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(54) **METHOD FOR DETECTION OF ISCHEMIC STROKES**

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(57) **ABSTRACT**

The present invention relates to the identification and use of diagnostic markers for ischemic stroke of the lacunar subtype. The invention relates to devices and kits for performing these methods.

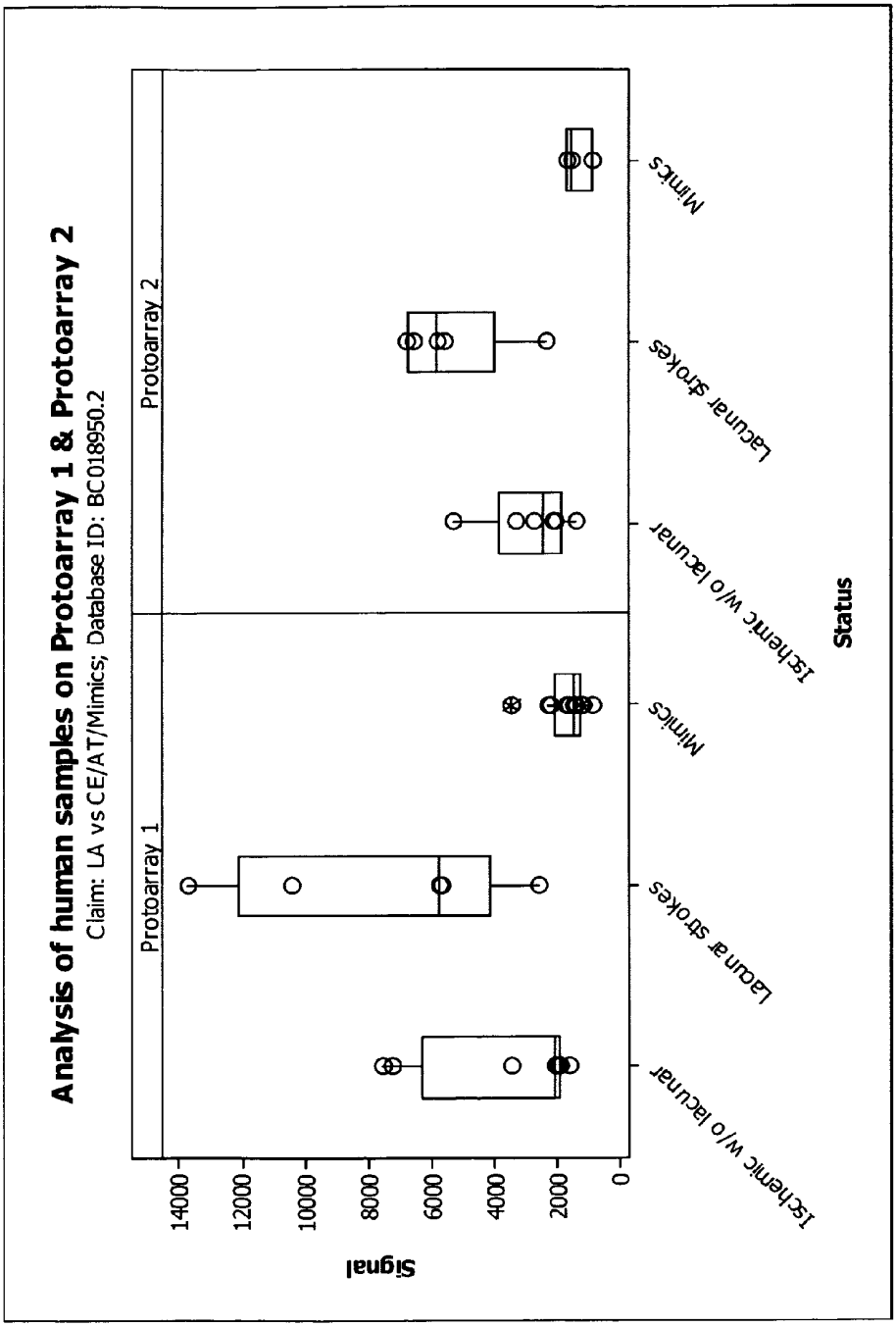
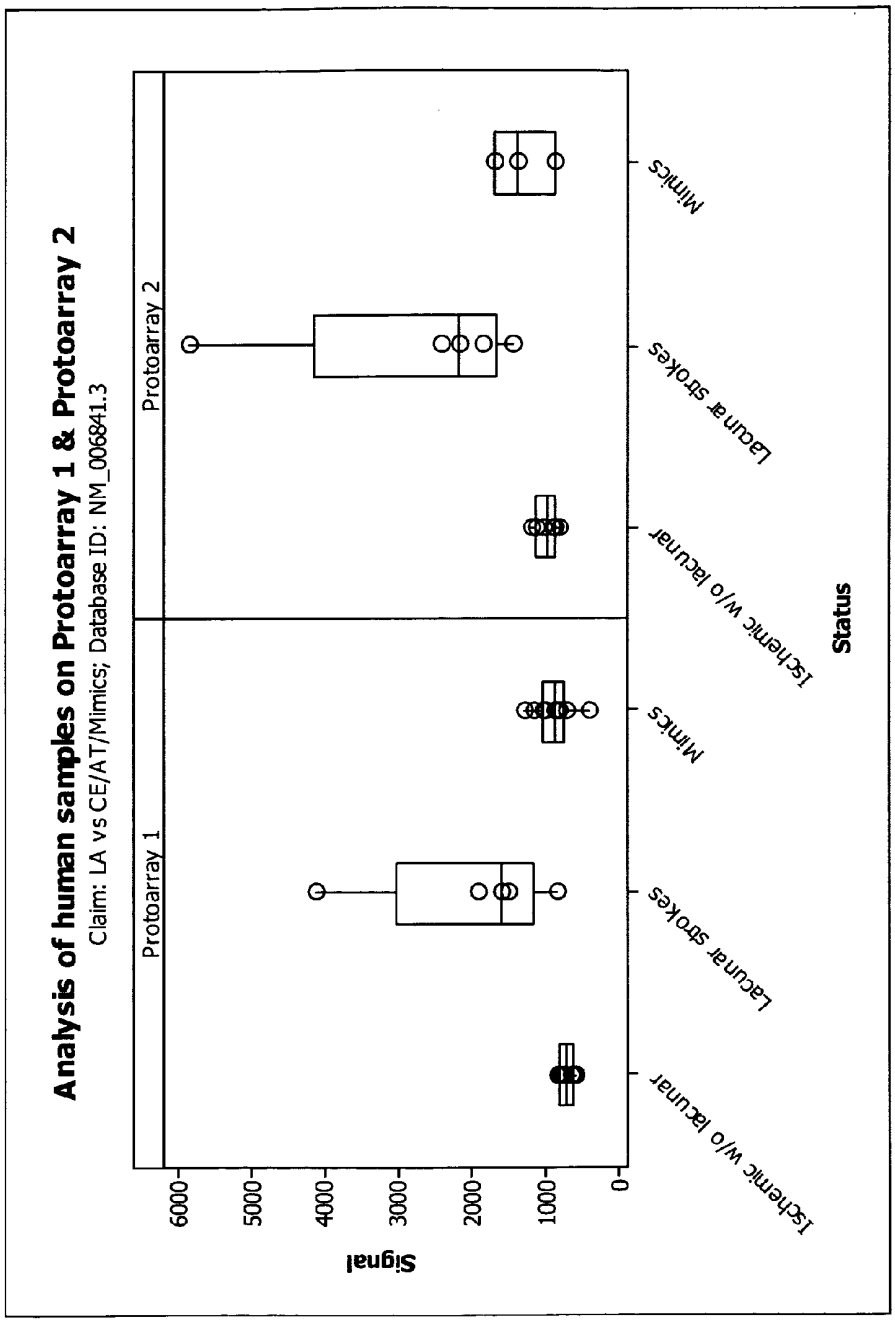

Analysis of human samples on Protoarray 1 & Protoarray 2
Claim: LA vs CE/AT/Mimics; Database ID: BC018950.2
Protoarray 1
Protoarray 2
Signal
14000
12000
10000
8000
6000
4000
2000
0
Ischemic W/o lacunar
Lacunar strokes
Mimics
Status

