs.661673	---	---
229487_art	1.88689	EBF1	early B-cell factor 1	W73890	1879	Hs.573143	NM_024007	NP_076870
230494_at	-1.20819	SLC20A1	Solute carrier family transporter), member 1	AI671885	6574	Hs.187946	NM_005415	NP_005406
230710_at	-1.20654	---	---	W05495	---	Hs.446388	---	---

(continued)

Table 14. Biomarkers that differentiate carotid stenosis from atrial fibrillation								
231021_at	-1.20317	SLC6A19	solute carrier family (neutral amino acid transporter), member 19	AI627358	340024	Hs.481478	NM_001003841	NP_001003841
231411_at	1.55003	LHFP	Lipoma HMGIC fusion partner	BE674089	10186	Hs.507798	NM_005780	NP_005771
232329_at	1.40571	RANBP10	RAN binding protein 10	AV717059	57610	Hs.368569	NM_020850	NP_065901
233621_s_at	-2.0246	ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12	AL137456.1	23365	Hs.24598	NM_015313	NP_056128
233742_at	-1.29936	C16orf68	Chromosome 16 open reading frame 68	AK000114.1	79091	Hs.306380	NM_024109	NP_077014
235874_at	1.2001	PRSS35	protease, serine, 35	AL574912	167681	Hs.98381	NM_153362	NP_699193
236548_at	-1.25287	GIPC2	GIPC PDZ domain containing family, member 2	AL044570	54810	Hs.659356	NM_017655	NP_060125
236963_at	1.70511	---	---	AV700946	---	Hs.432337	---	---
238360_s_at	-1.28837	---	---	AI885665	---	Hs.634043	---	---
238557_at	-1.25224	LOC100144603	hypothetical transcript	R58282	100144603	Hs.657275	NR_021492	---
238827_art	-1.36024	---	---	BE843544	---	Hs.666833	---	---
239977_at	1.26675	C12orf42	chromosome 12 open reading frame 42	AI638494	374470	Hs.534649	NM_001099336 /// NM_198521	NP_001092806 /// NP_940923
242462_at	1.23474	LOC100127980	hypothetical protein LOC100127980	BE218570	100127980	Hs.595153	XM_001720119 /// XM_001722650	XP_001720171 /// XP_001722702

Table 15. A list of 40 genes that differentiate atrial fibrillation from non-atrial fibrillation ($p < 0.005$, fold change >|1.2|).

Table 15. Biomarkers that differentiate atrial fibrillation from non-atrial fibrillation								
Probe Set ID	**Fold-Change (Afib vs. NonAfib)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
1553730_x_at	-1.20376	LRRC43	leucine rich repeat containing 43	NM_152759.1	254050	Hs.374856	NM_001098519 /// NM_152759	NP_001091989 /// NP_689972
1555677_s_at	1.21973	SMC1A	structural maintenance of chromosomes 1A	BC046147.1	8243	Hs.211602	NM_006306	NP_006297
1558540_s_at	-1.25589	MIF /// SLC2A11	macrophage migration inhibitory factor (glycosylation-inhibiting factor) /// solute carrier family 2 (facilitated glucose transporter), member 11	AK055523.1	4282 /// 66035	Hs.407995	NM_001024938 /// NM_001024939 /// NM_002415 /// NM_030807	NP_001020109 /// NP_001020110 /// NP_002406 /// NP_110434
1560550_at	-1.2744	---	---	BC037972.1	---	Hs.589927	---	---
1561741_at	-1.29835	---	---	BC042016.1	---	Hs.639369	---	---
1562254_at	1.45766	---	---	AK024394.1	---	---	---	---
1566402_at	1.79094	SNORA68	small nucleolar RNA, H/ACA box 68	Y11162.1	26780	Hs.684118	NR_000012	---
1569609_at	-1.55035	---	---	BC028185.1	---	Hs.621293	---	---
1569701_at	-1.32035	PER3	Period homolog 3 (Drosophila)	BC036937.1	8863	Hs.162200	NM_016831	NP_058515

(continued)

Table 15. Biomarkers that differentiate atrial fibrillation from non-atrial fibrillation								
Probe Set ID	**Fold-Change (Afib vs. NonAfib)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
202046_s_at	1.28563	GRLF1	glucocorticoid receptor DNA binding factor 1	NM_004491.1	2909	Hs.509447	NM_004491	NP_004482
202071_at	1.43001	SDC4	syndecan 4	NM_002999.1	6385	Hs.632267	NM_002999	NP_002990
202494_at	-1.21636	PPIE	peptidylprolyl isomerase E (cyclophilin E)	NM_006112.1	10450	Hs.524690	NM_006112 /// NM_203456 /// NM_203457	NP_006103 /// NP_982281 /// NP_982282
211343_s_at	-1.90743	COL13A1	collagen, type XIII, alpha 1	M33653.1	1305	Hs.695934	NM_001130103 /// NM_005203 /// NM_080798 /// NM_080799 /// NM_080800 /// NM_080801 /// NM_080802 /// NM_080803 /// NM_080804 /// NM_080805 /// NM_080806 /// NM_080807 /// NM_080808 /// NM_080809 /// NM_080810 /// *NM_080811* /// *NM_080812* /// *NM_080813* /// *NM_080814* /// NM_080815	NP_001123575 /// NP_005194 /// NP_542988 /// NP_542989 /// NP_542990 /// NP_542991 /// NP_542992 /// NP_542993 /// NP_542994 /// NP_542995 /// NP_542996 /// NP_542997 /// NP_542998 /// NP_542999 /// NP_543000 /// *NP_543001* /// NP_543002 /// NP_543003 /// NP_543004 /// NP_543005
213747_at	1.25775	---	---	AA047234	---	---	---	---
214964_at	1.26326	---	---	AA554430	---	Hs.661763	---	---

(continued)

Table 15. Biomarkers that differentiate atrial fibrillation from non-atrial fibrillation								
Probe Set ID	**Fold-Change (Afib vs. NonAfib)**	**Gene Symbol**	**Gene Title**	**GenBank ID**	**Entrez Gene ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
224336_s_at	-1.24201	DUSP16	dual specificity phosphatase 16	AB052156.1	80824	Hs.536535	NM_030640	NP_085143
225097_at	1.25832	HIPK2	homeodomain interacting protein kinase 2	BF594155	28996	Hs.397465	NM_001113239 /// NM_022740 /// XM_001716827 /// XM_925800	NP_001106710 /// *NP_073577* /// XP_001716879 /// XP_930893
225214_at	1.20457	LOC100129034	hypothetical protein LOC100129034	AI762915	100129034	Hs.654980	NR_027406 /// XR_079577	-
227775_at	-1.45039	BRUNOL6	bruno-like 6, RNA binding protein (Drosophila)	BE467313	60677	Hs.348342	NM_052840	NP_443072
227846_at	-1.23421	GPR176	G protein-coupled receptor 176	AA526584	11245	Hs.37196	NM_007223	NP 009154
229074_at	1.25524	---	---	AI692267	---	Hs.598990	---	---
229189_s_at	-1.59286	---	---	BF672306	---	Hs.438950	---	---
229190_at	-1.67118	---	---	BF672306	---	Hs.438950	---	---
230506-at	-1.494	C6orf164	chromosome 6 open reading frame 164	NM_022084.1	63914	Hs.645177	NR_026784	---

(continued)

Table 15. Biomarkers that differentiate atrial fibrillation from non-atrial fibrillation

Probe Set ID	Fold-Change (Afib vs. NonAfib)	Gene Symbol	Gene Title	GenBank ID	Entrez Gene ID	UniGene ID	RefSeq Transcript ID	RefSeq Protein ID
231219-at	1.42855	CMTM1	CKLF-like MARVEL transmembrane domain containing 1	AI825627	113540	Hs.15159	NM_052999 /// NM_181268 /// NM_181269 /// NM_181270 /// NM_181271 /// NM_181272 /// NM_181283 /// NMJ181296	*NP_443725* /// NP_851785 /// NP_851786 /// NP_851787 /// NP_851788 /// NP_851789 /// NP_851800 /// NP_851813
234142_at	-1.20672	---	---	AK025053.1	---	Hs.612895	---	---
235480_at	-1.36329	MAP3K71P1	Mitogen-activated protein kinase kinase kinase 7 interactingprotein 1	AA063633	10454	Hs.507681	NM_006116 /// NM_153497	NP_006107 /// NP J05717
235843_at	1.46959	---	---	BF448158	---	Hs.710512	---	---
236963_at	-2.00452	---	---	AV700946	---	Hs.432337	---	---
237075_at	1.95707	---	---	AI191591	---	---	---	---
237816_at	1.38498	---	---	AA702582	---	Hs.687470	---	---
239069_s_at	-1.39094	---	---	BF691045	---	Hs.649155	---	---
239718_at	-1.31179	---	---	R42552	---	Hs.718467	---	---
240369_at	1.35893	TTC7A	Tetratricopeptide repeat domain 7A	AW195569	57217	Hs.370603	NM_020458	NP_065191
241797_at	1.21912	---	---	AI904095	---	Hs.687709	---	---
243603_at	1.21948	---	---	AI973041	---	Hs.672035	---	---
244646_at	-1.2136	---	---	AW972881	---	Hs.663316	---	---

Table 16. The 38 endogenous reference biomarkers stably expressed in blood for use in normalization and as control levels.

Table 16. Stably expressed endogenous reference biomarkers						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
201499_s_at	USP7	ubiquitin specific peptidase 7 (herpes virus-associated)	NM_003470.1	Hs.706830	NM_003470	NP_003461
202501_at	MAPRE2	microtubule-associated protein, RP/EB family, member 2	NM_014268.1	Hs.532824	NM_001143826 /// NM_001143827 /// NM_014268 /// NR_026570	NP_001137298 /// NP_001137299 /// NP_055083
202573_at	CSNK1G2	casein kinase 1, gamma 2	AL530441	Hs.651905	NM_001319	NP_001310
203280_at	SAFB2	scaffold attachment factor B2	NM_014649.1	Hs.655392	NM_014649	NP_055464
204842_x_at	PRKAR2A	protein kinase, cAMP-dependent, regulatory, type II, alpha	BC002763.1	Hs.631923	NM_004157	NP_004148
206138_s_at	PI4KB	phosphatidylinositol 4-kinase, catalytic, beta	NM_002651.1	Hs.632465	NM_002651	NP_002642
207159_x_at	CRTC1	CREB regulated transcription coactivator 1	NM_025021.1	Hs.371096	NM_001098482 /// NM_015321	NP_001091952 /// NP_056136
208630_at	HADHA	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), alpha subunit	AI972144	Hs.516032	NM_000182	NP_000173
208786_s_at	MAP1LC3B	microtubule-associated protein 1 light chain 3 beta	AF183417.1	Hs.356061	NM_022818	NP_073729
209192_x_at	KAT5	K(lysine) acetyltransferase 5	BC000166.2	Hs.397010	NM_006388 /// NM_182709 /// NM_182710	NP_006379 /// NP_874368 /// NP_874369
210474_s_at	CDC2L1 /// CDC2L2	cell division cycle 2-like 1 (PITSLRE proteins) /// cell division cycle 2-like 2 (PITSLRE proteins)	U04819.1	Hs.651228	NM_024011 /// NM_033486 /// NM_033487 /// NM_033488 /// NM_033489 /// NM_033492 NM_033493 /// NM_033529	NP_076916 /// NP_277021 /// NP_277022 /// NP_277023 /// NP_277024 /// NP_277027 /// NP_277028 /// NP_277071
211040_x_at	GTSE1	G-2 and S-phase expressed	BC006325.1	Hs.386189	NM_016426	NP_057510

(continued)

Table 16. Stably expressed endogenous reference biomarkers						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
211289_x_at	CDC2L1 /// CDC2L2	cell division cycle 2-like 1 (PITSLRE proteins) /// cell division cycle 2-like 2 (PITSLRE proteins)	AF067524.1	Hs.651228	NM_024011 /// NM_033486 /// NM_033487 /// NM_033488/// NM_033489 /// NM_033492 NM_033493 /// NM_033529	NP_076916 /// NP_277021 /// NP_277022 /// NP_277023 /// NP_277024 /// NP_277027 /// NP_277028 /// NP_277071
213311_s_at	TCF25	transcription factor 25 (basic helix-loop-helix)	BF000251	Hs.415342	NM_014972	NP_055787
214665_s_at	CHP	calcium binding protein P22	AK000095.1	Hs.406234	NM_007236	NP_009167
215063_x_at	LRRC40	leucine rich repeat	AL390149.1	Hs.147836	NM_017768	NP_060238
		containing 40				
215200_x_at	---	---	AK022362.1	Hs.663419	---	---
215568_x_at	hCG_2003956 /// LYPLA2 /// LYPLA2P1	hCG2003956 /// lysophospholipase II /// lysophospholipase II pseudogene 1	AL031295	Hs.533479	NM_007260 /// NR_001444	NP_009191
216038_x_at	DAXX	death-domain associated protein	BE965715	Hs.336916	NM_001141969 /// NM_001141970 /// NM_001350 /// NR_024517	NP_001135441 /// NP_001135442 /// NP_001341
217393_x_at	UBE2NL	ubiquitin-conjugating enzyme E2N-like	AL109622	Hs.585177	NM_001012989	NP_001013007
217549_at	-	-	AW574933	Hs.527860	---	---
217672_x_at	EIF1	eukaryotic translation initiation factor 1	BF114906	Hs.150580	NM_005801	NP_005792
217938_s_at	KCMF1	potassium channel modulatory factor 1	NM_020122.1	Hs.654968	NM_020122	NP_064507
218378_s_at	PRKRIP1	PRKR interacting protein 1 (IL11 inducible)	NM_024653.1	Hs.406395	NM_024653	NP_078929
218571_s_at	CHMP4A	chromatin modifying protein 4A	NM_014169.1	Hs.279761	NM_014169	NP_054888