Fig. 1


Analysis of human samples on Protoarray 1 & Protoarray 2
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Protoarray 1
Protoarray 2
Signal
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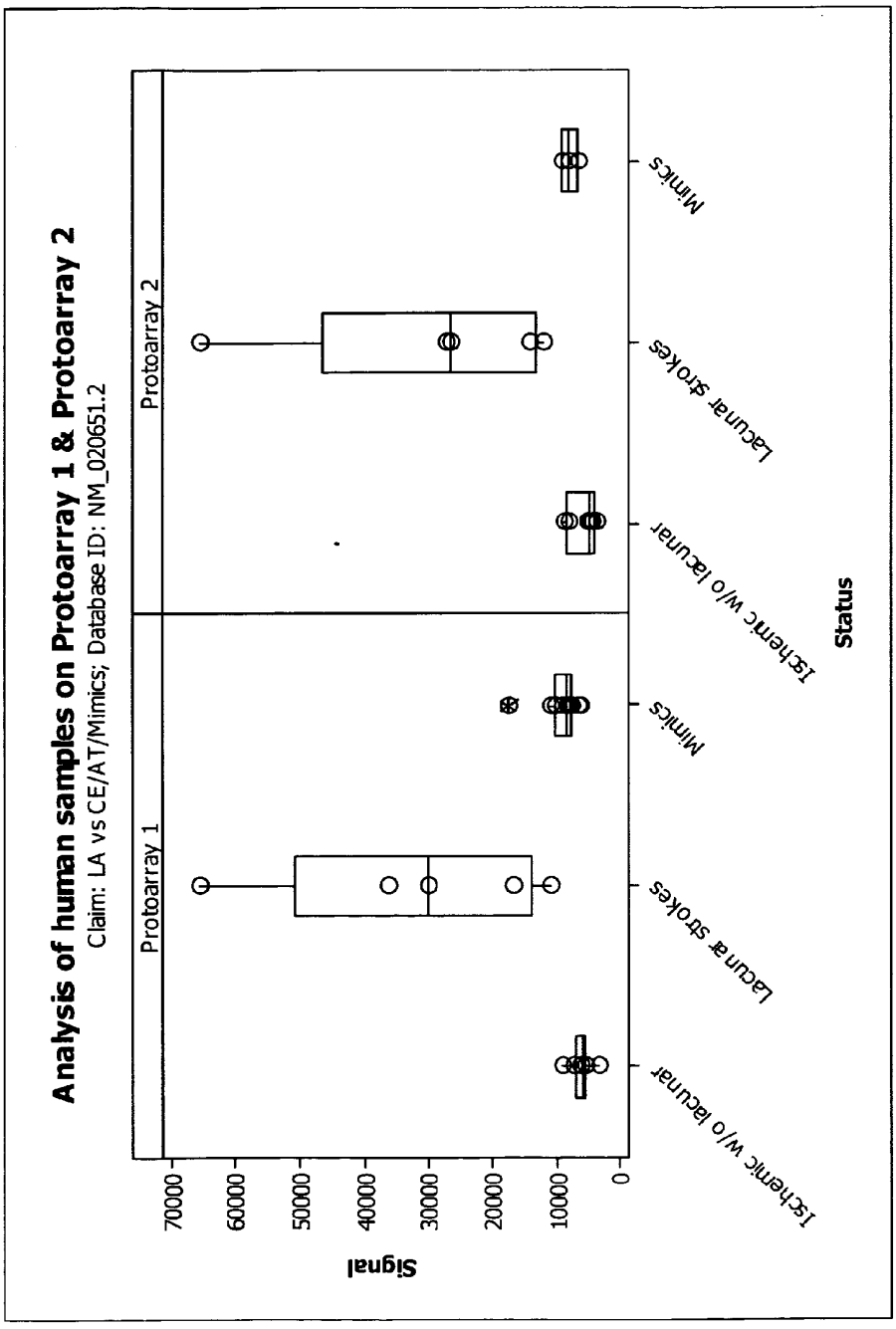
5000
4000
3000
2000
1000
0
Ischemic W/o lacunar
Lacunar strokes
Mimics
Status

Fig. 2


Analysis of human samples on Protoarray 1 & Protoarray 2
Claim: LA vs CE/AT/Mimics; Database ID: NM_020651.2
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Protoarray 2
Signal
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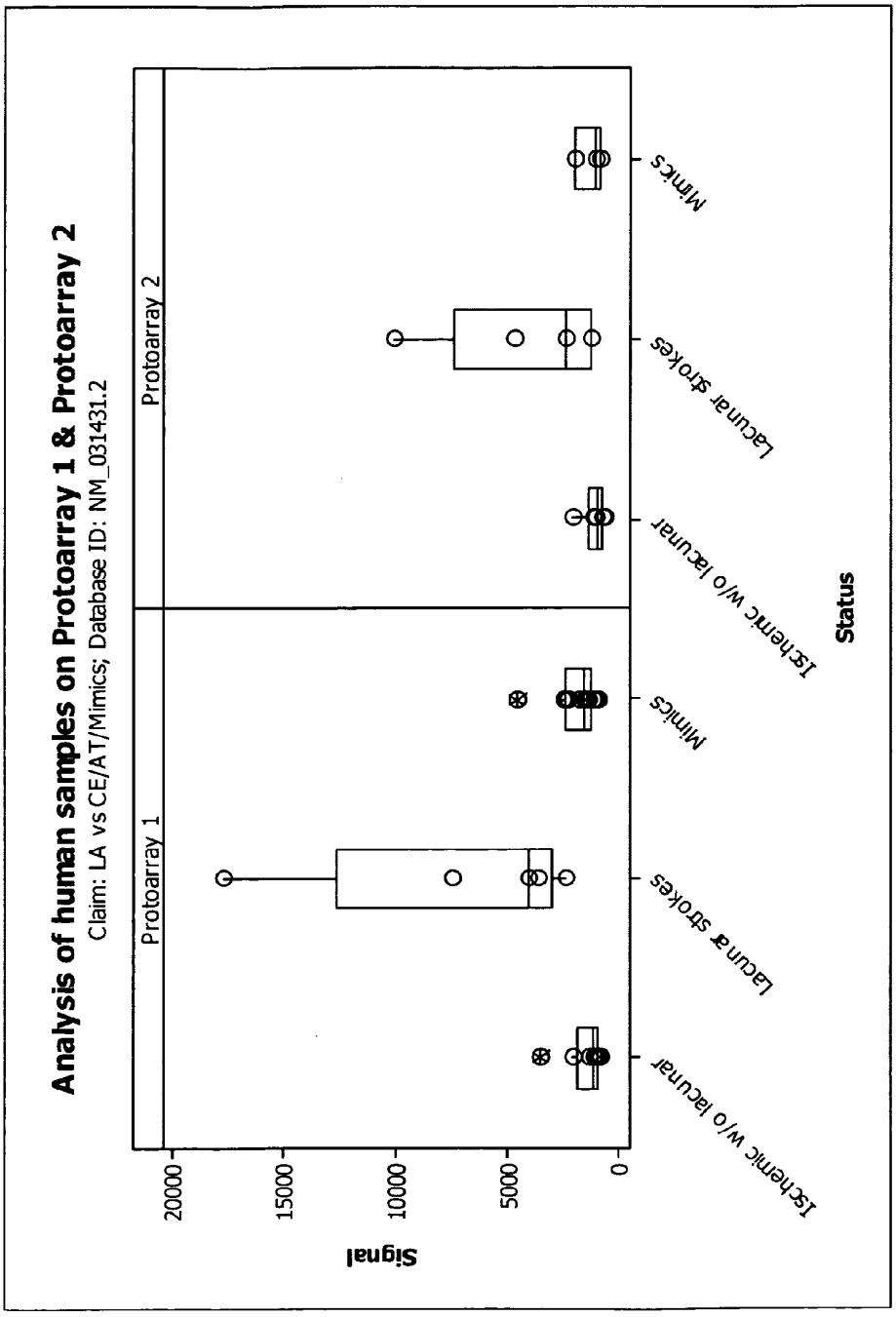
60000
50000
40000
30000
20000
10000
0
Ischemic w/o lacunar
Lacunar strokes
Mimics
Status

Fig. 3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Protoarray 1
Protoarray 2
Signal
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15000
10000
5000
0
Ischemic w/o lacunar
Lacunar strokes
Mimics
Status

Fig. 4

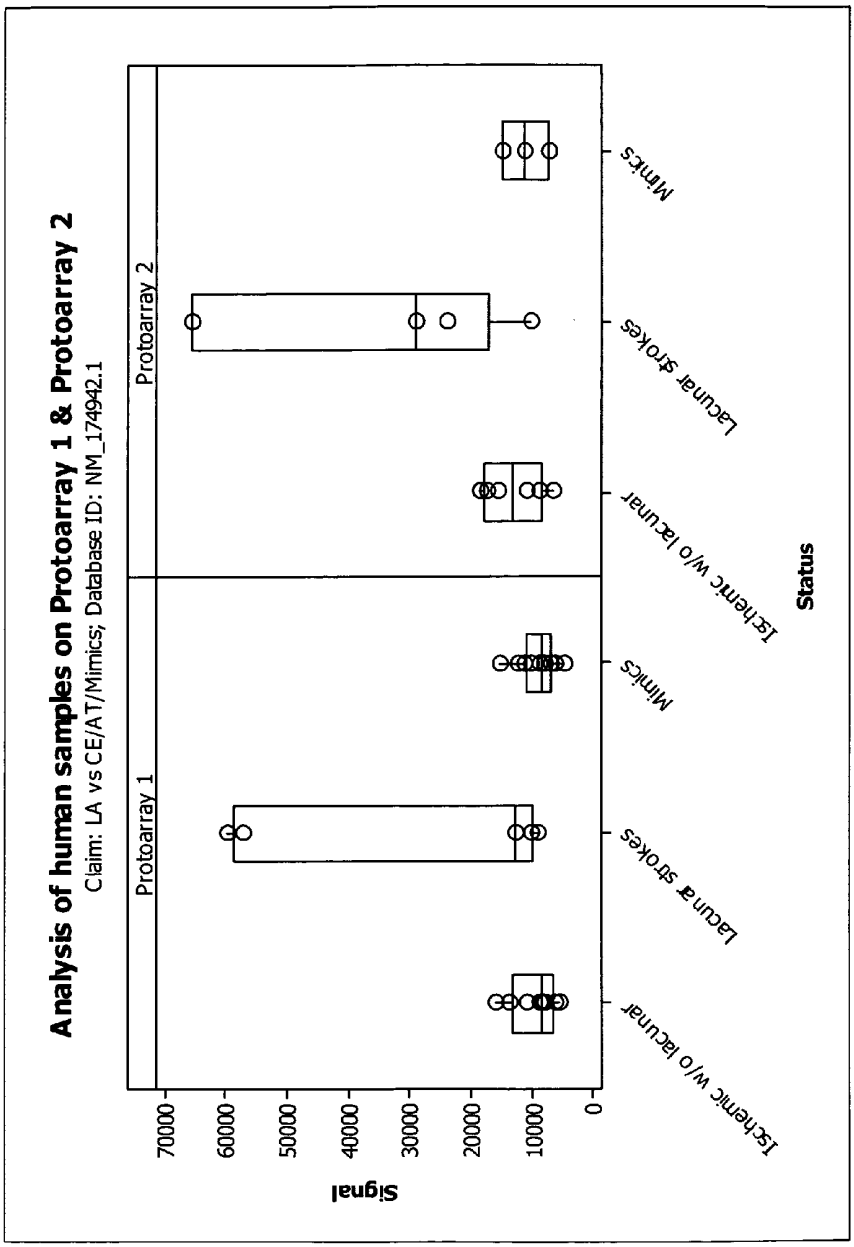
Analysis of human samples on Protoarray 1 & Protoarray 2
Claim: LA vs CE/AT/Mimics; Database ID: NM_174942.1
Protoarray 1
Protoarray 2
Signal
70000
60000
50000
40000
30000
20000
10000
0
Ischemic w/o lacunar
Lacunar strokes
Mimics
Status

Fig. 5

METHOD FOR DETECTION OF ISCHEMIC STROKES

FIELD OF THE INVENTION

[0001] The present invention relates to the identification and use of diagnostic markers for ischemic stroke especially of lacunar subtype. The invention relates to devices and kits for performing these methods.