(continued)

Table 16. Stably expressed endogenous reference biomarkers						
Probe Set ID	**Gene Symbol**	**Gene Title**	**GenBank ID**	**UniGene ID**	**RefSeq Transcript ID**	**RefSeq Protein ID**
219074_at	TMEM184C	transmembrane protein 184C	NM_018241.1	Hs.203896	NM_018241	NP_060711
220052_s_at	TINF2	TERF1 (TRF1)-interacting nuclear factor 2	NM_012461.1	Hs.496191	NM_001099274 /// NM_012461	NP_001092744 /// NP_036593
220411_x_at	PODNL1	podocan-like 1	NM_024825.1	Hs.448497	NM_001146254 /// NM_024825	NP_001139726 /// NP_001139727 /// NP_079101
221813_at	FBX042	F-box protein 42	AI129395	Hs.522384	NM_018994	NP_061867
222207_x_at	LOC441258	Williams Beuren syndrome chromosome region 19 pseudogene	AK024602.1	Hs.711232	---	---
222733_x_at	RRP1	ribosomal RNA processing homolog (S.	BC000380.1	Hs.110757	NM_003683	NP_003674
224667_x_at	C10orf104	chromosome 10 open reading frame 104	AK023981.1	Hs.426296	NM_173473	NP_775744
224858_at	ZDHHC5	zinc finger, DHHC-type containing 5	AK023130.1	Hs.27239	NM_015457	NP_056272
225403_at	C9orf23	chromosome 9 open reading frame 23	AL528391	Hs.15961	NM_48178 /// NM_148179	NP_680544 III NP_680545
226253_at	LRRC45	leucine rich repeat containing 45	BE965418	Hs.143774	NM_144999	NP_659436
227651_at	NACC1	nucleus accumbens associated 1, BEN and BTB (POZ) domain containing	AI498126	Hs.531614	NM_052876	NP_443108
232190_x_at	LOC100133445 /// LOC115110	hypothetical LOC100133445 /// hypothetical protein LOC115110	AI393958	Hs.132272	NR_026927 /// XR_036887 /// XR_038144	---
49878_at	PEX16	peroxisomal biogenesis factor 16	AA523441	Hs.100915	NM_004813 /// NM_057174	NP_004804 /// NP_476515

Example 3: Exemplary Flow Outline of Using Gene Expression Analysis for the Diagnosis of the Occurrence of Ischemic Stroke and the Cause of Ischemic Stroke

[0224] The following example provides an exemplary outline of using the biomarkers described herein for the diagnosis of the occurrence and cause of stroke in a patient suspected of having a stroke.

[0225] (1) Detection of biomarkers can be performed using a microarray, e.g., a microfluidics approach. cDNA from the patient's RNA in a blood sample is prepared and labeled (e.g., with a fluorophore). The labled cDNA is hybridized to probes on the array within the microfluidics device. The fluoresence of the bound cDNA is measured to provide a quantitative measure of the amount of RNA for each gene expressed in the blood of the patient.

[0226] (2) The amount of RNA for at least about 15 target genes is first measured in the blood sample. The amount of RNA for at least about 30 endogenous reference biomarkers is measured in the blood sample. The amounts of RNA for each target gene is normalized to the reference genes (geometric average) and a normalized expression value obtained for each target gene. The expression of all of the target genes (15 or more) is then used as input into a predictive equation (support vector machine - for example) that then determines whether the gene expression profile for the subject is most similar to that for stroke or control, and whether the gene expression profile for the subjects is most similar to cardioembolic stroke, atheroembolic stroke, or neither.

[0227] (3) Based upon the results of the testing for the above biomarkers, a regime for the prevention and/or treatment of stroke is prescribed and/or administered to the patient.

(a) Patients with a positive diagnosis of stroke, based on the biomarkers of Table 7A can be subject to further confirmatory diagnostic testing, e.g., MRI imaging of brain and vessels, blood tests, EKG, echocardiogram, others.
(b) Patients with a negative diagnosis of stroke, based on the biomarkers of Table 7A can be sent home, or subject to diagnostic analysis and/or testing for a differenct condition.
(c) Cryptogenic stroke - if determined to be cardioembolic, e.g., based on the biomarkers of Tables 13A and 15, an anticoagulant may be prescribed or administered.
(d) Cryptogenic stroke - if determined to be atherosclerotic, e.g., based on the biomarkers of Table 14, the patient can be subject to vascular imaging to image carotid and other brain vessels; an anti-platelet agent may be prescribed or administered.
(e) If a diagnosis of cardioembolic stroke, e.g., based on the biomarkers of Tables 13A and 15, an anticoagulant may be prescribed or administered.
(f) If a diagnosis of large vessel atheroembolic stroke, e.g., based on the biomarkers of Table 14, the patient can be subject to vascular imaging to image carotid and other brain vessels. An anti-platelet agent may be prescribed or administered, e.g., if stenosis <50% or if intracranial or aortic atherosclerosis. Recommend or perform carotid surgery if stenosis >50%.

[0228] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the scope of the appended claims.

Claims

1. A method for diagnosing ischemic stroke, the method comprising: determining a level of expression of ischemic stroke-associated biomarkers PGM5, CCDC144C /// LOC100134159 and at least one additional biomarker taken from a group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 and VAPA, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 and SH3GL3, in a biological sample from a patient,
wherein the patient has at least one vascular risk factor and a decreased expression level of PGM5 and CCDC144C /// LOC100134159 together with an increased expression level of at least one ischemic stroke-associated biomarker taken from a sub-group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 and VAPA, and/or a decreased expression level of at least one ischemic stroke-associated biomarker taken from a sub-group consisting of LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR,

OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 and SH3GL3 compared to a control indicates that the patient suffers from ischemic stroke.

2. The method of claim 1, wherein the control is the expression level of a stably expressed endogenous reference biomarker.

3. The method of any one of claims 1 to 2, further comprising diagnosing the cause of ischemic stroke or a predisposition for developing ischemic stroke, the method comprising:

a) determining a level of expression of at least 15 ischemic stroke-associated biomarkers in a biological sample from a patient, wherein the biomarkers comprise a plurality of biomarkers selected from Table 13A, a plurality of biomarkers selected from Table 14 and a plurality of biomarkers selected from Table 15; and
b) comparing the level of expression of the ischemic stroke-associated biomarkers to the expression level of a plurality of stably expressed endogenous reference biomarkers,

wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 13A compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing cardioembolic stroke;
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 14 compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing carotid stenosis; and
wherein an increase or decrease of the expression level of the plurality of biomarkers selected from Table 15 compared to the expression level of the plurality of endogenous reference biomarkers indicates that the patient suffers from or is at risk of developing atrial fibrillation, thereby diagnosing the occurrence and cause of ischemic stroke or the predisposition for developing ischemic stroke.

4. The method of any one of claims 1 to 3, further comprising determining the level of expression of one or biomarkers listed in Table 7B.

5. The method of any one of claims 1 to 4, wherein the expression levels of the biomarkers are concurrently or sequentially determined.

6. The method of any one of claims 1 to 5, wherein the biological sample is blood, serum or plasma.

7. The method of any one of claims 1 to 6, wherein the plurality of stably expressed endogenous reference biomarkers is selected from Table 16.

8. The method of any one of claims 1 to 7, wherein

a) the determining step is performed at 3 or fewer hours after a suspected ischemic event; and/or
b) the determining step is performed within 6, 12, 18, 24, 36 or 48 hours of a suspected ischemic event.

9. The method of any one of claims 1 to 7, wherein

a) the determining step is performed at least 3 hours after a suspected ischemic event; and/or
b) the determining step is performed at least 24 hours after a suspected ischemic event.

10. The method of any one of claims 1 to 9, wherein the ischemic stroke is a member selected from the group consisting of: embolic stroke, thrombotic stroke, transient ischemic attack, cardioembolic stroke and atherothrombotic stroke.

11. The method of any one of claims 1 to 10, further comprising the step of determining the cause of ischemic stroke if a diagnosis of ischemic stroke or risk of ischemic stroke is determined.

12. The method of claim 11, further comprising the step of recommending a treatment or prevention regime appropriate to the determined cause of ischemic stroke.

13. The method of any one of claims 3 to 12, wherein:

a) an increased expression level of one or more ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A /// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP and LRRC37A3 indicates that the patient has experienced or is at risk for cardioembolic stroke;
b) a decreased expression level of one or more ischemic stroke-associated biomarkers of Table 13A selected from the group consisting of LOC284751, CD46, ENPP2, C19orf28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, C16orf68, TFDP1 and GSTK1 indicates that the patient has experienced or is at risk for cardioembolic stroke;
c) an increased expression level of one or more ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12orf42 and LOC100127980 indicates that the patient has experienced or is at risk for carotid stenosis;
d) a decreased expression level of one or more ischemic stroke-associated biomarkers of Table 14 selected from the group consisting of FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880, SLC20A1, SLC6A19, ARHGEF12, C16orf68, GIPC2 and LOC100144603 indicates that the patient has experienced or is at risk for carotid stenosis;
e) an increased expression level of one or more ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of SMC1A, SNORA68, GRLF1, SDC4, HIPK2, LOC100129034, CMTM1 and TTC7A indicates that the patient has experienced or is at risk for atrial fibrillation; and/or
f) a decreased expression level of one or more ischemic stroke-associated biomarkers of Table 15 selected from the group consisting of LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6orf164 and MAP3K7IP1 indicates that the patient has experienced or is at risk for atrial fibrillation.

14. The method of any one of claims 1 to 12, wherein:

a) the level of expression of the biomarker is determined at the transcriptional level;
b) the level of expression is determined by detecting hybridization of an ischemic stroke-associated gene probe to gene transcripts of the biomarkers in the biological sample; and/or
c) the level of expression is determined by amplification of gene transcripts of the biomarkers.

15. The method of any one of claims 2 to 14, wherein the level of expression of at least 15 biomarkers is determined.

16. The use of a solid support comprising nucleic acids that hybridize to ischemic stroke-associated biomarkers PGM5 and CCDC144C /// LOC100134159 and at least one additional biomarker taken from a group consisting of RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 VAPA, PGM5, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 and SH3GL3 for carrying out the method of any of the preceding claims.

17. The use of claim 16, further comprising a plurality of nucleic acids that hybridize to a plurality of the genes set forth in Table 13A, a plurality of nucleic acids that hybridize to a plurality of the genes set forth in Table 14 and a plurality of nucleic acids that hybridize to a plurality of the genes set forth in Table 15.

18. The use of any one of claims 16 to 17, further comprising a plurality of nucleic acids that hybridize to a plurality of the genes set forth in:

a) Tables 13A and 13B selected from the group consisting of EBF1, GRM5, TSKS, ENPP2, AP3S2, LRRC37A3, C16orf68, LOC284751, IRF6, LHFP, BANK1, ARHGEF5, TNF254, TFDP1, COL13A1, GSTK1, ADAMTSL4, P2RX5, LHFP, PIK3C2B, CHURC1, EXT2, HLA-DOA, OOEP, ZNF185, TMEM19, FCRL1, FLJ40125, ARHGEF12, CLEC18A, CD46, PTPN20A /// PTPN20B, and C19orf28;
b) Table 14 selected from the group consisting of EBF1, FLJ31945, C16orf68, SLC20A1, DOPEY2, COL13A1, LHFP, LOC284751, GRM5, LOC100144603, MTBP, SHOX2, ARHGEF5, RNF7, CLASP2, GIPC2, RANBP10,

CMBL, LOC100127980, CYTH3, PROCR, LOC146880, SLC6A19, ICAM4, C12orf42, ARHGEF12, PRSS35, NT5E, LOC100271832, LHFP, NT5E and AKR1C3;
c) Table 15 selected from the group consisting of CMTM1, COL13A1, SDC4, C6orf164, GPR176, BRUNOL6, SNORA68, MIF /// SLC2A11, DUSP16, HIPK2, TTC7A, PPIE, GRLF1, MAP3K7IP1, LOC100129034, PER3, SMC1A, and LRRC43; and/or
d) Table 16 selected from the group consisting of USP7, MAPRE2, CSNK1G2, SAFB2, PRKAR2A, PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF1, KCMF1, PRKRIP1, CHMP4A, TMEM184C, TINF2, PODNL1, FBXO42, LOC441258, RRP1, C10orf104, ZDHHC5, C9orf23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16.

19. The use of any one of claims 16 to 18, wherein the solid support is a microarray.

Patentansprüche

1. Verfahren zur Diagnose von ischämischem Schlaganfall, wobei das Verfahren Folgendes umfasst: Bestimmen eines Expressionsniveaus von mit ischämischem Schlaganfall assoziierten Biomarkern PGM5, CCDC144C /// LOC100134159 und mindestens eines zusätzlichen Biomarkers stammend aus einer Gruppe bestehend aus RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, OKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 und VAPA, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 und SH3GL3, in einer biologischen Probe von einem Patienten,
worin der Patient mindestens einen vaskulären Risikofaktor und ein vermindertes Expressionsniveau von PGM5 und CCDC144C /// LOC100134159 zusammen mit einem erhöhten Expressionsniveau von mindestens einem mit ischämischem Schlaganfall assoziierten Biomarker stammend aus einer Untergruppe bestehend aus RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, OKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 und VAPA und/oder ein vermindertes Expressionsniveau von mindestens einem mit ischämischen Schlaganfall assoziierten Biomarker stammend aus einer Untergruppe bestehend aus LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 und SH3GL3 im Vergleich zu einer Kontrolle aufweist, was darauf hindeutet, dass der Patient an ischämischem Schlaganfall leidet.