BACKGROUND OF THE INVENTION

[0002] Ischemic Stroke is a manifestation of vascular injury to the brain which is commonly secondary to atherosclerosis or hypertension.

[0003] Ischemic stroke encompasses atherothrombotic, cardioembolic and lacunar types of strokes. Thrombi are occlusions of arteries created in situ within the brain, while emboli are occlusions caused by material from a distant source, such as the heart and major vessels, often dislodged due to myocardial infarct or atrial fibrillation. Less frequently, thrombi may also result from vascular inflammation due to disorders such as meningitis. Thrombi or emboli can result from atherosclerosis or other disorders, for example, arteritis, and lead to physical obstruction of arterial blood supply to the brain. Lacunar stroke refers to an infarct within non-cortical regions of the brain.

[0004] The onset of ischemic stroke is often abrupt, and can become an "evolving stroke" manifested by neurologic deficits that worsen over a 24-48 hour period. In evolving stroke, "stroke-associated symptom(s)" commonly include unilateral neurologic dysfunction that extends progressively, without producing headache or fever. Evolving stroke may also become a "completed stroke", in which symptoms develop rapidly and are maximal within a few minutes.

[0005] Ischemic stroke was thought to cause permanent injury to brain tissues. However, the similarity of the acute clinical syndromes of ischemic stroke to other diseases made it difficult to differentiate them. These are also termed "stroke mimics".

[0006] Examples for such mimics are epileptic seizure, brain tumour or migraine. Therefore an ischemic stroke may remain undetected. On the other hand a falsely detected ischemic stroke may lead to a wrong treatment.

[0007] Immediate diagnosis and care of a patient experiencing stroke can be critical. For example, tissue plasminogen activator (tPA) given within three hours of symptom onset in ischemic stroke is beneficial for selected acute stroke patients. Alternatively, patients may benefit from anticoagulants (e.g., heparin) if they are not candidates for TPA therapy. Delays in the confirmation of stroke diagnosis and the identification of stroke type limit the number of patients that may benefit from early intervention therapy.

[0008] Accordingly, there is a present need in the art for a rapid, sensitive and specific diagnostic assay for stroke. Such a diagnostic assay would greatly increase the number of patients that can receive beneficial stroke treatment and therapy, and reduce the costs associated with incorrect stroke diagnosis. It is especially beneficial if the subtype of the ischemic stroke can be identified in order to adjust the treatment and/or medication.

[0009] Some diagnosis methods require expensive machines and instruments like a computer tomograph (CT). The sensitivity of these methods for ischemic stroke in the first hours after stroke is limited and therefore the additional testing of a blood biomarker with a better sensitivity would add diagnostic value.

[0010] It is, therefore, an object of the present invention to provide a method for diagnosing ischemic stroke, preferably of lacunar subtype, especially to differentiate these ischemic strokes from stroke mimics. Further it is an object of the present invention to provide a device and a kit to carry out the methods.

[0011] This aim is achieved by the inventions as claimed in the independent claims. Advantageous embodiments are described in the dependent claims.

[0012] Even if no multiple back-referenced claims are drawn, all reasonable combinations of the features in the claims shall be disclosed.

[0013] The object of the invention is also achieved by a method. In what follows, individual steps of a method will be described in more detail. The steps do not necessarily have to be performed in the order given in the text. Also, further steps not explicitly stated may be part of the method.

[0014] The problem is solved by a method of diagnosing ischemic stroke in a subject, comprising the steps: detecting the presence and/or amount of at least one marker or at least one antibody against at least one marker in a sample from said subject, wherein the at least one marker is selected from the group consisting of Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3) and correlating the result to the occurrence or non-occurrence of a stroke in said subject.

[0015] In a preferred embodiment ischemic stroke of lacunar subtype is diagnosed. Then the result is correlated to the occurrence or non-occurrence of a lacunar stroke (ischemic stroke of lacunar subtype) in said subject.

[0016] The methods comprise analyzing a sample obtained from a subject for the presence or amount of one or more markers or one or more antibodies against markers. These methods can comprise identifying one or more markers or antibodies, the presence or amount of which is associated with the diagnosis, prognosis, or differentiation of ischemic strokes. Once such marker(s) are identified, the level of such marker(s) in a sample obtained from a subject of interest can be measured. In certain embodiments, these markers can be compared to a level that is associated with the diagnosis, prognosis, or differentiation of ischemic strokes. By correlating the subject's marker level(s) to the diagnostic marker level(s) or the correlating with classification methods, the presence or absence of ischemic strokes and TIAs the probability of future adverse outcomes (early risk assessment, screening), etc., in a patient or apparently healthy individuum may be rapidly and accurately determined.

[0017] The method of the invention is especially suited for determining the presence or absence of a disease in a subject that is exhibiting a perceptible change in one or more physical characteristics (that is, one or more "symptoms") that are indicative of a plurality of possible etiologies underlying the observed symptom(s), one of which is ischemic stroke. These methods comprise analyzing a sample obtained from the subject for the presence or amount of one or more markers or one or more antibodies against markers selected to rule in or out stroke, or one or more types of stroke, as a possible etiology of the observed symptom(s). Etiologies other than stroke that

are within the differential diagnosis of the symptom(s) observed are referred to herein as "stroke mimics". The presence or amount of such marker(s) in a sample obtained from the subject can be used to rule in or rule out stroke, preferably lacunar stroke, thereby either providing a diagnosis (rule-in) and/or excluding a diagnosis (rule-out).

[0018] The term "marker" as used herein refers to proteins or polypeptides to be used as targets for screening samples obtained from subjects. "Proteins or polypeptides" used as markers in the present invention are contemplated to include any fragments thereof, in particular, immunologically detectable fragments. One skilled in the art would recognize that minor variation of the sequence not necessarily affect the affinity of an antibody to that protein. Therefore markers that show a sequence similarity of 80%, 85%, 90%, 92%, 95%, 97%, 98%, 99% or 99.9% to the protein sequences presented in this application are also regarded as marker as long as they bind the same antibodies. This also includes variants which differ due to posttranslational modifications such as phosphorylation or myristylation. The same is valid for nucleic acid sequences encoding the markers.

[0019] If the antibodies against the markers are to be detected the marker used for the detection of the antibodies may include modifications to the protein, which are required to perform the detection of the presence and/or the amount of the antibodies, e.g. expression as a fusion protein, e.g. as N-terminal glutathione S-transferase (GST) fusion protein. The binding of an antibody to a marker does not necessarily mean that the antibody will bind the same protein or antigen within its normal function. The method merely identifies the affinity of an antibody present in the sample to the marker in the measurement.

[0020] First, the amount of the antibody may be measured directly by the binding of the antibody against the marker, e.g. by nuclear magnetic resonance (NMR) or surface plasmon resonance.

[0021] Second, the bound antibody may be detected by the binding of another ligand specific to the bound antibody. This may be a second antibody. This ligand may be coupled covalently or non-covalently to a label allowing detection and measurement of the ligand. Labeling may be done by direct or indirect methods. Direct labeling involves coupling of the label directly (covalently or non-covalently) to the ligand. Indirect labeling involves binding (covalently or non-covalently) of a secondary ligand to the first ligand. The secondary ligand should specifically bind to the first ligand. Said secondary ligand may be coupled with a suitable label and/or be the target (receptor) of tertiary ligand binding to the secondary ligand. The use of secondary, tertiary or even higher order ligands is often used to increase the signal. Suitable secondary and higher order ligands may include antibodies, secondary antibodies, and the well-known streptavidin-biotin system (Vector Laboratories, Inc.). The ligand may also be "tagged" with one or more tags as known in the art. Such tags may then be targets for higher order ligands. Suitable tags include biotin, digoxygenin, His-Tag, Glutathion-S-Transferase, FLAG, GFP, myc-tag, influenza A virus haemagglutinin (HA), maltose binding protein, and the like. In the case of a peptide or polypeptide, the tag is preferably at the N-terminus and/or C-terminus. Suitable labels are any labels detectable by an appropriate detection method. Typical labels include gold particles, latex beads, acridan ester, acridinium esters, luminol, ruthenium, enzymatically active labels, radioactive labels, magnetic labels ("e.g. magnetic beads", including paramagnetic and superparamagnetic labels), and fluorescent labels. Enzymatically active labels include e.g. horseradish peroxidase, alkaline phosphatase, beta-Galactosidase, Luciferase, and derivatives thereof. Suitable substrates for detection include di-amino-benzidine (DAB), 3,3'-5,5'-tetramethylbenzidine, NBT- BCIP (4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate, available as ready-made stock solution from Roche Diagnostics), CDP-Star(™) (Amersham Biosciences), ECF (™) (Amersham Biosciences). A suitable enzyme-substrate combination may result in a colored reaction product, fluorescence or chemo luminescence, which can be measured according to methods known in the art (e.g. using a light-sensitive film or a suitable camera system). As for measuring the enzymatic reaction, the criteria given above apply analogously. Typical fluorescent labels include fluorescent proteins (such as GFP and its derivatives), Cy3, Cy5, Texas Red, Fluorescein, and the Alexa dyes (e.g. Alexa 568, Alexa 647). Further fluorescent labels are available e.g. from Molecular Probes (Oregon). Also the use of quantum dots as fluorescent labels is contemplated. Typical radioactive labels include 35S, ^{125}I, ^{32}P, ^{33}P and the like. A radioactive label can be detected by any method known and appropriate, e.g. a light-sensitive film or a phosphor imager. Suitable measurement methods according the present invention also include precipitation (particularly immunoprecipitation), electrochemiluminescence (electro-generated chemiluminescence), RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), sandwich enzyme immune tests, electrochemiluminescence sandwich immunoassays (ECLIA), dissociation-enhanced lanthanide fluoro immuno assay (DELFIA), scintillation proximity assay (SPA), turbidimetry, nephelometry, latexenhanced turbidimetry or nephelometry, or solid phase immune tests. Further methods known in the art (such as gel electrophoresis, 2D gel electrophoresis, SDS polyacrylamid gel electrophoresis (SDS-PAGE), Western Blotting, and mass spectrometry), can be used alone or in combination with labelling or other detection methods as described above.

[0022] The markers described herein may be used individually, or in combination with other markers.

[0023] The at least one antibody against the at least one marker as described herein can be a monoclonal or a polyclonal antibody. In a preferred embodiment the antibody is an autoantibody present in the sample. The detection of the presence and/or amount of at least one antibody in the sample corresponds to measurement of an antibody profile of the sample against the marker(s). The measurement of autoantibodies is preferred, more preferred the measurement of the amount of autoantibodies, because these autoantibodies are produced by the immune system and may be better correlated to ischemic stroke and the risk for ischemic stroke than the proteins. Furthermore the autoantibodies may be distributed better in bodily fluids since the proteins may be attached to cell surfaces.

[0024] The term "sample" as used herein refers to a sample of bodily fluid obtained for the purpose of diagnosis, prognosis, or evaluation of a subject of interest, such as a patient. In certain embodiments, such a sample may be obtained for the purpose of determining the outcome of an ongoing condition or the effect of a treatment regimen on a condition. Preferred test samples include blood, serum, plasma, cerebrospinal fluid, urine and saliva. In addition, one of skill in the art would realize that some test samples would be more

readily analyzed following a fractionation or purification procedure, for example, separation of whole blood into serum or plasma components.

[0025] In a preferred embodiment of the invention the sample is a sample of blood, serum, or plasma from said subject.

[0026] The term "subject" as used herein relates to animals, preferably mammals, and, more preferably, humans.

[0027] The term "amount" as used herein encompasses the absolute amount (e.g., of a marker or an antibody against a marker), the relative amount or concentration (e.g., of a marker or an antibody against a marker) as well as any value or parameter which correlates thereto. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the said markers or antibodies against a marker by direct measurements, e.g., intensity values in mass spectra or NMR spectra. Moreover, encompassed are all values or parameters which are obtained by indirect measurements specified elsewhere in this description, e.g., expression levels determined from biological read out systems in response to the peptides or intensity signals obtained from specifically bound ligands. It is to be understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

[0028] In another embodiment of the invention the presence and/or amount of at least one further marker or at least one antibody against at least one further marker in the sample is detected, wherein the at least one further marker is selected from the group consisting of Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3) and is different from the marker already measured.

[0029] These further markers may be detected together with the other markers. Groups of markers may be identified using statistical and optimizing methods for example multivariate predictive modelling, wherein statistical learning algorithms are used.

[0030] The method is also suited for acute stroke subjects. For purposes of the present invention, the term "acute stroke" refers to a stroke that has occurred within the prior 12 hours, more preferably within the prior 6 hours, and most preferably within the prior 3 hours.

[0031] Another object of the invention is the differentiation of lacunar strokes from other stroke subtypes (cardioembolic or atherothrombotic) or stroke mimics. There the same markers may be used.

[0032] In a preferred embodiment of the invention the presence and/or amount of antibodies against the at least one marker is detected, more preferably the amount. It is also possible that from the same sample presence and/or amount of a marker and antibody against the same or a different marker is determined.

[0033] In a preferred embodiment of the invention the marker is selected from the group consisting of Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3) and the presence of at least one marker or at least one antibody against at least one marker is detected and correlated to the occurrence or non-occurrence of an ischemic stroke of the lacunar subtype, preferable for the differentiation of ischemic strokes of the lacunar subtype against ischemic strokes of the cardioembolic and atherothrombotic subtype or ischemic stroke mimics.

[0034] In another preferred embodiment of the invention the marker is selected from the group consisting of Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3) and the presence of at least one marker or at least one antibody against at least one marker is detected and correlated to the occurrence or non-occurence of ischemic strokes of the lacunar sub-type, preferably correlated to the occurrence or non-occurrence of ischemic strokes of the lacunar subtype against stroke mimics.

[0035] The markers can be used on their own or in combination of 2, 3, 4 or 5 markers or in combination with other markers. It is also possible to derive signatures of specific combination of markers to increase sensitivity and specificity of the method.

[0036] Another object of the invention is the use of at least one at least one marker or at least one antibody against the at least one marker in a sample from a subject, wherein the at least one marker is selected from the group consisting of Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3) for the detection of ischemic stroke, preferably for the differentiation of ischemic strokes of lacunar subtype from ischemic stroke mimics, more preferably for the differentiation of ischemic strokes of lacunar subtype from ischemic stroke mimics and ischemic stroke of the cardioembolic or atherothrombotic subtypes.

[0037] In a preferred embodiment the use also encompasses the use of the further markers for the detection of ischemic strokes. In an especially preferred embodiment the use comprises the use of at least one of the previous described markers or antibodies against these markers for the detection of ischemic strokes of lacunar subtype.

[0038] In another preferred embodiment the use encompasses the use of the markers or antibodies against the markers previously described for the method for the occurrence or non-occurrence of ischemic strokes of the ischemic stroke of the lacunar subtype for the detection of ischemic strokes of the lacunar subtype, preferably for the differentiation of ischemic strokes of the lacunar subtype against stroke mimics.

[0039] Another object of the invention is a device for the detection of ischemic strokes comprising:

[0040] a) means for determining the amount and/or presence of at least one marker or at least one antibody against at least one marker in a sample from a subject, wherein the at least one marker is selected from the group consisting of

[0041] Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3); and

[0042] b) means for analyzing the amounts and/or presence of the marker(s) or antibodies against the marker(s) against a reference value and/or by classification methods.

[0043] In a preferred embodiment the device is a device for the detection of ischemic strokes of lacunar subtype, more preferably for the differentiation of ischemic strokes of lacunar subtype against stroke mimcs and ischemic strokes of cardioembolic or atherothrombotic subtypes.

[0044] In a preferred embodiment the device further comprises means for determining the amount and/or presence of the further markers or antibodies against the further markers. In an especially preferred embodiment the device comprises means for determining the amount and/or presence of at least one of the previously described markers and/or antibodies against these markers.

[0045] In a preferred embodiment the device further comprises means for determining the amount and/or presence of the previously described markers or antibodies against the markers for the method for the occurrence or non-occurrence of ischemic strokes of the ischemic stroke of the lacunar subtype.

[0046] In a preferred embodiment the means for determining the amount and/or presence are single- or multiplex detection methods, where the markers are immobilized on the solid surface. The device then comprises means to determine the amount of at least one antibody against the immobilized marker as described previously. Preferably these means are a labelled secondary antibody.

[0047] Such devices may further comprise a computer unit for analyzing and processing the measured data. Preferably a computer, which is programmed to perform classification methods or to evaluate the measurements.

[0048] In another embodiment such devices may comprise a database, wherein reference values and/or the classification methods or their parameters are stored for different analysis methods.

[0049] In another embodiment such devices may comprise displaying means to display the data.

[0050] In another embodiment the means for analyzing the measured data may analyse the data in various aspects, e.g. ischemic stroke as well as the subtype of ischemic stroke.

[0051] Preferred means for determining the amount and/or presence are disclosed in connection with embodiments relating to the method of the invention above. In such a case, the means are operatively linked in that the user of the system brings together the result of the determination of the amount and the diagnostic or prognostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further ado. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or plates or electronic devices which merely require loading with a sample. The results may be given as output of raw data which need interpretation by the clinician. Preferably, the output of the device is, however, processed, i.e. evaluated, raw data the interpretation of which does not require a clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands, Plasmon surface resonance devices, NMR spectrometers, mass-spectrometers, fluorescence readers etc.) or evaluation units/devices referred to above in accordance with the method of the invention.

[0052] Another object of the invention relates to a kit adapted for carrying out the previously described method of diagnosing ischemic stroke in a subject comprising

[0053] a) means for determining the amount and/or presence of at least one marker or antibody against at least one marker in a sample from a subject, wherein the at least one marker a marker as used in the previously described method of diagnosing ischemic strokes in a subject; and

[0054] b) means for analyzing the amounts and/or presence of the at least one marker or antibodies against the at least one marker against a reference value and/or by classification methods.

[0055] In a preferred embodiment the ischemic stroke diagnosed is an ischemic stroke of lacunar subtype.

[0056] In a preferred embodiment the kit further comprises means for determining the amount and/or presence of the previously described further markers or antibodies against further markers.

[0057] In a preferred embodiment the kit further comprises means for determining the amount and/or presence of the previously described markers or antibodies against the markers for the method for the occurrence or non-occurrence of ischemic strokes of the ischemic stroke of the lacunar subtype.

[0058] The means for determining the amount/or presence of antibodies against the markers may be single- or multiplex detection methods, where the markers are immobilized, e.g. on a plate or on particles on which surface the markers are placed, e.g. immobilized or present in different spots, e.g. drops of solvent. These plates may be produced by contact printing.

[0059] The term "kit" as used herein refers to a collection of the aforementioned means, preferably, provided separately or within a single container. The kit may in addition comprise means for determining the amount and/or presence of at least one marker or at least one antibody against at least one marker, preferable at least one antibody against at least one marker. Optionally, the kit may additionally comprise a user's manual for interpreting the results of any measurement(s) with respect to diagnosing ischemic stroke or its subtypes in a subject as defined in the present invention. Particularly, such manual may include information about what determined amounts and/or presences corresponds to what kind of diagnosis. This is outlined in detail elsewhere in this specification. Additionally, such user's manual may provide instructions about correctly using the components of the kit for determining the amount of the respective markers or antibodies against the respective marker.