2. Verfahren nach Anspruch 1, worin die Kontrolle das Expressionsniveau eines stabil exprimierten endogenen Referenz-Biomarkers ist.

3. Verfahren nach irgendeinem Anspruch 1 bis 2, ferner umfassend die Diagnose der Ursache von ischämischem Schlaganfall oder einer Prädisposition für die Entwicklung von ischämischem Schlaganfall, wobei das Verfahren Folgendes umfasst:

a) Bestimmen eines Expressionsniveaus von mindestens 15 mit ischämischem Schlaganfall assoziierten Biomarkern in einer biologischen Probe von einem Patienten, worin die Biomarker eine Mehrzahl von aus Tabelle 13A ausgewählten Biomarkern, eine Mehrzahl von aus Tabelle 14 ausgewählten Biomarkern und eine Mehrzahl von aus Tabelle 15 ausgewählten Biomarkern umfassen; und
b) Vergleichen des Expressionsniveaus der mit ischämischem Schlaganfall assoziierten Biomarker mit dem Expressionsniveau einer Mehrzahl von stabil exprimierten endogenen Referenz-Biomarkern,

worin eine Zunahme oder Abnahme des Expressionsniveaus der Mehrzahl von aus Tabelle 13A ausgewählten Biomarkern im Vergleich zum Expressionsniveau der Mehrzahl von endogenen Referenz-Biomarkern darauf hindeutet, dass der Patient an kardioembolischem Schlaganfall leidet oder bei ihm das Risiko seines Auftretens besteht; worin eine Zunahme oder Abnahme des Expressionsniveaus der Mehrzahl von aus Tabelle 14 ausgewählten Biomarkern im Vergleich zum Expressionsniveau der Mehrzahl von endogenen Referenz-Biomarkern darauf hindeutet,

dass der Patient an Carotisstenose leidet oder bei ihm das Risiko ihres Auftretens besteht; und
worin eine Zunahme oder Abnahme des Expressionsniveaus der Mehrzahl von aus Tabelle 15 ausgewählten Biomarkern im Vergleich zum Expressionsniveau der Mehrzahl von endogenen Referenz-Biomarkern darauf hindeutet, dass der Patient an Vorhofflimmern leidet oder bei ihm das Risiko seines Auftretens besteht, wodurch das Auftreten und die Ursache von ischämischem Schlaganfall oder die Prädisposition für die Entwicklung von ischämischem Schlaganfall diagnostiziert werden oder wird.

4. Verfahren nach irgendeinem Anspruch 1 bis 3, ferner umfassend das Bestimmen des Expressionsniveaus eines oder mehrerer der in Tabelle 7B aufgeführten Biomarker.

5. Verfahren nach irgendeinem Anspruch 1 bis 4, worin die Expressionsniveaus der Biomarker gleichzeitig oder sequenziell bestimmt werden.

6. Verfahren nach irgendeinem Anspruch 1 bis 5, worin die biologische Probe Blut, Serum oder Plasma ist.

7. Verfahren nach irgendeinem Anspruch 1 bis 6, worin die Mehrzahl von stabil exprimierten endogenen Referenz-Biomarkern aus Tabelle 16 ausgewählt wird.

8. Verfahren nach irgendeinem Anspruch 1 bis 7, worin

a) der Bestimmungsschritt 3 oder weniger Stunden nach einem vermuteten ischämischen Ereignis durchgeführt wird; und/oder
b) der Bestimmungsschritt innerhalb von 6, 12, 18, 24, 36 oder 48 Stunden nach einem vermuteten ischämischen Ereignis durchgeführt wird.

9. Verfahren nach irgendeinem Anspruch 1 bis 7, worin

a) der Bestimmungsschritt mindestens 3 Stunden nach einem vermuteten ischämischen Ereignis durchgeführt wird;
b) der Bestimmungsschritt mindestens 24 Stunden nach einem vermuteten ischämischen Ereignis durchgeführt wird.

10. Verfahren nach irgendeinem Anspruch 1 bis 9, worin der ischämische Schlaganfall zu der aus embolischem Schlaganfall, thrombotischem Schlaganfall, transientem ischämischem Anfall, kardioembolischem Schlaganfall und atherothrombotischem Schlaganfall bestehenden Gruppe gehört und aus ihr ausgewählt wird.

11. Verfahren nach irgendeinem Anspruch 1 bis 10, ferner umfassend den Schritt des Bestimmens der Ursache von ischämischem Schlaganfall, wenn ischämischer Schlaganfall oder Gefahr von ischämischem Schlaganfall als Diagnose gestellt wird.

12. Verfahren nach Anspruch 11, ferner umfassend den Schritt des Empfehlens eines für die ermittelte Ursache von ischämischem Schlaganfall angemessenen Behandlungs- oder Vorbeugungsprogramms.

13. Verfahren nach irgendeinem Anspruch 3 bis 12, worin:

a) ein erhöhtes Expressionsniveau eines oder mehrerer mit ischämischem Schlaganfall assoziierter Biomarker von Tabelle 13A, die aus der aus IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A /// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP und LRRC37A3 bestehenden Gruppe ausgewählt werden, darauf hindeutet, dass der Patient einen kardioembolischen Schlaganfall erlitten hat oder bei ihm das Risiko seines Auftretens besteht;
b) ein vermindertes Expressionsniveau eines oder mehrerer mit ischämischem Schlaganfall assoziierter Biomarker von Tabelle 13A, die aus der aus LOC284751, CD46, ENPP2, C19orf28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, C16orf68, TFDP1 und GSTK1 bestehenden Gruppe ausgewählt werden, darauf hindeutet, dass der Patient einen kardioembolischen Schlaganfall erlitten hat oder bei ihm das Risiko seines Auftretens besteht;
c) ein erhöhtes Expressionsniveau eines oder mehrerer mit ischämischem Schlaganfall assoziierter Biomarker von Tabelle 14, die aus der aus NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12orf42 und LOC100127980 bestehenden Gruppe ausgewählt

werden, darauf hindeutet, dass der Patient eine Carotisstenose erlitten hat oder bei ihm das Risiko ihres Auftretens besteht;
d) ein vermindertes Expressionsniveau eines oder mehrerer mit ischämischem Schlaganfall assoziierter Biomarker von Tabelle 14, die aus der Gruppe aus FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880, SLC20A1, SLC6A19, ARHGEF12, C16orf68, GIPC2 und LOC100144603 bestehenden Gruppe ausgewählt werden, darauf hindeutet, dass der Patient eine Carotisstenose erlitten hat oder bei ihm das Risiko ihres Auftretens besteht;
e) ein erhöhtes Expressionsniveau eines oder mehrerer mit ischämischem Schlaganfall assoziierter Biomarker von Tabelle 15, die aus der aus SMC1A, SNORA68, GRLF1, SDC4, HIPK2, LOC100129034, CMTM1 und TTC7A bestehenden Gruppe ausgewählt werden, darauf hindeutet, dass der Patient ein Vorhofflimmern erlitten hat oder bei ihm das Risiko seines Auftretens besteht; und/oder
f) ein vermindertes Expressionsniveau eines oder mehrerer mit ischämischem Schlaganfall assoziierter Biomarker von Tabelle 15, die aus der Gruppe aus LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6orf164 und MAP3K7IP1 bestehenden Gruppe ausgewählt werden, darauf hindeutet, dass der Patient ein Vorhofflimmern erlitten hat oder bei ihm das Risiko seines Auftretens besteht

14. Verfahren nach irgendeinem Anspruch 1 bis 12, worin:

a) das Expressionsniveaus des Biomarkers auf der transkriptionalen Ebene bestimmt wird;
b) das Expressionsniveau durch Detektieren von Hybridisierung einer mit ischämischem Schlaganfall assoziierten Gensonde zu Gentranskripten der Biomarker in der biologischen Probe bestimmt wird; und/oder
c) das Expressionsniveau durch Amplifikation von Gentranskripten der Biomarker bestimmt wird.

15. Verfahren nach irgendeinem Anspruch 2 bis 14, worin das Expressionsniveau von mindestens 15 Biomarkern bestimmt wird.

16. Verwendung eines festen Trägers umfassend Nukleinsäuren, die zu mit ischämischem Schlaganfall assoziierten Biomarkern PGM5 und CCDC144C /// LOC100134159 und mindestens einem zusätzlichen Biomarker stammend aus einer Gruppe bestehend aus RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5, VAPA, PGM5, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 und SH3GL3, zum Durchführen des Verfahrens nach irgendeinem der vorhergehenden Ansprüche, hybridisieren.

17. Verwendung nach Anspruch 16, ferner umfassend eine Mehrzahl von Nukleinsäuren, die zu einer Mehrzahl der in Tabelle 13A dargelegten Gene hybridisieren, eine Mehrzahl von Nukleinsäuren, die zu einer Mehrzahl der in Tabelle 14 dargelegten Gene hybridisieren, und eine Mehrzahl von Nukleinsäuren, die zu einer Mehrzahl der in Tabelle 15 dargelegten Gene hybridisieren.

18. Verwendung nach irgendeinem Anspruch 16 bis 17, ferner umfassend eine Mehrzahl von Nukleinsäuren, die zu einer Mehrzahl der in Folgendem dargelegten Gene hybridisieren:

a) Tabelle 13A und 13B, ausgewählt aus der Gruppe bestehend aus EBF1, GRM5, TSKS, ENPP2, AP3S2, LRRC37A3, C16orf68, LOC284751, IRF6, LHFP, BANK1, ARHGEF5, ZNF254, TFDP1, COL13A1, GSTK1, ADAMTSL4, P2RX5, LHFP, PIK3C2B, CHURC1, EXT2, HLA-DOA, OOEP, ZNF185, TMEM19, FCRL1, FLJ40125, ARHGEF12, CLEC18A, CD46, PTPN20A /// PTPN20B und C19orf28;
b) Tabelle 14, ausgewählt aus der Gruppe bestehend aus EBF1, FLJ31945, C16orf68, SLC20A1, DOPEY2, COL13A1, LHFP, LOC284751, GRM5, LOC100144603, MTBP, SHOX2, ARHGEF5, RNF7, CLASP2, GIPC2, RANBP10, CMBL, LOC100127980, CYTH3, PROCR, LOC146880, SLC6A19, ICAM4, C12orf42, ARHGEF12, PRSS35, NT5E, LOC100271832, LHFP, NT5E und AKR1 C3;
c) Tabelle 15, ausgewählt aus der Gruppe bestehend aus CMTM1, COL13A, SDC4, C6orf164, GPR176, BRUNOL6, SNORA68, MIF /// SLC2A11, DUSP16, HIPK2, TTC7A, PPIE, GRLF1, MAP3K7IP1, LOC100129034, PER3, SMC1A und LRRC43; und/oder
d) Tabelle 16, ausgewählt aus der Gruppe bestehend aus USP7, MAPRE2, CSNK1 G2, SAFB2, PRKAR2A,

PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF1, KCMF1, PRKRIP1, CHMP4A, TMEM184C, TINF2, PODNL1, FBX042, LOC441258, RRP1, C10orf104, ZDHHC5, C9orf23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16.

19. Verwendung nach irgendeinem Anspruch 16 bis 18, worin der feste Träger ein Mikroarray ist.

Revendications

1. Procédé de diagnostic d'un accident ischémique, le procédé consistant à :

déterminer un niveau d'expression de biomarqueurs PGM5, CCDC144C /// LOC100134159 associés à un accident ischémique et d'au moins un biomarqueur supplémentaire choisi dans le groupe constitué de RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, K1AA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 et VAPA, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 et SH3GL3, dans un échantillon biologique provenant d'un patient,
dans lequel le fait que le patient présente au moins un facteur de risque vasculaire et un niveau d'expression diminué de PGM5 et CCDC144C /// LOC100134159 conjointement avec un niveau d'expression accru d'au moins un biomarqueur associé à un accident ischémique choisi dans un sous-groupe constitué de RNF141, CLEC4E, TIMP2, PIHTF1, GKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPD1, BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCH02, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 et VAPA, et/ou un niveau d'expression diminué d'au moins un biomarqueur associé à un accident ischémique choisi dans un sous-groupe constitué de LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 et SH3GL3 par rapport à un témoin indique que le patient souffre d'un accident ischémique.

2. Procédé selon la revendication 1, dans lequel le témoin est le niveau d'expression d'un biomarqueur endogène de référence exprimé de façon stable.

3. Procédé selon l'une quelconque des revendications 1 à 2, consistant en outre à diagnostiquer la cause d'un accident ischémique ou une prédisposition au développement d'un accident ischémique, le procédé consistant à :

a) déterminer un niveau d'expression d'au moins 15 biomarqueurs associés à un accident ischémique dans un échantillon biologique provenant d'un patient, les biomarqueurs consistant en une pluralité de biomarqueurs choisis dans le tableau 13A, une pluralité de biomarqueurs choisis dans le tableau 14 et une pluralité de biomarqueurs choisis dans le tableau 15 ; et
b) comparer le niveau d'expression des biomarqueurs associés à un accident ischémique au niveau d'expression d'une pluralité de biomarqueurs endogènes de référence exprimés de façon stable,

dans lequel une augmentation ou une diminution du niveau d'expression de la pluralité de biomarqueurs choisis dans le tableau 13A par comparaison avec le niveau d'expression de la pluralité de biomarqueurs endogènes de référence indique que le patient souffre d'un accident embolique d'origine cardiaque ou risque d'en développer un ;
dans lequel une augmentation ou une diminution du niveau d'expression de la pluralité de biomarqueurs choisis dans le tableau 14 par comparaison avec le niveau d'expression de la pluralité de biomarqueurs endogènes de référence indique que le patient souffre d'une sténose carotidienne ou risque d'en développer une ; et
dans lequel une augmentation ou une diminution du niveau d'expression de la pluralité de biomarqueurs choisis dans le tableau 15 par comparaison avec le niveau d'expression de la pluralité de biomarqueurs endogènes de référence indique que le patient souffre d'une fibrillation auriculaire ou risque d'en développer une ; ce qui permet de diagnostiquer l'apparition et la cause d'un accident ischémique ou la prédisposition au développement d'un accident ischémique.

4. Procédé selon l'une quelconque des revendications 1 à 3, consistant en outre à déterminer le niveau d'expression de l'un des biomarqueurs énumérés dans le tableau 7B.

5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel les niveaux d'expression des biomarqueurs sont déterminés de façon simultanée ou consécutive.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel l'échantillon biologique est du sang, du sérum ou du plasma.

7. Procédé selon l'une quelconque des revendications 1 à 6, dans lequel la pluralité de biomarqueurs endogènes de référence exprimés de façon stable est choisie dans le tableau 16.

8. Procédé selon l'une quelconque des revendications 1 à 7, dans lequel :

a) l'étape de détermination est réalisée 3 heures maximum après un événement ischémique suspect ; et/ou
b) l'étape de détermination est réalisée 6, 12, 18, 24, 36 ou 48 heures maximum après un événement ischémique suspect.

9. Procédé selon l'une quelconque des revendications 1 à 7, dans lequel :

a) l'étape de détermination est réalisée au moins 3 heures après un événement ischémique suspect ;
b) l'étape de détermination est réalisée au moins 24 heures après un événement ischémique suspect.

10. Procédé selon l'une quelconque des revendications 1 à 9, dans lequel l'accident ischémique est un accident choisi dans le groupe constitué de : une embolie, un accident thrombotique, une attaque ischémique transitoire, accident embolique d'origine cardiaque et un accident athérothrombotique.

11. Procédé selon l'une quelconque des revendications 1 à 10, comprenant en outre l'étape consistant à déterminer la cause d'un accident ischémique si un diagnostic d'un accident ischémique ou si le risque d'un accident ischémique est déterminé.

12. Procédé selon la revendication 11, comprenant en outre l'étape consistant à recommander un traitement ou un régime de prévention approprié à la cause déterminée d'un accident ischémique.

13. Procédé selon l'une quelconque des revendications 3 à 12, dans lequel :

a) un niveau d'expression accru d'un ou plusieurs biomarqueurs associés à un accident ischémique du tableau 13A choisis dans le groupe constitué de IRF6, ZNF254, GRM5, EXT2, AP3S2, PIK3C2B, ARHGEF5, COL13A1, PTPN20A/// PTPN20B, LHFP, BANK1, HLA-DOA, EBF1, TMEM19, LHFP, FCRL1, OOEP et LRRC37A3 indique que le patient a souffert d'un accident embolique d'origine cardiaque ou risque d'en souffrir un ;
b) un niveau d'expression diminué d'un ou plusieurs biomarqueurs associés à un accident ischémique du tableau 13A choisis dans le groupe constitué de LOC284751, CD46, ENPP2, C19 ou f28, TSKS, CHURC1, ADAMTSL4, FLJ40125, CLEC18A, ARHGEF12, CT16 ou f68, TFDP1 et GSTK1 indique que le patient a souffert d'un accident embolique d'origine cardiaque ou risque d'en souffrir un ;
c) un niveau d'expression accru d'un ou plusieurs biomarqueurs associés à un accident ischémique du tableau 14 choisis dans le groupe constitué de NT5E, CLASP2, GRM5, PROCR, ARHGEF5, AKR1C3, COL13A1, LHFP, RNF7, CYTH3, EBF1, RANBP10, PRSS35, C12 ou f42 et LOC100127980 indique que le patient a souffert d'une sténose carotidienne ou risque d'en souffrir une ;
d) un niveau d'expression diminué d'un ou plusieurs biomarqueurs associés à un accident ischémique du tableau 14 choisis dans le groupe constitué de FLJ31945, LOC284751, LOC100271832, MTBP, ICAM4, SHOX2, DOPEY2, CMBL, LOC146880, SLC20A1, SLC6A19, ARHGEF12, C16 ou f68, GIPC2 et LOC100144603 indique que le patient a souffert d'une sténose carotidienne ou risque d'en souffrir une ;
e) un niveau d'expression accru d'un ou plusieurs biomarqueurs associés à un accident ischémique du tableau 15 choisis dans le groupe constitué de SMC1A, SNORA68, GRLF1, SDC4, HIPK2, LOC100129034, CMTM1 et TTC7A indique que le patient a souffert d'une fibrillation auriculaire ou risque d'en souffrir une ; et/ou
f) un niveau d'expression diminué d'un ou plusieurs biomarqueurs associés à un accident ischémique du tableau 15 choisis dans le groupe constitué de LRRC43, MIF /// SLC2A11, PER3, PPIE, COL13A1, DUSP16, LOC100129034, BRUNOL6, GPR176, C6 ou f164 et MAP3K7IP1 indique que le patient a souffert d'une fibrilla-

tion auriculaire ou risque d'en souffrir une.

14. Procédé selon l'une quelconque des revendications 1 à 12, dans lequel :

a) le niveau d'expression du biomarqueur est déterminé au niveau transcriptionnel ;
b) le niveau d'expression est déterminé en détectant l'hybridation d'une sonde génétique associée à un accident ischémique avec des transcrits de gènes des biomarqueurs dans l'échantillon biologique ; et/ou
c) le niveau d'expression est déterminé par des amplifications des transcrits de gènes des biomarqueurs.

15. Procédé selon l'une quelconque des revendications 2 à 14, dans lequel le niveau d'expression d'au moins 15 biomarqueurs est déterminé.

16. Utilisation d'un support solide comprenant des acides nucléiques qui s'hybrident avec des biomarqueurs associés à un accident ischémique PGM5 et CCDC144C /// LOC100134159 et au moins un biomarqueur supplémentaire choisi dans le groupe constitué de RNF141, CLEC4E, TIMP2, PHTF1, CKLF, RRAGD, CLEC4E, FGD4, CPEB2, LOC100290882, UBXN2B, ENTPDL BST1, LTB4R, F5, IFRD1, KIAA0319, CHMP1B, MCTP1, VNN3, AMN1, LAMP2, FCHO2, ZNF608, REM2, QKI, RBM25, FAR2, ST3GAL6, HNRNPH2, GAB1, UBR5 VAPA, PGM5, LECT2, SHOX, TBX5, SPTLC3, SNIP, RBMS3, P704P, THSD4, FAT3, SNRPN, GLYATL1, GADL1, CXADR, OVOL2, SPIB, BXDC5, UNC5B, ASTN2, FLJ35934, ANKRD28, CCDC144A, TIMM8A, ALDOAP2, LDB3, PTPRD, LOC729222 /// PPFIBP1, CCRL1, HNRNPUL2, FCRL4, ELAVL2, PRTG, DLX6, FOXA2, SCD5, GABRB2, GYPA, LOC283027, LOC344595, LOC100129488, RPL22 et SH3GL3, pour la réalisation du procédé selon l'une quelconque des revendications précédentes.

17. Utilisation selon la revendication 16, comprenant en outre une pluralité d'acides nucléiques qui s'hybrident avec une pluralité des gènes énumérés dans le tableau 13A, une pluralité d'acides nucléiques qui s'hybrident avec une pluralité des gènes énumérés dans le tableau 14, et une pluralité d'acides nucléiques qui s'hybrident avec une pluralité des gènes énumérés dans le tableau 15.

18. Utilisation selon l'une quelconque des revendications 16 à 17, comprenant en outre une pluralité d'acides nucléiques qui s'hybrident avec une pluralité des gènes énumérés dans :

a) les tableaux 13A et 13B et choisis dans le groupe constitué de EBF1, GRM5, TSKS, ENPP2, AP3S2, LRRC37A3, C16 ou f68, LOC284751, IRF6, LHFP, BANK1, ARHGEF5, ZNF254, TFDP1, COL13A1, GSTK1, ADAMTSL4, P2RX5, LHFP, PIK3C2B, CHURC1, EXT2, HLA-DOA, OOEP, ZNF185, TMEM19, FCRL1,FLJ40125, ARHGEF12, CLEC18A, CD46, PTPN20A /// PTPN20B et C19 ou f28 ;
b) le tableau 14 et choisis dans le groupe constitué de EBF1, FLJ31945, C16 ou f68, SLC20A1, DOPEY2, COL13A1, LHFP, LOC284751, GRM5, LOC100144603, MTBP, SHOX2, ARHGEF5, RNF7, CLASP2, GIPC2, RANBP10, CMBL, LOC100127980, CYTH3, PROCR, LOC146880, SLC6A19, ICAM4, C12 ou f42, ARHGEF12, PRSS35, NT5E, LOC100271832, LHFP, NT5E et AKR1C3 ;
c) le tableau 15 et choisis dans le groupe constitué de CMTM1, COL13A1, SDC4, C6 ou f164, GPR176, BRUNOL6, SNORA68, MIF /// SLC2A11, DUSP16, HIPK2, TTC7A, PPIE, GRLF1, MAP3K71P1, LOC100129034, PER3, SMC1A et LRRC43 ; et/ou
d) le tableau 16 et choisis dans le groupe constitué de USP7, MAPRE2, CSNK1G2, SAFB2, PRKAR2A, PI4KB, CRTC1, HADHA, MAP1LC3B, KAT5, CDC2L1 /// CDC2L2, GTSE1, TCF25, CHP, LRRC40, hCG_2003956 /// LYPLA2 /// LYPLA2P1, DAXX, UBE2NL, EIF1, KCMF1, PRKRIP1, CHMP4A, TMEM184C, TINF2, PODNL1, FBXO42, LOC441258, RRP1, C10 ou f104, ZDHHC5, C9 ou f23, LRRC45, NACC1, LOC100133445 /// LOC115110, PEX16.

19. Utilisation selon l'une quelconque des revendications 16 à 18, dans laquelle le support solide est un microréseau.

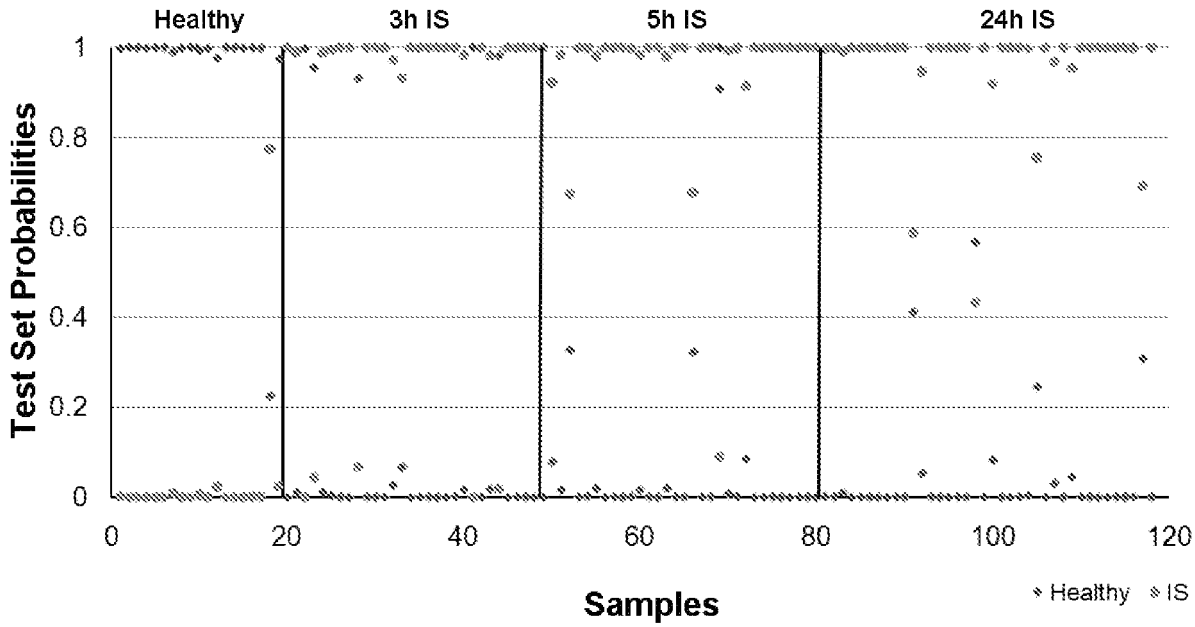

Healthy
3h IS
5h IS
24h IS
Test Set Probabilities
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
Samples
Healthy
IS