[0060] The kit may further contain the recipes some or all of the buffers needed or may contain premixed ingredients.

[0061] The kit may further comprise a data carrier with a software for the analysis of the measured data.

[0062] Another object of the invention is the combination of any of the described methods for ruling in or out ischemic stroke with imaging methods (e.g. CT, NMR).

[0063] Another object of the invention is the use of the described method for the prediction of lacunar stroke. The markers or combination of markers described herein may also be used for the prediction of lacunar stroke.

BRIEF DESCRIPTION OF THE FIGURES

[0064] Other objects and advantages of the present invention may be ascertained from a reading of the specification and appended claims in conjunction with the drawings and tables therein.

[0065] For a more complete understanding of the present invention, reference is established to the following description made in connection with accompanying drawings in which:

[0066] FIG. **1**: Measured data of ischemic strokes without lacunar subtype (cardioembolic and atherothrombotic subtypes; left column), lacunar strokes and stroke mimics for marker BC018950.2 for two protoarrays.

[0067] FIG. **2**: Measured data of ischemic strokes without lacunar subtype (cardioembolic and atherothrombotic subtypes; left column), lacunar strokes and stroke mimics for marker NM_006841.3 for two protoarrays.

[0068] FIG. **3**: Measured data of ischemic strokes without lacunar subtype (cardioembolic and atherothrombotic subtypes; left column), lacunar strokes and stroke mimics for marker NM_020651.2 for two protoarrays.

[0069] FIG. **4**: Measured data of ischemic strokes without lacunar subtype (cardioembolic and atherothrombotic subtypes; left column), lacunar strokes and stroke mimics for marker NM_031431.2 for two protoarrays.

[0070] FIG. **5**: Measured data of ischemic strokes without lacunar subtype (cardioembolic and atherothrombotic subtypes; left column), lacunar strokes and stroke mimics for marker NM_174942.1 for two protoarrays.

DETAILED DESCRIPTION OF THE INVENTION

[0071] As already described the method of diagnosing ischemic stroke in a subject, comprising the step of detecting the presence and/or amount of at least one marker or at least one antibody against at least one marker in a sample from said subject, wherein the at least one marker is selected from the group of in total 5 proteins and polypeptides. In a preferred embodiment the markers derived from proteins and polypeptides, which are encoded by the sequences with the Database number shown in the column "Sequence" in table 1. In a more preferred embodiments the markers are derived from proteins and polypeptides, which are encoded by the sequences with the database ID shown in the column "Database ID" in table 1.

[0072] In a preferred embodiment of the invention the presence and/or presence of at least one antibody against the at least one marker is detected. This is preferably done in a protein micro array, e.g. ProtoArray from Invitrogen. In such arrays the protein or polypeptide to be bound by the antibody is printed on a plate, mostly a nitrocellulose coated glass plate. It may be necessary to express the protein or polypeptide as a fusion protein, e.g. GST fusion protein. The plate is then incubated with the sample fluid, wherein the antibody (primary antibody) it so be detected. After a washing step the antibodies bound to the protein or polypeptide are detected by incubating the array with a secondary antibody, which binds the primary antibody. This secondary antibody is labelled with a detectable tag. This can be a fluorescent tag, an enzymatic tag like horseradish peroxidase, a ligand binding tag. The amount of fluorescence is proportional to the amount of primary antibody. It may be necessary to correct the value by the amount of marker present in the array.

[0073] Next to one of the markers also other markers may be measured and analyzed at the same time.

[0074] In a preferred embodiment the method is a method to distinguish ischemic stroke of lacunar subtype against other ischemic strokes and/or stroke mimics, preferably ischemic stroke of the atherothrombic and cardioembolic subtype and/or stroke mimics.

[0075] Preferred markers are shown in the tables 1 or 2. Table 2 also gives the Seq-ID-Nos of the preferred markers for the invention.

[0076] The sequences of the markers are available from public databases. The corresponding protein sequences are listed in the sequence protocol.

[0077] Sample Preparation

[0078] The measurements were preformed using the Invitrogen ProtoArray.

[0079] Samples from 12 controls (stroke mimics) and 13 ischemic stroke patients were obtained. From the stroke patients were 5 of the lacunar subtype, 4 of the cardioembolic subtype and 4 of the atherothrombotic subtype. The stroke mimics can be further classified as epileptic seizure (4 samples), Hypoglicemia (1 sample), brain tumor (3 samples), vertigo (1 sample), radial palsy (1 sample), migraine (1 sample) and syncope (1 sample).

[0080] For the measurement the ProtoArray from Invitrogen was used (www.invitrogen.com/protoarray) following the standard protocol from the manufacturer. 9500 native human proteins were expressed in baculovirus and immobilized on a chip by contact type printing. 3 additional proteins were expressed and included in the array (2 NMDA subunits). The proteins are expressed as GST fusion protein in order to allow quality control by probing the microarray with a GST-antibody.

[0081] 9501 proteins (auto-antibodies against these proteins) were measured, out of which 9411 were unique. Some proteins were tested twice in different chip locations. All 9501 proteins were measured in duplicates.

[0082] For the measurement the array is incubated with the sample, optionally in a probing buffer, typically for 2 hours at 4° C. Any antibodies present in the sample will bind to the different proteins presented in the array. The sample is decanted and the array is washed several times. Then the array is incubated with the secondary antibody. This is typically a fluorescent labelled antibody, e.g. anti-mouse or anti-rabbit Alexa Fluor 647 from Invitrogen). This incubation is also normalty done for two hours and at 4° C. The array is then washed several times and dried. Then the microarray is scanned with a fluorescent scanner (GenePix 4000B Fluorescent Scanner; Molecular Devices) and the data was acquired with GenePix Pro software (Molecular Devices) and processed using the ProtoArray Proscpector tool developed by Invitrogen, which performs the normalizing and processing of the data.

[0083] In analysing the measured data either the Minimum (Min) or Average (Avg) of the duplicates was considered.

[0084] The data was further normalized in respect to control proteins and background using either Linear Normalization (LN) or Quantile Normalization (QN).

[0085] This resulted in a total for four combinations of datasets: MinLN, MinQN, AvgLN and AvgQN.

[0086] Negative values were converted to zero. The data was further log-transformed (after adding 1 to account for zeros).

[0087] All analysis was done with all four combinations of datasets. The following results were obtained using the Minimum Quantile Normalized data (MinQN).

[0088] Univariate Analysis

[0089] In this analysis the proteins that are significant on their own were identified using Permutation Test Statistic and Kruskal-Wallis Testing correcting for the small sample size issue.

[0090] Statistical significance is reported in terms of the False Positive Rate (p-value) and the Family Wise Error Rate (FWER).

[0091] Stroke Subtypes Analysis

[0092] For this analysis the data processed and normalized with the quantile normalization method was used.

[0093] In order to obtain the significant proteins the data was analyzed using the Permutation Test Statistic and Kruskal-Wallis Testing. The data of 2 samples were excluded because these samples were redraw samples from 2 patients which were taken one week after hospitalization of the patients. Only samples drawn within 3 hours after onset of the symptoms were included in analysis.

[0094] Graphs were generated for the candidates which were identified by the statistical analysis and were then visually evaluated. The 5 most promising candidate markers were selected visually.

[0095] The analysis of the data in regard of stroke patients of lacunar subtype versus stroke mimics revealed 5 antibodies against proteins as marker of particular significance. These proteins are shown in table 1. The graphs of the data of these proteins are shown in FIGS. **1** to **5**.

[0096] In further analysis the data was analyze with regard to different subtypes of ischemic stroke.

[0097] The most significant proteins identified to differentiate the atherothrombic and cardioembolic subtype and stroke mimics from lacunar ischemic stroke are shown in table 1. The corresponding graphs are displayed in FIGS. **1** to **5**.

[0098] While the present inventions have been described and illustrated in conjunction with a number of specific embodiments, those skilled in the art will appreciate that variations and modifications may be made without departing from the principles of the inventions as herein illustrated, as described and claimed. The present inventions may be embodied in other specific forms without departing from their spirit or essential characteristics. The described embodiments are considered in all respects to be illustrative and not restrictive. The scope of the inventions are, therefore, indicated by the appended claims, rather than by the foregoing description. All changes which come within the meaning and range of equivalence of the claims are to be embraced within their scope.

TABLE 1

#	Sequence	Database ID	Description
1	BC018950	BC018950.2	TNF receptor-associated protein 1 (TRAP1)
2	NM_020651	NM_020651.2	Pellino homolog 1 (*Drosophila*) (PELI1) (IOH26519)
3	NM_031431	NM_031431.2	Conserved oligomeric Golgi complex subunit 3 (COG3)
4	NM_006841	NM_006841.3	*Homo sapiens* solute carrier family 38, member 3 (SLC38A3)
5	NM_174942	NM_174942.1	*Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3)

TABLE 2

Accession Number	Seq-ID-No.	Length (amino acids)
BC018950.2	1	704
NM_020651.2	2	418
NM_031431.2	3	828
NM_006841.3	4	504
NM_174942.1	5	694

SEQUENCE LISTING

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<223> OTHER INFORMATION: /mol_type="protein"
/organism="Homo sapiens"

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Pro Leu Leu Arg Ala Pro Ala Leu Ala Ala Val Pro Gly Gly Lys Pro
            20                  25                  30

Ile Leu Cys Pro Arg Arg Thr Thr Ala Gln Leu Gly Pro Arg Arg Asn
        35                  40                  45
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Pro Ala Trp Ser Leu Gln Ala Gly Arg Leu Phe Ser Thr Gln Thr Ala
    50                  55                  60

Glu Asp Lys Glu Glu Pro Leu His Ser Ile Ile Ser Ser Thr Glu Ser
65                  70                  75                  80

Val Gln Gly Ser Thr Ser Lys His Glu Phe Gln Ala Glu Thr Lys Lys
                85                  90                  95