Figure 1

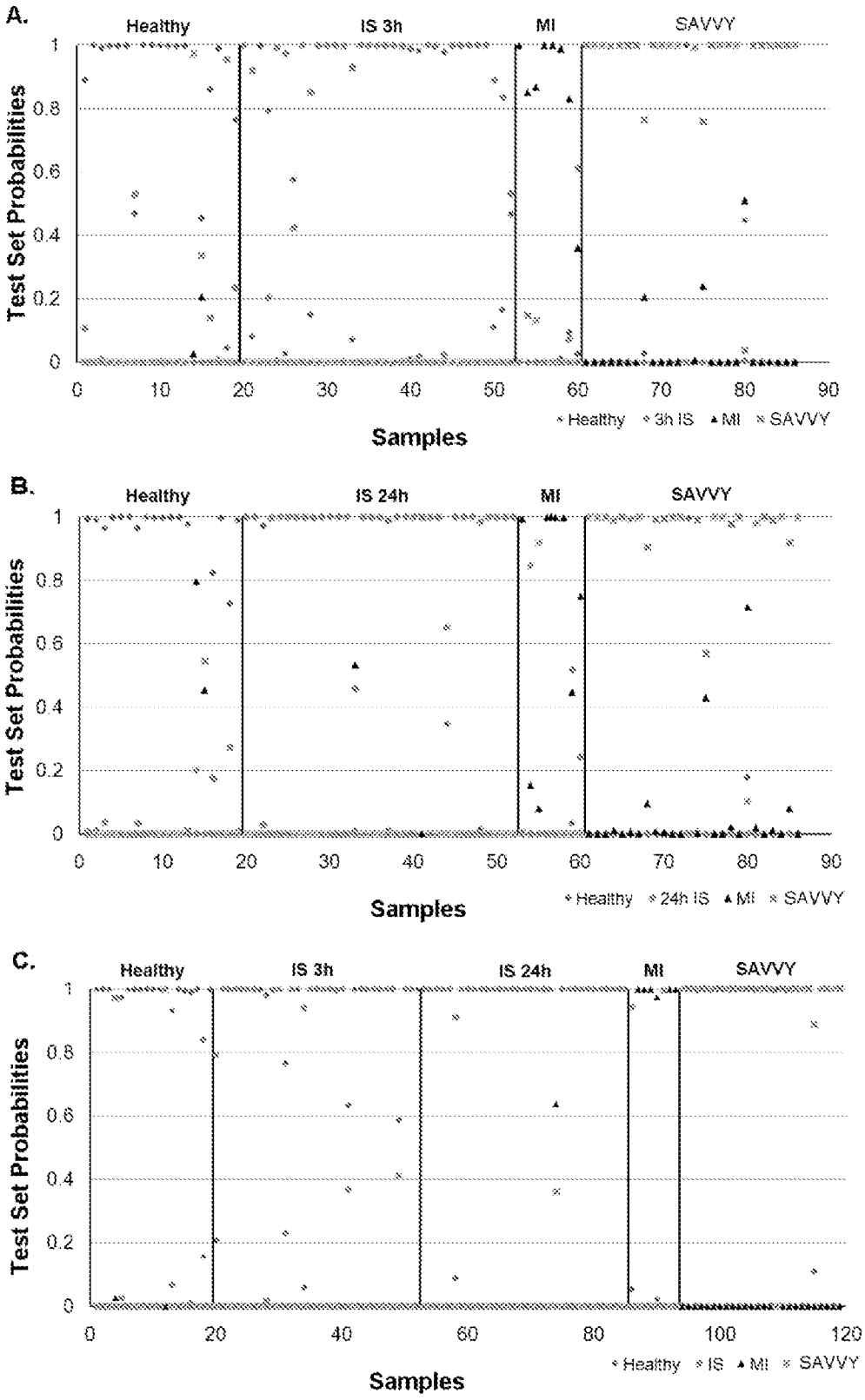

A.
Healthy
IS 3h
MI
SAVVY
Test Set Probabilities
Samples
Healthy 3h IS MI SAVVY
B.
Healthy
IS 24h
MI
SAVVY
Test Set Probabilities
Samples
Healthy 24h IS MI SAVVY
C.
Healthy
IS 3h
IS 24h
MI
SAVVY
Test Set Probabilities
Samples
Healthy IS MI SAVVY

Figure 2

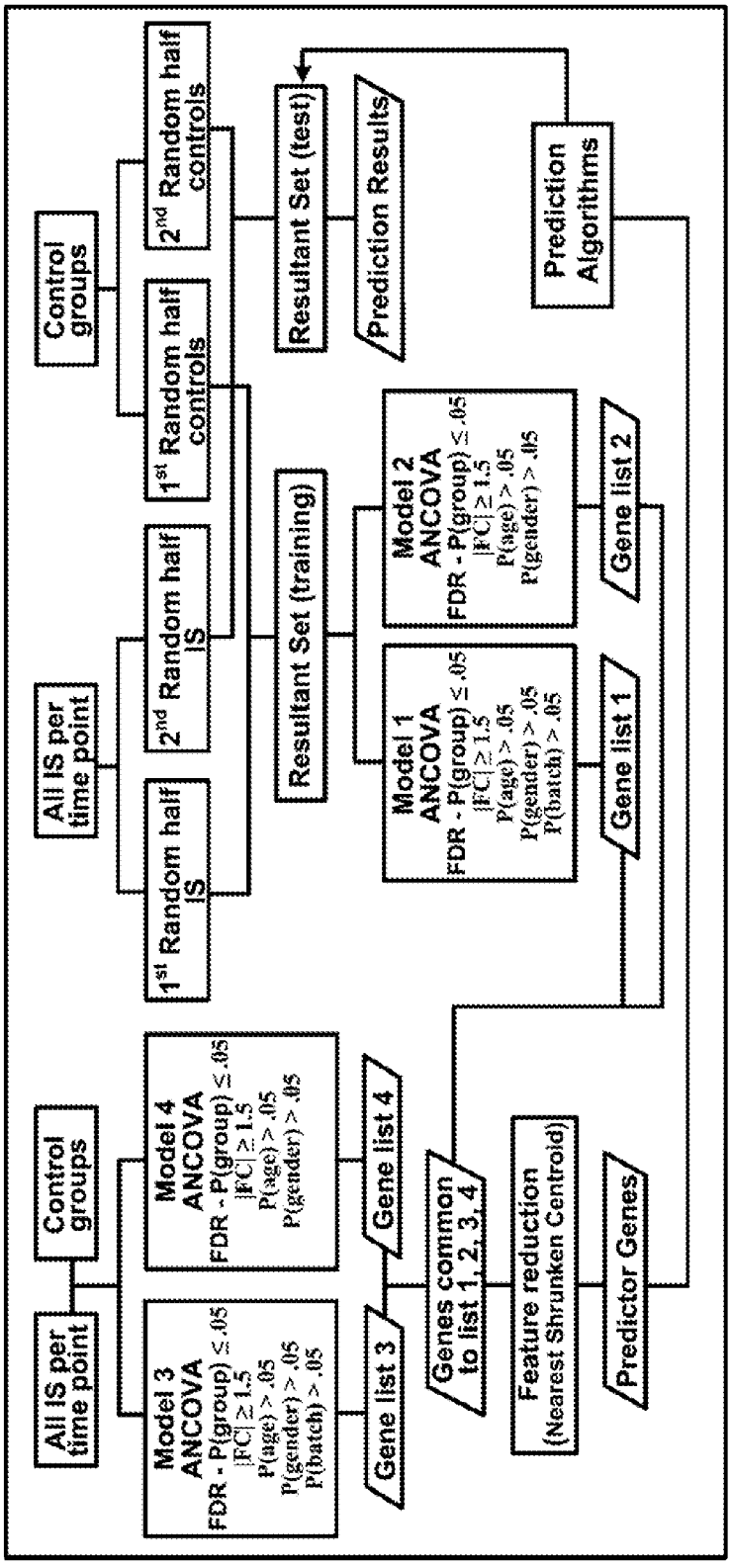

All IS per time point
Control groups
Model 3 ANCOVA
FDR - P(group) ≤ .05
|FC| ≥ 1.5
P(age) > .05
P(gender) > .05
P(batch) > .05
Gene list 3
Model 4 ANCOVA
FDR - P(group) ≤ .05
|FC| ≥ 1.5
P(age) > .05
P(gender) > .05
Gene list 4
Genes common to list 1, 2, 3, 4
Feature reduction (Nearest Shrunken Centroid)
Predictor Genes
All IS per time point
1st Random half IS
2nd Random half IS
Resultant Set (training)
Model 1 ANCOVA
FDR - P(group) ≤ .05
|FC| ≥ 1.5
P(age) > .05
P(gender) > .05
P(batch) > .05
Model 2 ANCOVA
FDR - P(group) ≤ .05
|FC| ≥ 1.5
P(age) > .05
P(gender) > .05
Gene list 1
Gene list 2
Control groups
1st Random half controls
2nd Random half controls
Resultant Set (test)
Prediction Results
Prediction Algorithms

Figure 3

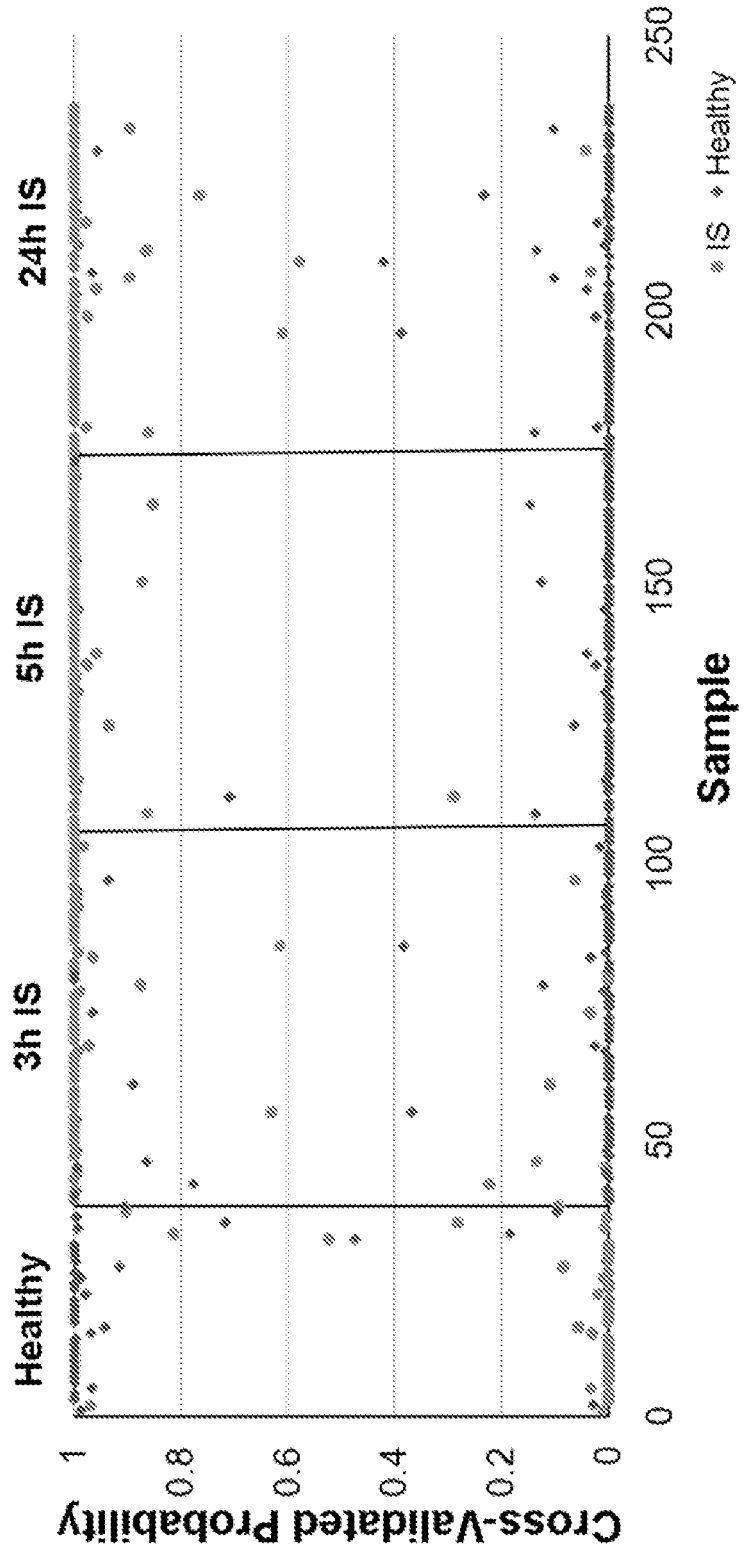
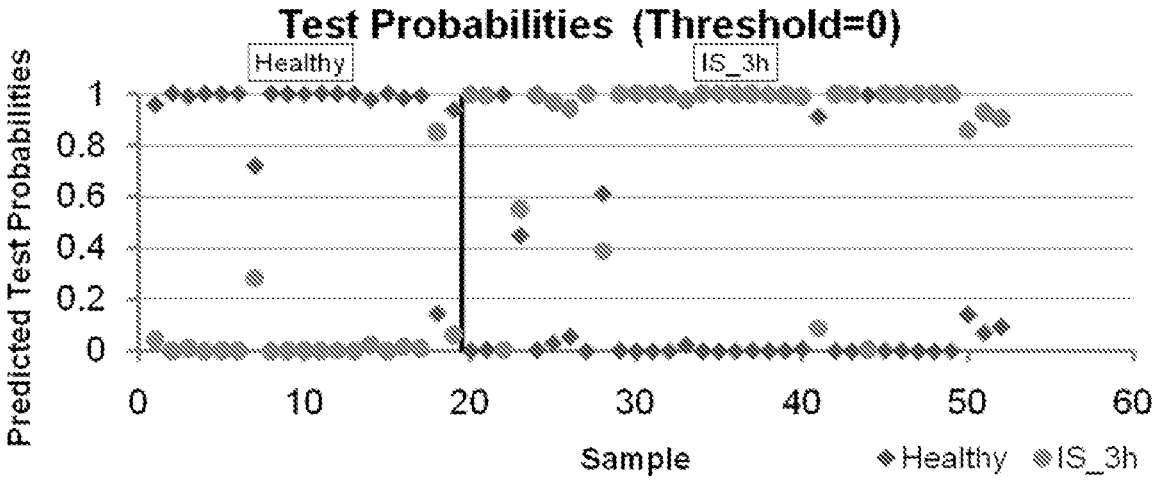

Healthy
3h IS
5h IS
24h IS
Cross-Validated Probability
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
Sample
IS
Healthy

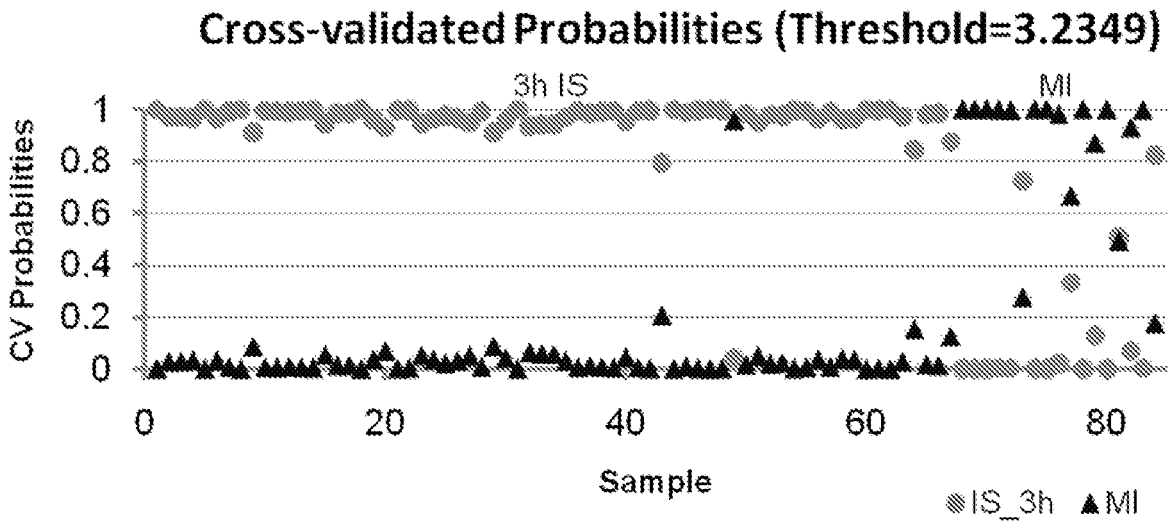
Figure 4



Test set Prediction Confusion Matrix (Threshold=0)

True\Predicted	Healthy	IS_3h	Correct Classification, %
Healthy	18	1	94.73
IS_3h	4	29	87.9

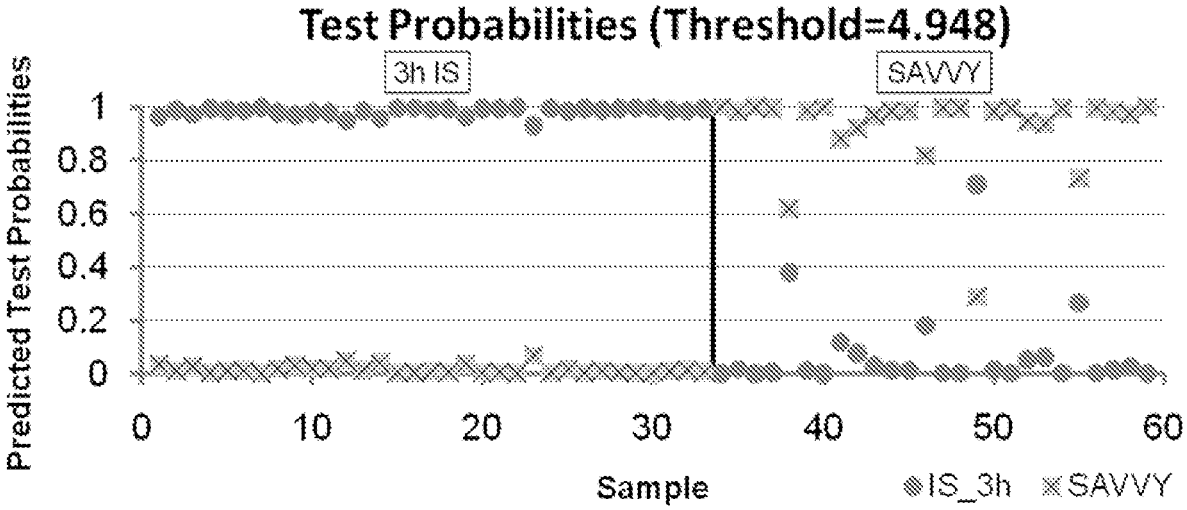
Figure 5



CV Confusion Matrix (Threshold=3.23495)

True\Predicted	IS_3h	MI	Correct Classification, %
IS_3h	66	1	98.5
MI	3	14	82.4

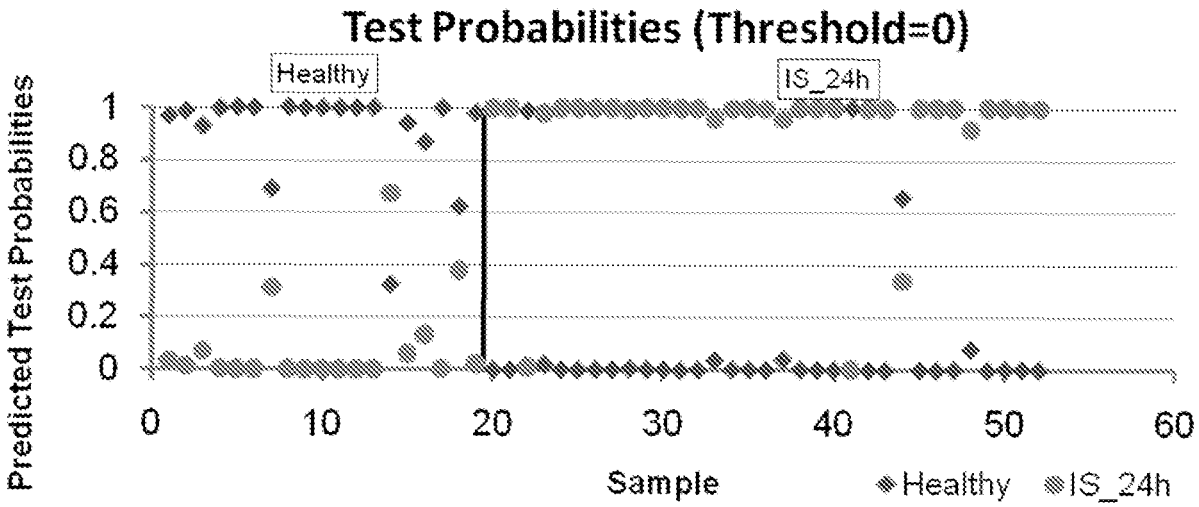
Figure 6



Test set Prediction Confusion Matrix (Threshold=4.948)

True\Predicted	IS_3h	SAVVY	Correct Classification, %
IS_3h	33	0	100
SAVVY	1	25	96.2

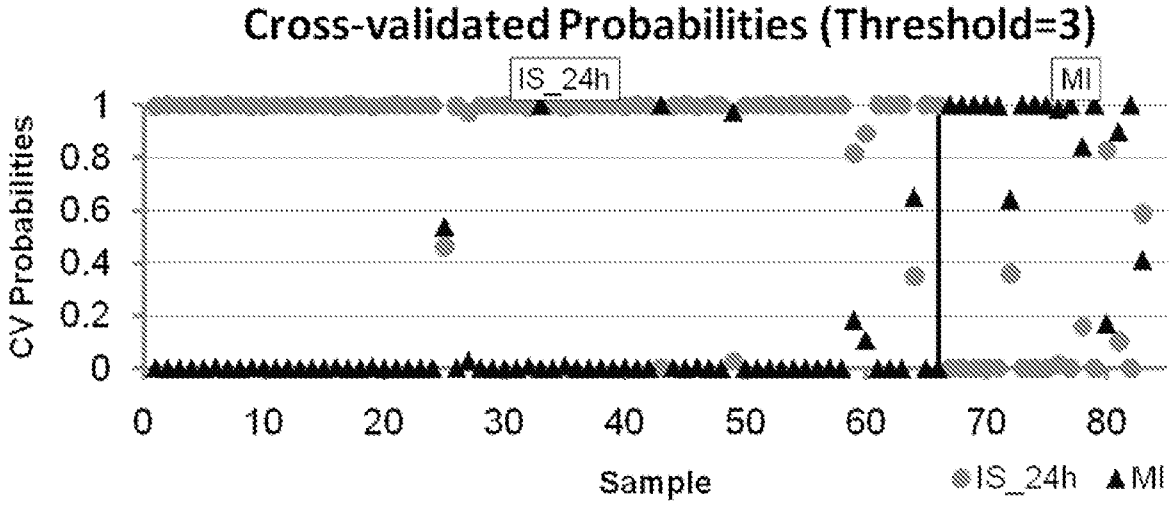
Figure 7



Test set Prediction Confusion Matrix (Threshold=0)

True\Predicted	Healthy	IS_24h	Correct Classification, %
Healthy	18	1	94.7
IS_24h	3	30	90.9

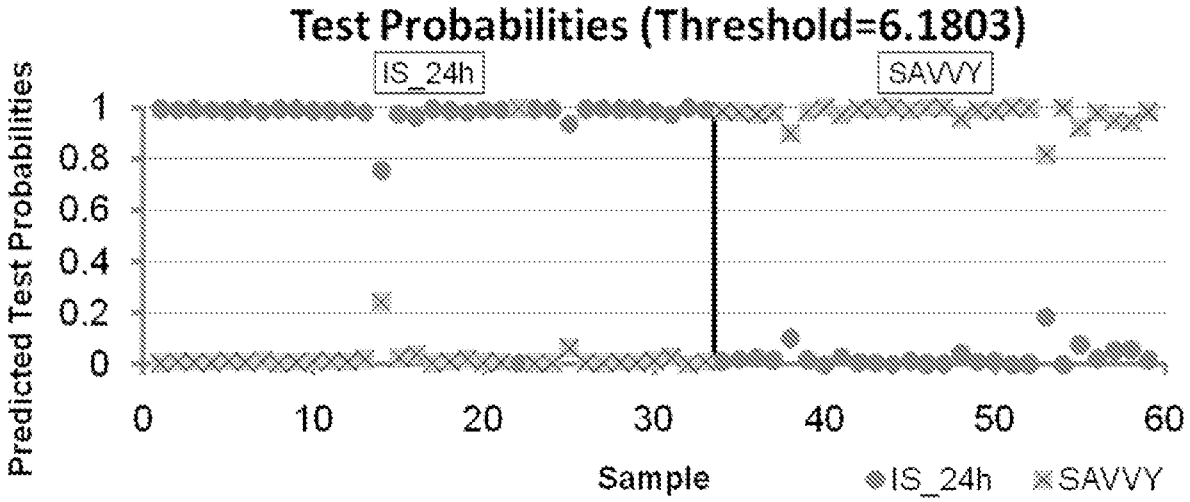
Figure 8



CV Confusion Matrix (Threshold=2.92544)

True\Predicted	IS_24h	MI	Correct Classification, %
IS_24h	62	4	93.9
MI	2	15	88.2

Figure 9



Test set Prediction Confusion Matrix (Threshold=6.1803)

True\Predicted	IS_24h	SAVVY	Correct Classification, %
IS_24h	32	1	97
SAVVY	0	26	100