Leu Leu Asp Ile Val Ala Arg Ser Leu Tyr Ser Glu Lys Glu Val Phe
            100                 105                 110

Ile Arg Glu Leu Ile Ser Asn Ala Ser Asp Ala Leu Glu Lys Leu Arg
        115                 120                 125

His Lys Leu Val Ser Asp Gly Gln Ala Leu Pro Glu Met Glu Ile His
    130                 135                 140

Leu Gln Thr Asn Ala Glu Lys Gly Thr Ile Thr Ile Gln Asp Thr Gly
145                 150                 155                 160

Ile Gly Met Thr Gln Glu Glu Leu Val Ser Asn Leu Gly Thr Ile Ala
                165                 170                 175

Arg Ser Gly Ser Lys Ala Phe Leu Asp Ala Leu Gln Asn Gln Ala Glu
            180                 185                 190

Ala Ser Ser Lys Ile Ile Gly Gln Phe Gly Val Gly Phe Tyr Ser Ala
        195                 200                 205

Phe Met Val Ala Asp Arg Val Glu Val Tyr Ser Arg Ser Ala Ala Pro
    210                 215                 220

Gly Ser Leu Gly Tyr Gln Trp Leu Ser Asp Gly Ser Gly Val Phe Glu
225                 230                 235                 240

Ile Ala Glu Ala Ser Gly Val Arg Thr Gly Thr Lys Ile Ile Ile His
                245                 250                 255

Leu Lys Ser Asp Cys Lys Glu Phe Ser Ser Glu Ala Arg Val Arg Asp
            260                 265                 270

Val Val Thr Lys Tyr Ser Asn Phe Val Ser Phe Pro Leu Tyr Leu Asn
        275                 280                 285

Gly Arg Arg Met Asn Thr Leu Gln Ala Ile Trp Met Met Asp Pro Lys
    290                 295                 300

Asp Val Arg Glu Trp Gln His Glu Glu Phe Tyr Arg Tyr Val Ala Gln
305                 310                 315                 320

Ala His Asp Lys Pro Arg Tyr Thr Leu His Tyr Lys Thr Asp Ala Pro
                325                 330                 335

Leu Asn Ile Arg Ser Ile Phe Tyr Val Pro Asp Met Lys Pro Ser Met
            340                 345                 350

Phe Asp Val Ser Arg Glu Leu Gly Ser Ser Val Ala Leu Tyr Ser Arg
        355                 360                 365

Lys Val Leu Ile Gln Thr Lys Ala Thr Asp Ile Leu Pro Lys Trp Leu
    370                 375                 380

Arg Phe Ile Arg Gly Val Val Asp Ser Glu Asp Ile Pro Leu Asn Leu
385                 390                 395                 400

Ser Arg Glu Leu Leu Gln Glu Ser Ala Leu Ile Arg Lys Leu Arg Asp
                405                 410                 415

Val Leu Gln Gln Arg Leu Ile Lys Phe Phe Ile Asp Gln Ser Lys Lys
            420                 425                 430

Asp Ala Glu Lys Tyr Ala Lys Phe Phe Glu Asp Tyr Gly Leu Phe Met
        435                 440                 445

Arg Glu Gly Ile Val Thr Ala Thr Glu Gln Glu Val Lys Glu Asp Ile
    450                 455                 460
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Ala Lys Leu Leu Arg Tyr Glu Ser Ser Ala Leu Pro Ser Gly Gln Leu
465                 470                 475                 480

Thr Ser Leu Ser Glu Tyr Ala Ser Arg Met Arg Ala Gly Thr Arg Asn
                485                 490                 495

Ile Tyr Tyr Leu Cys Ala Pro Asn Arg His Leu Ala Glu His Ser Pro
            500                 505                 510

Tyr Tyr Glu Ala Met Lys Lys Lys Asp Thr Glu Val Leu Phe Cys Phe
        515                 520                 525

Glu Gln Phe Asp Glu Leu Thr Leu Leu His Leu Arg Glu Phe Asp Lys
    530                 535                 540

Lys Lys Leu Ile Ser Val Glu Thr Asp Ile Val Val Asp His Tyr Lys
545                 550                 555                 560

Glu Glu Lys Phe Glu Asp Arg Ser Pro Ala Ala Glu Cys Leu Ser Glu
                565                 570                 575

Lys Glu Thr Glu Glu Leu Met Ala Trp Met Arg Asn Val Leu Gly Ser
            580                 585                 590

Arg Val Thr Asn Val Lys Val Thr Leu Arg Leu Asp Thr His Pro Ala
        595                 600                 605

Met Val Thr Val Leu Glu Met Gly Ala Ala Arg His Phe Leu Arg Met
    610                 615                 620

Gln Gln Leu Ala Lys Thr Gln Glu Glu Arg Ala Gln Leu Leu Gln Pro
625                 630                 635                 640

Thr Leu Glu Ile Asn Pro Arg His Ala Leu Ile Lys Lys Leu Asn Gln
                645                 650                 655

Leu Arg Ala Ser Glu Pro Gly Leu Ala Gln Leu Leu Val Asp Gln Ile
            660                 665                 670

Tyr Glu Asn Ala Met Ile Ala Ala Gly Leu Val Asp Asp Pro Arg Ala
        675                 680                 685

Met Val Gly Arg Leu Asn Glu Leu Leu Val Lys Ala Leu Glu Arg His
    690                 695                 700


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Met Phe Ser Pro Asp Gln Glu Asn His Pro Ser Lys Ala Pro Val Lys
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Tyr Gly Glu Leu Ile Val Leu Gly Tyr Asn Gly Ser Leu Pro Asn Gly
            20                  25                  30

Asp Arg Gly Arg Arg Lys Ser Arg Phe Ala Leu Phe Lys Arg Pro Lys
        35                  40                  45

Ala Asn Gly Val Lys Pro Ser Thr Val His Ile Ala Cys Thr Pro Gln
    50                  55                  60

Ala Ala Lys Ala Ile Ser Asn Lys Asp Gln His Ser Ile Ser Tyr Thr
65                  70                  75                  80

Leu Ser Arg Ala Gln Thr Val Val Val Glu Tyr Thr His Asp Ser Asn
                85                  90                  95
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```
Thr Asp Met Phe Gln Ile Gly Arg Ser Thr Glu Ser Pro Ile Asp Phe
            100                 105                 110

Val Val Thr Asp Thr Val Pro Gly Ser Gln Ser Asn Ser Asp Thr Gln
        115                 120                 125

Ser Val Gln Ser Thr Ile Ser Arg Phe Ala Cys Arg Ile Ile Cys Glu
    130                 135                 140

Arg Asn Pro Pro Phe Thr Ala Arg Ile Tyr Ala Ala Gly Phe Asp Ser
145                 150                 155                 160

Ser Lys Asn Ile Phe Leu Gly Glu Lys Ala Ala Lys Trp Lys Thr Ser
                165                 170                 175

Asp Gly Gln Met Asp Gly Leu Thr Thr Asn Gly Val Leu Val Met His
            180                 185                 190

Pro Arg Asn Gly Phe Thr Glu Asp Ser Lys Pro Gly Ile Trp Arg Glu
        195                 200                 205

Ile Ser Val Cys Gly Asn Val Phe Ser Leu Arg Glu Thr Arg Ser Ala
    210                 215                 220

Gln Gln Arg Gly Lys Met Val Glu Ile Glu Thr Asn Gln Leu Gln Asp
225                 230                 235                 240

Gly Ser Leu Ile Asp Leu Cys Gly Ala Thr Leu Leu Trp Arg Thr Ala
                245                 250                 255

Glu Gly Leu Ser His Thr Pro Thr Val Lys His Leu Glu Ala Leu Arg
            260                 265                 270

Gln Glu Ile Asn Ala Ala Arg Pro Gln Cys Pro Val Gly Phe Asn Thr
        275                 280                 285

Leu Ala Phe Pro Ser Met Lys Arg Lys Asp Val Val Asp Glu Lys Gln
    290                 295                 300

Pro Trp Val Tyr Leu Asn Cys Gly His Val His Gly Tyr His Asn Trp
305                 310                 315                 320

Gly Asn Lys Glu Glu Arg Asp Gly Lys Asp Arg Glu Cys Pro Met Cys
                325                 330                 335

Arg Ser Val Gly Pro Tyr Val Pro Leu Trp Leu Gly Cys Glu Ala Gly
            340                 345                 350

Phe Tyr Val Asp Ala Gly Pro Pro Thr His Ala Phe Ser Pro Cys Gly
        355                 360                 365

His Val Cys Ser Glu Lys Thr Thr Ala Tyr Trp Ser Gln Ile Pro Leu
    370                 375                 380

Pro His Gly Thr His Thr Phe His Ala Ala Cys Pro Phe Cys Ala His
385                 390                 395                 400

Gln Leu Ala Gly Glu Gln Gly Tyr Ile Arg Leu Ile Phe Gln Gly Pro
                405                 410                 415

Leu Asp


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<222> LOCATION: (1)..(828)
<223> OTHER INFORMATION: /mol_type="protein"
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1               5                   10                  15
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Asp Ala Arg Glu Lys Leu Ala Leu Trp Asp Arg Arg Pro Asp Thr Thr
            20                  25                  30

Ala Pro Leu Thr Asp Arg Gln Thr Asp Ser Val Leu Glu Leu Lys Ala
        35                  40                  45

Ala Ala Glu Asn Leu Pro Val Pro Ala Glu Leu Pro Ile Glu Asp Leu
    50                  55                  60

Cys Ser Leu Thr Ser Gln Ser Leu Pro Ile Glu Leu Thr Ser Val Val
65                  70                  75                  80

Pro Glu Ser Thr Glu Asp Ile Leu Leu Lys Gly Phe Thr Ser Leu Gly
                85                  90                  95

Met Glu Glu Glu Arg Ile Glu Thr Ala Gln Gln Phe Phe Ser Trp Phe
            100                 105                 110

Ala Lys Leu Gln Thr Gln Met Asp Gln Asp Glu Gly Thr Lys Tyr Arg
        115                 120                 125