Figure 10

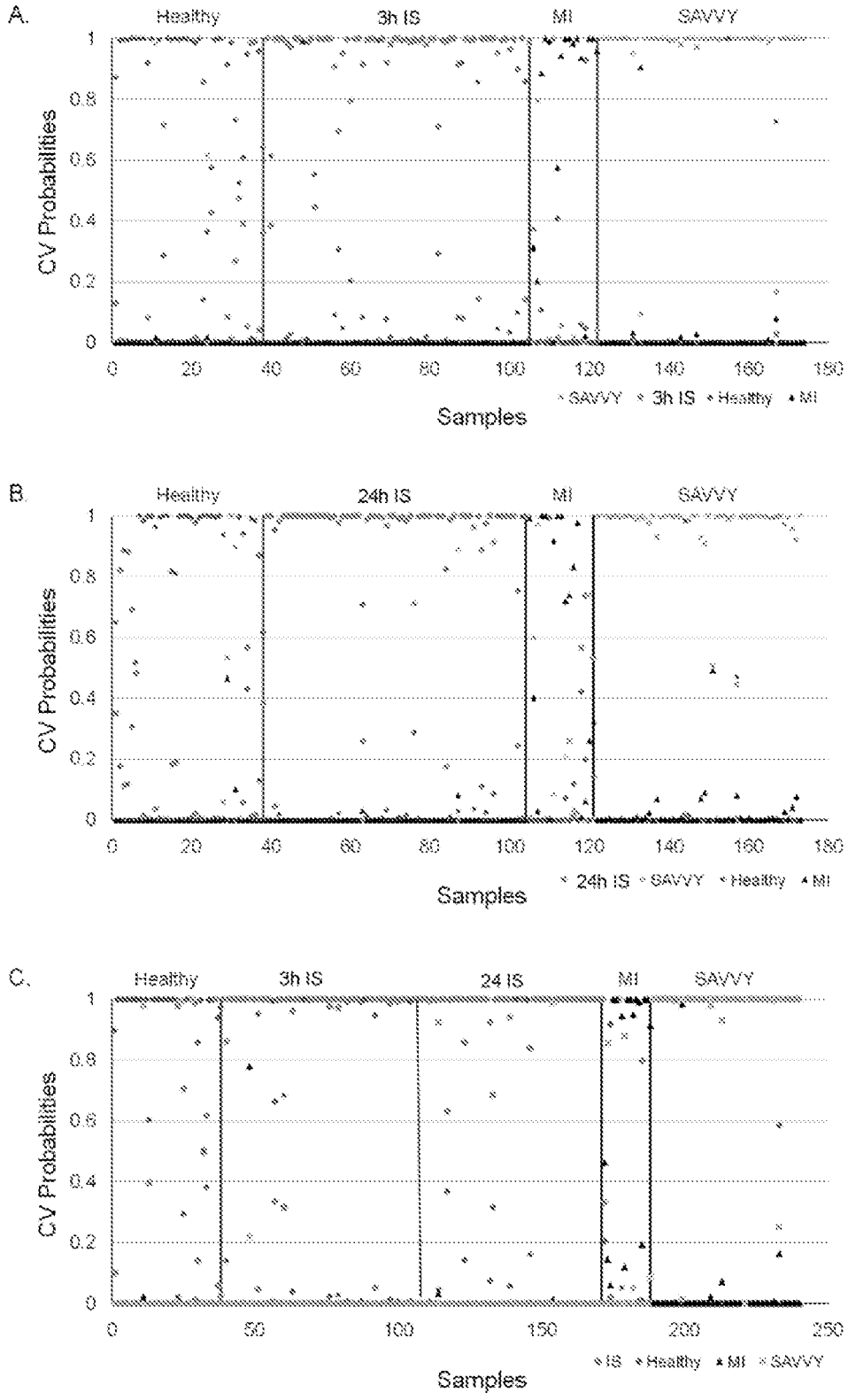
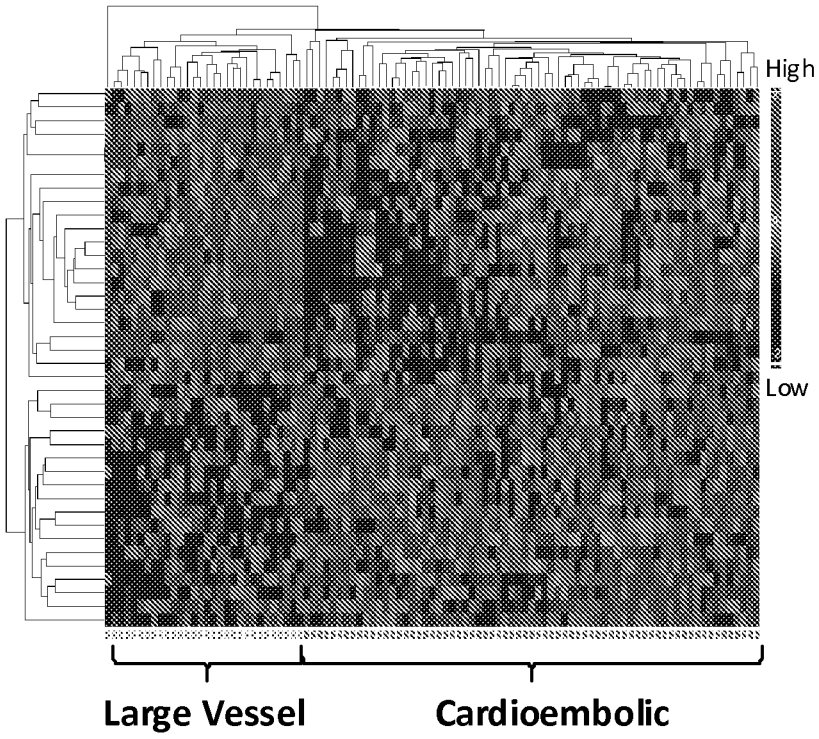

A.
Healthy
3h IS
MI
SAVVY
CV Probabilities
Samples
SAVVY
3h IS
Healthy
MI
B.
Healthy
24h IS
MI
SAVVY
CV Probabilities
Samples
24h IS
SAVVY
Healthy
MI
C.
Healthy
3h IS
24 IS
MI
SAVVY
CV Probabilities
Samples
IS
Healthy
MI
SAVVY

Figure 11

A


High
Low
Large Vessel
Cardioembolic

B

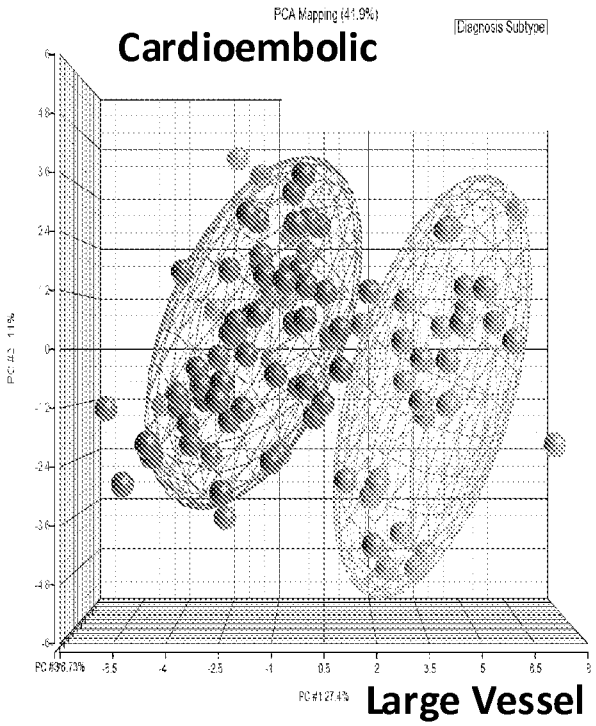

PCA Mapping (41.9%)
Diagnosis Subtype
Cardioembolic
Large Vessel

Figure 12

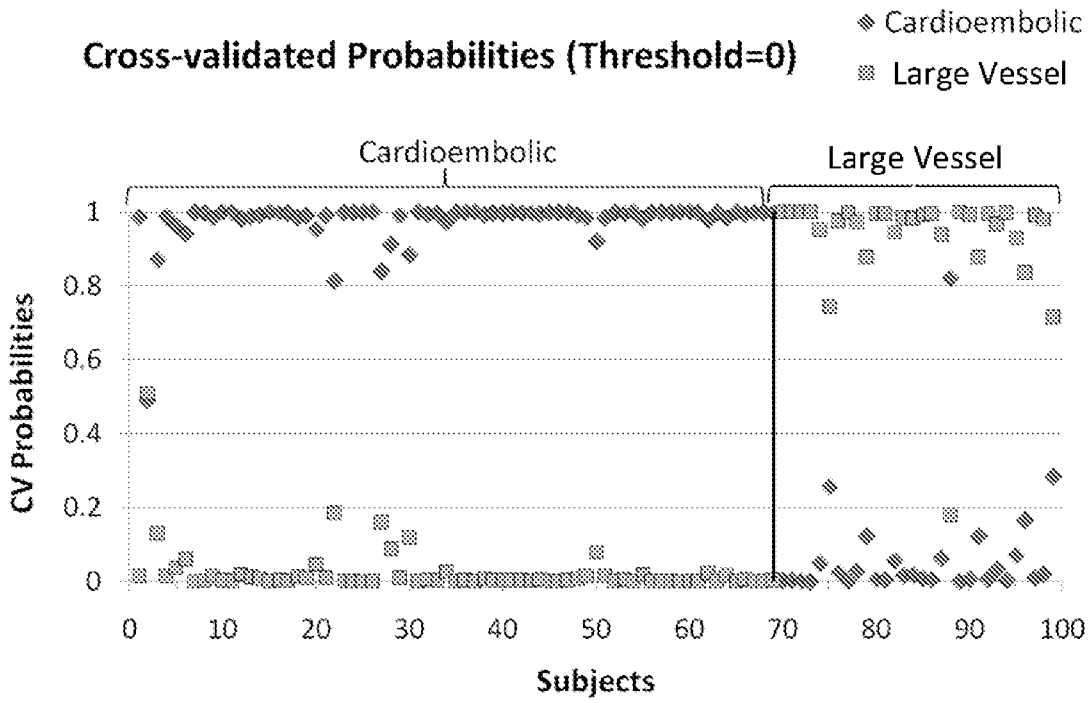

Cross-validated Probabilities (Threshold=0)
Cardioembolic
Large Vessel
Cardioembolic
Large Vessel
CV Probabilities
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
80
90
100
Subjects

Figure 13

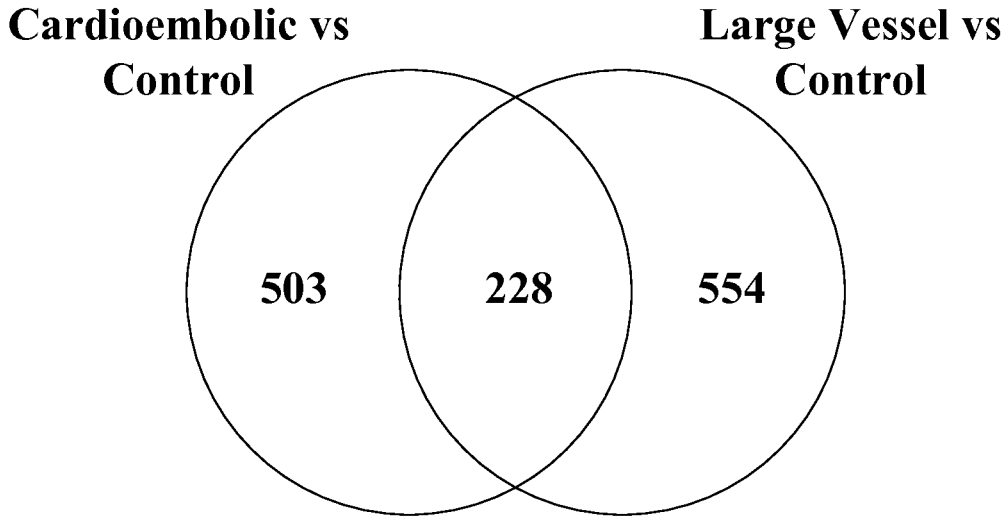
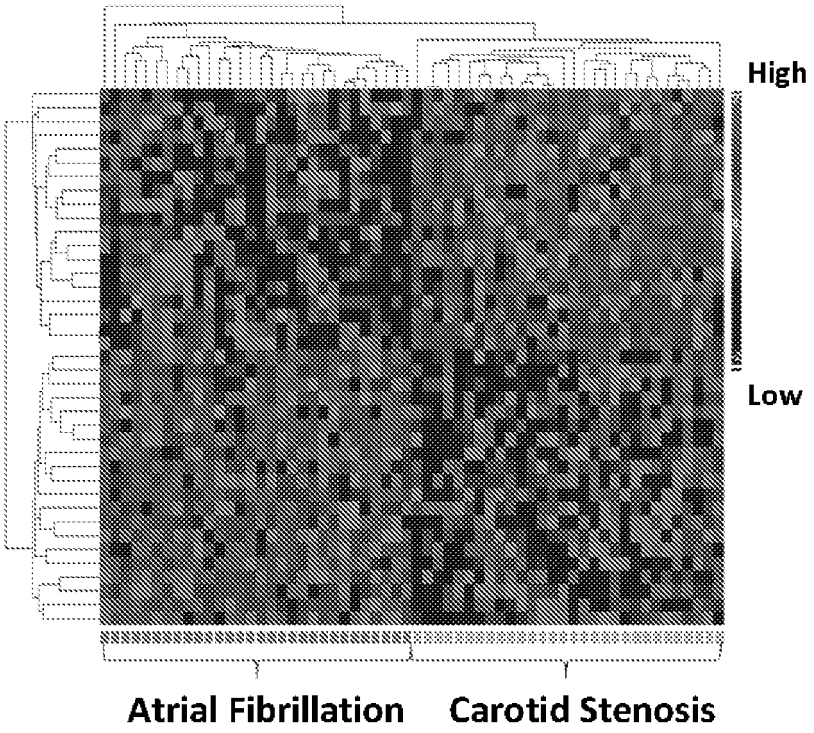

Cardioembolic vs Control
Large Vessel vs Control
503
228
554

Figure 14

A


High
Low
Atrial Fibrillation
Carotid Stenosis

B

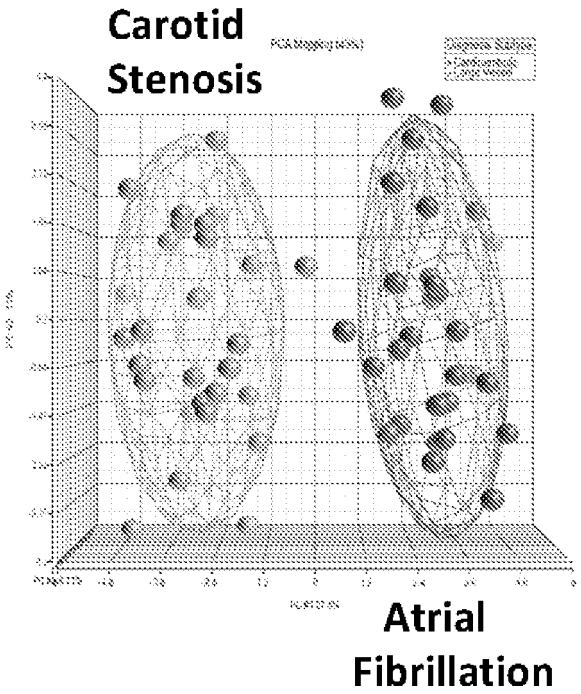

Carotid
Stenosis
Atrial
Fibrillation

Figure 15

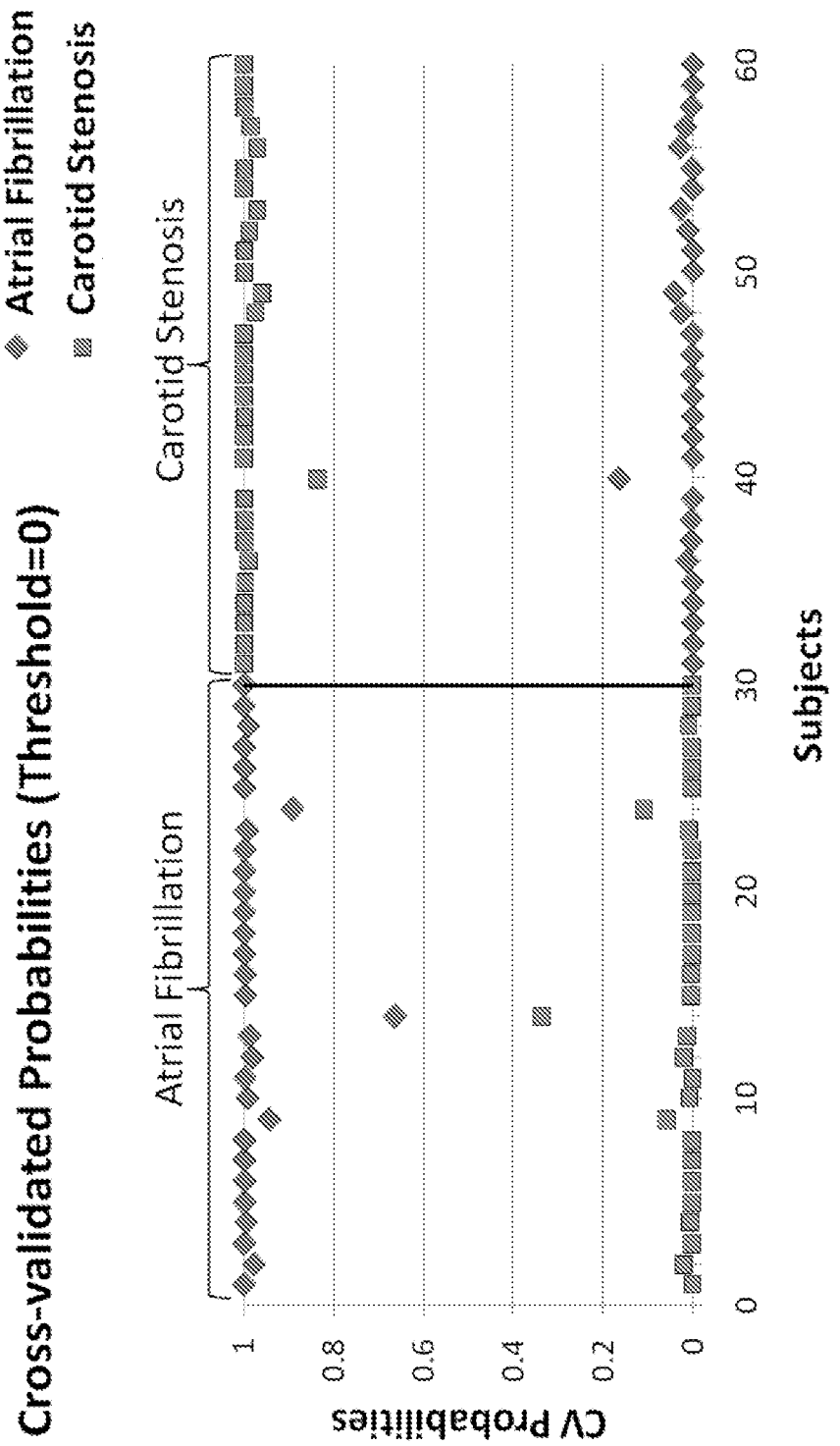

Cross-validated Probabilities (Threshold=0)
Atrial Fibrillation
Carotid Stenosis
Atrial Fibrillation
Carotid Stenosis
CV Probabilities
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
Subjects

Figure 16

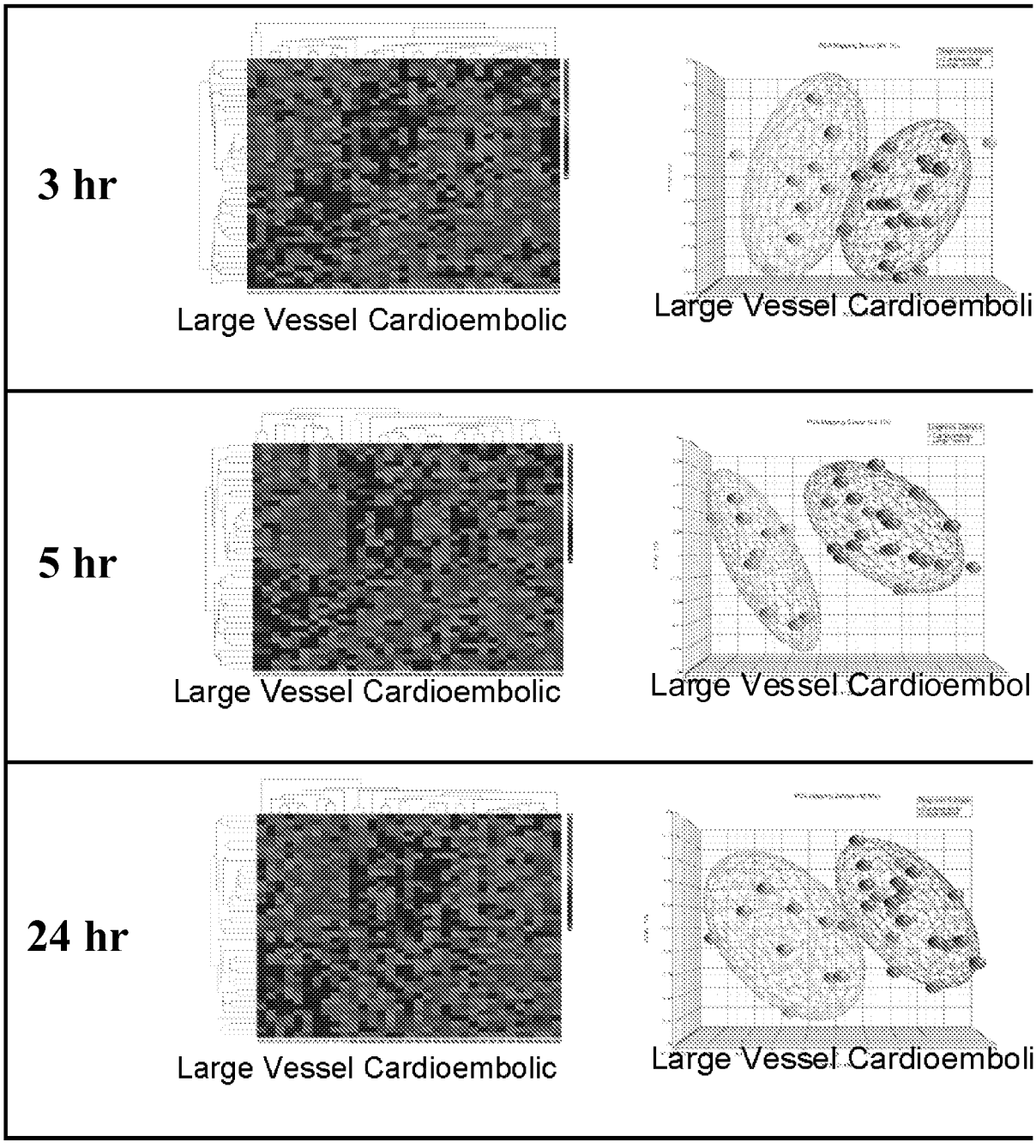

3 hr
Large Vessel Cardioembolic
Large Vessel Cardioembol
5 hr
Large Vessel Cardioembolic
Large Vessel Cardioembol
24 hr
Large Vessel Cardioembolic
Large Vessel Cardioembol

Figure 17

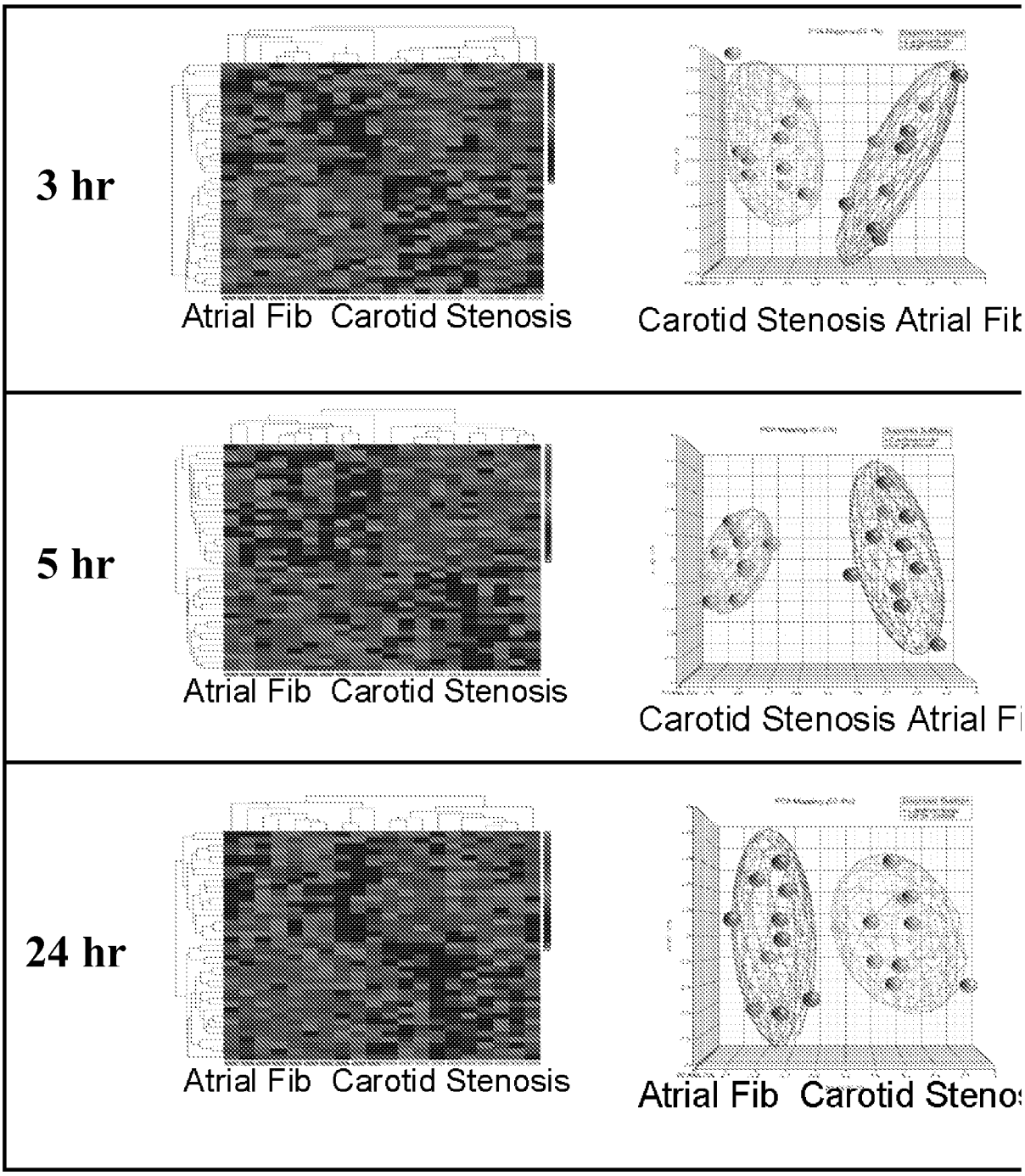

3 hr
Atrial Fib Carotid Stenosis
Carotid Stenosis Atrial Fit
5 hr
Atrial Fib Carotid Stenosis
Carotid Stenosis Atrial Fi
24 hr
Atrial Fib Carotid Stenosis
Atrial Fib Carotid Steno

Figure 18

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	用于诊断中风及其原因的生物标志物		
公开(公告)号	EP2593567B1	公开(公告)日	2016-04-20
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当前申请(专利权)人(译)	加利福尼亚大学董事会		
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摘要(译)

本发明提供了用于诊断中风发生和原因的组合物和方法。

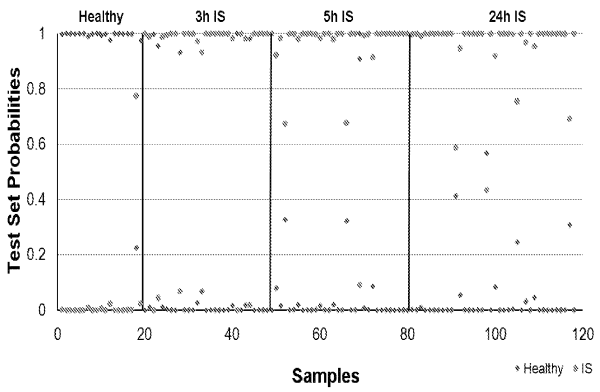

Figure 1