Gln Met Arg Asp Tyr Leu Ser Gly Phe Gln Glu Gln Cys Asp Ala Ile
    130                 135                 140

Leu Asn Asp Val Asn Ser Ala Leu Gln His Leu Glu Ser Leu Gln Lys
145                 150                 155                 160

Gln Tyr Leu Phe Val Ser Asn Lys Thr Gly Thr Leu His Glu Ala Cys
                165                 170                 175

Glu Gln Leu Leu Lys Glu Gln Ser Glu Leu Val Asp Leu Ala Glu Asn
            180                 185                 190

Ile Gln Gln Lys Leu Ser Tyr Phe Asn Glu Leu Glu Thr Ile Asn Thr
        195                 200                 205

Lys Leu Asn Ser Pro Thr Leu Ser Val Asn Ser Asp Gly Phe Ile Pro
    210                 215                 220

Met Leu Ala Lys Leu Asp Asp Cys Ile Thr Tyr Ile Ser Ser His Pro
225                 230                 235                 240

Asn Phe Lys Asp Tyr Pro Ile Tyr Leu Leu Lys Phe Lys Gln Cys Leu
                245                 250                 255

Ser Lys Ala Leu His Leu Met Lys Thr Tyr Thr Val Asn Thr Leu Gln
            260                 265                 270

Thr Leu Thr Ser Gln Leu Leu Lys Arg Asp Pro Ser Ser Val Pro Asn
        275                 280                 285

Ala Asp Asn Ala Phe Thr Leu Phe Tyr Val Lys Phe Arg Ala Ala Ala
    290                 295                 300

Pro Lys Val Arg Thr Leu Ile Glu Gln Ile Glu Leu Arg Ser Glu Lys
305                 310                 315                 320

Ile Pro Glu Tyr Gln Gln Leu Leu Asn Asp Ile His Gln Cys Tyr Leu
                325                 330                 335

Asp Gln Arg Glu Leu Leu Leu Gly Pro Ser Ile Ala Cys Thr Val Ala
            340                 345                 350

Glu Leu Thr Ser Gln Asn Asn Arg Asp His Cys Ala Leu Val Arg Ser
        355                 360                 365

Gly Cys Ala Phe Met Val His Val Cys Gln Asp Glu His Gln Leu Tyr
    370                 375                 380

Asn Glu Phe Phe Thr Lys Pro Thr Ser Lys Leu Asp Glu Leu Leu Glu
385                 390                 395                 400

Lys Leu Cys Val Ser Leu Tyr Asp Val Phe Arg Pro Leu Ile Ile His
                405                 410                 415

Val Ile His Leu Glu Thr Leu Ser Glu Leu Cys Gly Ile Leu Lys Asn
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            420                 425                 430

Glu Val Leu Glu Asp His Val Gln Asn Asn Ala Glu Gln Leu Gly Ala
        435                 440                 445

Phe Ala Ala Gly Val Lys Gln Met Leu Glu Asp Val Gln Glu Arg Leu
    450                 455                 460

Val Tyr Arg Thr His Ile Tyr Ile Gln Thr Asp Ile Thr Gly Tyr Lys
465                 470                 475                 480

Pro Ala Pro Gly Asp Leu Ala Tyr Pro Asp Lys Leu Val Met Met Glu
                485                 490                 495

Gln Ile Ala Gln Ser Leu Lys Asp Glu Gln Lys Lys Val Pro Ser Glu
            500                 505                 510

Ala Ser Phe Ser Asp Val His Leu Glu Glu Gly Glu Ser Asn Ser Leu
        515                 520                 525

Thr Lys Ser Gly Ser Thr Glu Ser Leu Asn Pro Arg Pro Gln Thr Thr
    530                 535                 540

Ile Ser Pro Ala Asp Leu His Gly Met Trp Tyr Pro Thr Val Arg Arg
545                 550                 555                 560

Thr Leu Val Cys Leu Ser Lys Leu Tyr Arg Cys Ile Asp Arg Ala Val
                565                 570                 575

Phe Gln Gly Leu Ser Gln Glu Ala Leu Ser Ala Cys Ile Gln Ser Leu
            580                 585                 590

Leu Gly Ala Ser Glu Ser Ile Ser Lys Asn Lys Thr Gln Ile Asp Gly
        595                 600                 605

Gln Leu Phe Leu Ile Lys His Leu Leu Ile Leu Arg Glu Gln Ile Ala
    610                 615                 620

Pro Phe His Thr Glu Phe Thr Ile Lys Glu Ile Ser Leu Asp Leu Lys
625                 630                 635                 640

Lys Thr Arg Asp Ala Ala Phe Lys Ile Leu Asn Pro Met Thr Val Pro
                645                 650                 655

Arg Phe Phe Arg Leu Asn Ser Asn Asn Ala Leu Ile Glu Phe Leu Leu
            660                 665                 670

Glu Gly Thr Pro Glu Ile Arg Glu His Tyr Leu Asp Ser Lys Lys Asp
        675                 680                 685

Val Asp Arg His Leu Lys Ser Ala Cys Glu Gln Phe Ile Gln Gln Gln
    690                 695                 700

Thr Lys Leu Phe Val Glu Gln Leu Glu Glu Phe Met Thr Lys Val Ser
705                 710                 715                 720

Ala Leu Lys Thr Met Ala Ser Gln Gly Gly Pro Lys Tyr Thr Leu Ser
                725                 730                 735

Gln Gln Pro Trp Ala Gln Pro Ala Lys Val Asn Asp Leu Ala Ala Thr
            740                 745                 750

Ala Tyr Lys Thr Ile Lys Thr Lys Leu Pro Val Thr Leu Arg Ser Met
        755                 760                 765

Ser Leu Tyr Leu Ser Asn Lys Asp Thr Glu Phe Ile Leu Phe Lys Pro
    770                 775                 780

Val Arg Asn Asn Ile Gln Gln Val Phe Gln Lys Phe His Ala Leu Leu
785                 790                 795                 800

Lys Glu Glu Phe Ser Pro Glu Asp Ile Gln Ile Ile Ala Cys Pro Ser
                805                 810                 815

Met Glu Gln Leu Ser Leu Leu Leu Ser Val Ser Lys
            820                 825
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Lys His Ser Glu Gly Leu Leu Pro Val Ile Thr Pro Met Ala Gly Asn
20 25 30

Gln Arg Val Glu Asp Pro Ala Arg Ser Cys Met Glu Gly Lys Ser Phe
35 40 45

Leu Gln Lys Ser Pro Ser Lys Glu Pro His Phe Thr Asp Phe Glu Gly
50 55 60

Lys Thr Ser Phe Gly Met Ser Val Phe Asn Leu Ser Asn Ala Ile Met
65 70 75 80

Gly Ser Gly Ile Leu Gly Leu Ala Tyr Ala Met Ala Asn Thr Gly Ile
85 90 95

Ile Leu Phe Leu Phe Leu Leu Thr Ala Val Ala Leu Leu Ser Ser Tyr
100 105 110

Ser Ile His Leu Leu Leu Lys Ser Ser Gly Val Val Gly Ile Arg Ala
115 120 125

Tyr Glu Gln Leu Gly Tyr Arg Ala Phe Gly Thr Pro Gly Lys Leu Ala
130 135 140

Ala Ala Leu Ala Ile Thr Leu Gln Asn Ile Gly Ala Met Ser Ser Tyr
145 150 155 160

Leu Tyr Ile Ile Lys Ser Glu Leu Pro Leu Val Ile Gln Thr Phe Leu
165 170 175

Asn Leu Glu Glu Lys Thr Ser Asp Trp Tyr Met Asn Gly Asn Tyr Leu
180 185 190

Val Ile Leu Val Ser Val Thr Ile Ile Leu Pro Leu Ala Leu Met Arg
195 200 205

Gln Leu Gly Tyr Leu Gly Tyr Ser Ser Gly Phe Ser Leu Ser Cys Met
210 215 220

Val Phe Phe Leu Ile Ala Val Ile Tyr Lys Lys Phe His Val Pro Cys
225 230 235 240

Pro Leu Pro Pro Asn Phe Asn Asn Thr Thr Gly Asn Phe Ser His Val
245 250 255

Glu Ile Val Lys Glu Lys Val Gln Leu Gln Val Glu Pro Glu Ala Ser
260 265 270

Ala Phe Cys Thr Pro Ser Tyr Phe Thr Leu Asn Ser Gln Thr Ala Tyr
275 280 285

Thr Ile Pro Ile Met Ala Phe Ala Phe Val Cys His Pro Glu Val Leu
290 295 300

Pro Ile Tyr Thr Glu Leu Lys Asp Pro Ser Lys Lys Lys Met Gln His
305 310 315 320

Ile Ser Asn Leu Ser Ile Ala Val Met Tyr Ile Met Tyr Phe Leu Ala
325 330 335

Ala Leu Phe Gly Tyr Leu Thr Phe Tyr Asn Gly Val Glu Ser Glu Leu

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            340                 345                 350

Leu His Thr Tyr Ser Lys Val Asp Pro Phe Asp Val Leu Ile Leu Cys
        355                 360                 365

Val Arg Val Ala Val Leu Thr Ala Val Thr Leu Thr Val Pro Ile Val
    370                 375                 380

Leu Phe Pro Val Arg Arg Ala Ile Gln Gln Met Leu Phe Pro Asn Gln
385                 390                 395                 400

Glu Phe Ser Trp Leu Arg His Val Leu Ile Ala Val Gly Leu Leu Thr
                405                 410                 415

Cys Ile Asn Leu Leu Val Ile Phe Ala Pro Asn Ile Leu Gly Ile Phe
            420                 425                 430

Gly Val Ile Gly Ala Thr Ser Ala Pro Phe Leu Ile Phe Ile Phe Pro
        435                 440                 445

Ala Ile Phe Tyr Phe Arg Ile Met Pro Thr Glu Lys Glu Pro Ala Arg
    450                 455                 460

Ser Thr Pro Lys Ile Leu Ala Leu Cys Phe Ala Met Leu Gly Phe Leu
465                 470                 475                 480

Leu Met Thr Met Ser Leu Ser Phe Ile Ile Ile Asp Trp Ala Ser Gly
                485                 490                 495

Thr Ser Arg His Gly Gly Asn His
            500


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Pro Arg Ser Pro Leu Thr Pro Arg His Gly Pro Gly Leu Ala Asn Val
            20                  25                  30

Cys Gln Tyr Asp Glu Trp Ile Ala Val Arg His Glu Ala Thr Leu Leu
        35                  40                  45

Pro Met Gln Glu Asp Leu Ser Ile Trp Leu Ser Gly Leu Leu Gly Ile
    50                  55                  60

Lys Val Lys Ala Glu Lys Leu Leu Glu Glu Leu Asp Asn Gly Val Leu
65                  70                  75                  80

Leu Cys Gln Leu Ile Asp Val Leu Gln Asn Met Val Lys Thr Cys Asn
                85                  90                  95

Ser Glu Glu Ser Gly Asn Phe Pro Met Arg Lys Val Pro Cys Lys Lys
            100                 105                 110

Asp Ala Ala Ser Gly Ser Phe Phe Ala Arg Asp Asn Thr Ala Asn Phe
        115                 120                 125

Leu His Trp Cys Arg Asp Ile Gly Val Asp Glu Thr Tyr Leu Phe Glu
    130                 135                 140

Ser Glu Gly Leu Val Leu His Lys Asp Pro Arg Gln Val Tyr Leu Cys
145                 150                 155                 160

Leu Leu Glu Ile Gly Arg Ile Val Ser Arg Tyr Gly Val Glu Pro Pro
                165                 170                 175
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Val Leu Val Lys Leu Glu Lys Glu Ile Glu Leu Glu Glu Thr Leu Leu
            180                 185                 190

Asn Thr Ser Gly Pro Glu Asp Ser Ile Ser Ile Pro Lys Ser Cys Cys
        195                 200                 205

Arg His Glu Glu Leu His Glu Ala Val Lys His Ile Ala Glu Asp Pro
    210                 215                 220

Pro Cys Ser Cys Ser His Arg Phe Ser Ile Glu Tyr Leu Ser Glu Gly
225                 230                 235                 240

Arg Tyr Arg Leu Gly Asp Lys Ile Leu Phe Ile Arg Met Leu His Gly
                245                 250                 255

Lys His Val Met Val Arg Val Gly Gly Gly Trp Asp Thr Leu Gln Gly
            260                 265                 270

Phe Leu Leu Lys Tyr Asp Pro Cys Arg Ile Leu Gln Phe Ala Thr Leu
        275                 280                 285

Glu Gln Lys Ile Leu Ala Phe Gln Lys Gly Val Ser Asn Glu Ser Val
    290                 295                 300

Pro Asp Ser Pro Ala Arg Thr Pro Gln Pro Pro Glu Met Asn Pro Leu
305                 310                 315                 320

Ser Ala Val Asn Met Phe Gln Lys Gln Asn Ser Lys Pro Ser Val Pro
                325                 330                 335

Val Ser Ile Pro Lys Ser Lys Glu Lys Gln Gly Arg Pro Pro Gly Ala
            340                 345                 350

Leu Val Pro Ala Ser Ser Leu Lys Gly Gly Asn Leu Gly Ser Met Ser
        355                 360                 365

Val Arg Ser Lys Leu Pro Asn Ser Pro Ala Ala Ser Ser His Pro Lys
    370                 375                 380

Leu Lys Ser Ser Lys Gly Ile Thr Lys Lys Pro Gln Ala Pro Ser Asn
385                 390                 395                 400

Asn Ala Ser Ser Ser Leu Ala Ser Leu Asn Pro Val Gly Lys Asn Thr
                405                 410                 415

Ser Ser Pro Ala Leu Pro Arg Thr Ala Pro Cys Ile Ser Glu Ser Pro
            420                 425                 430

Arg Lys Cys Ile Ser Ser Pro Asn Thr Pro Lys Ala Lys Val Ile Pro
        435                 440                 445

Ala Gln Asn Ser Ala Asp Leu Pro Glu Ser Thr Leu Leu Pro Asn Lys
    450                 455                 460

Cys Ser Gly Lys Thr Gln Pro Lys Tyr Leu Lys His Asn His Ile Ser
465                 470                 475                 480

Ser Arg Asp Asn Ala Val Ser His Leu Ala Ala His Ser Asn Ser Ser
                485                 490                 495

Ser Lys Cys Pro Lys Leu Pro Lys Ala Asn Ile Pro Val Arg Pro Lys
            500                 505                 510

Pro Ser Phe Gln Ser Ser Ala Lys Met Thr Lys Thr Ser Ser Lys Thr
        515                 520                 525

Ile Ala Thr Gly Leu Gly Thr Gln Ser Gln Pro Ser Asp Gly Ala Pro
    530                 535                 540

Gln Ala Lys Pro Val Pro Ala Gln Lys Leu Lys Ser Ala Leu Asn Leu
545                 550                 555                 560

Asn Gln Pro Val Ser Val Ser Ser Val Ser Pro Val Lys Ala Thr Gln
                565                 570                 575

Lys Ser Lys Asp Lys Asn Ile Val Ser Ala Thr Lys Lys Gln Pro Gln
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-continued

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            580                 585                 590

Asn Lys Ser Ala Phe Gln Lys Thr Gly Pro Ser Ser Leu Lys Ser Pro
        595                 600                 605

Gly Arg Thr Pro Leu Ser Ile Val Ser Leu Pro Gln Ser Ser Thr Lys
    610                 615                 620

Thr Gln Thr Ala Pro Lys Ser Ala Gln Thr Val Ala Lys Ser Gln His
625                 630                 635                 640

Ser Thr Lys Gly Pro Pro Arg Ser Gly Lys Thr Pro Ala Ser Ile Arg
                645                 650                 655

Lys Pro Pro Ser Ser Val Lys Asp Ala Asp Ser Gly Asp Lys Lys Pro
            660                 665                 670

Thr Ala Lys Lys Lys Glu Asp Asp Asp His Tyr Phe Val Met Thr Gly
        675                 680                 685

Ser Lys Lys Pro Arg Lys
    690
```

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

detecting the presence and/or amount of at least one marker or at least one antibody against at least one marker in a sample from said subject, wherein the at least one marker is selected from the group consisting of Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), and *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3); and

correlating the result to the occurrence or non-occurrence of ischemic stroke in said subject.

2. The method of claim **1**, wherein the ischemic stroke comprises an ischemic stroke of lacunar subtype.

3. The method of claim **1**, wherein the sample comprises a sample of blood, serum and/or plasma from said subject.

4. The method of claim **1**, wherein the presence and/or amount of at least one antibody against the at least one marker is detected.

5-6. (canceled)

7. A device for the detection of ischemic stroke, comprising:

a) means for determining the amount and/or presence of at least one marker or at least one antibody against at least one marker in a sample from a subject, wherein the at least one marker is selected from the group consisting of:

Pellino homolog 1 (*Drosophila*) (PELI1), TNF receptor-associated protein 1 (TRAP1), Conserved oligomeric Golgi complex subunit 3 (COG3), *Homo sapiens* solute carrier family 38 member 3 (SLC38A3), and *Homo sapiens* growth arrest-specific 2 like 3 (GAS2L3); and

b) means for analyzing the amounts and/or presence of the marker(s) or antibodies against the marker(s) against a reference value.

8. Device of claim **7**, wherein the ischemic stroke detected comprises an ischemic stroke of lacunar subtype.

9. A kit adapted for carrying out the method of claim **1** comprising:

a) means for determining the amount and/or presence of the at least one marker or antibody against the at least one marker in a sample from a subject; and

b) means for analyzing the amounts and/or presence of the at least one marker or antibodies against the at least one marker against a reference value.

10-11. (canceled)

12. A method of diagnosing ischemic stroke in a subject, comprising:

detecting the presence and/or amount of at least one marker or at least one antibody against at least one marker in a sample from said subject, wherein the at least one marker has more than 80% identity to a protein sequence consisting of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, or SEQ ID NO. 5; and

correlating the result to the occurrence or non-occurrence of ischemic stroke in said subject.

* * * * *

专利名称(译)	检测缺血性中风的方法		
公开(公告)号	US20130122519A1	公开(公告)日	2013-05-16
申请号	US13/812163	申请日	2011-07-28
[标]申请(专利权)人(译)	CURDT INGO BLINCKO STUART DHEIN JENS DEVANARAYAN VISWANATH 比利亚隆加琼作为		
申请(专利权)人(译)	CURDT，INGO BLINCKO，STUART DHEIN，JENS DEVANARAYAN，VISWANATH 比利亚隆加，JOAN蒙塔内尔		
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[标]发明人	CURDT INGO BLINCKO STUART DHEIN JENS DEVANARAYAN VISWANATH VILLALONGA JOAN MONTANER		
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IPC分类号	G01N33/53		
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优先权	2010007854 2010-07-28 EP		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及腔隙亚型的缺血性中风的诊断标志物的鉴定和用途。本发明涉及用于执行这些方法的装置和套件。

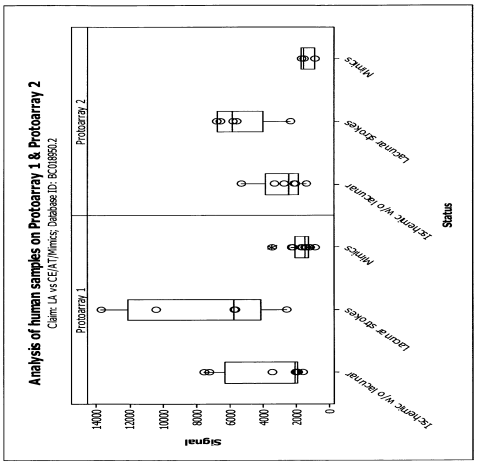

Fig. 1