

DESCRIPTION

TITLE OF INVENTION:

Determination Method using Genetic Variants

5 TECHNICAL FIELD

[0001] The present invention relates to a determination method using genetic variant.

BACKGROUND ART

[0002] In Japan, 110 thousand people die of stroke every year, and 70 to 80 percent of the death is caused by cerebral infarction, which is a major cause of hemiplegia, dementia, and the like. Cerebral infarction is a disease where brain cells die due to occlusion of a blood vessel that carries blood to the brain cells. Cerebral infarction is mainly classified into atherothrombotic cerebral infarction mainly caused by arteriosclerosis of a relatively large blood vessel in the brain, lacunar infarction caused by occlusion of a small blood vessel in the brain, and cardiogenic embolism in which a thrombus formed in the heart due to a heart disease, such as atrial fibrillation, flows from the heart to obstruct a large vessel in the brain.

[0003] Japanese Patent Laying-Open No. 2020-092660 (PTL 1) discloses a method for predicting a risk for a subject without cerebral infarction to develop cerebral infarction, based on information about the presence or absence of the RNF213 p.R4810K gene variant in a sample collected from the subject and information about the sex of the subject. International Patent Laying-Open No. WO 2020/121489 (PTL 2) discloses that when a sample collected from a subject without cerebral infarction has the RNF213 p.R4810K gene variant, determination can be made that the subject can develop cerebral infarction earlier than normal.

25 CITATION LIST

PATENT LITERATURE

[0004] PTL 1: Japanese Patent Laying-Open No. 2020-092660

PTL 2: International Patent Laying-Open No. WO 2020/121489

SUMMARY OF INVENTION

TECHNICAL PROBLEM

[0005] When a patient developed cerebral infarction, MRI (Magnetic Resonance Imaging) examination or CT (Computed Tomography) examination can be performed to identify the type of the cerebral infarction and the location of the abnormal blood vessel in the brain. Treatments that can be mainly performed for cerebral infarction include thrombolytic therapy/tPA (tissue Plasminogen Activator) which involves using a drug to lyse a thrombus obstructing a blood vessel, and endovascular therapy which involves inserting a narrow tube called a catheter into a blood vessel at the groin and/or the like to directly remove a thrombus located at the lesion in the brain.

[0006] Some patients who underwent endovascular therapy for cerebral infarction experience reocclusion of the treated blood vessel. Because of this, the doctor needs to monitor for reocclusion regularly for several months after the procedure, and there are also other burdens on the doctor including home visit services for the patient. The present invention aims at providing a method that makes it easy to predict the risk of reocclusion of a blood vessel after endovascular therapy.

SOLUTION TO PROBLEM

[0007] An embodiment of the present invention relates to

a method for predicting reocclusion of an intracranial cerebral blood vessel, the method comprising:

detecting whether or not a subject has an RNF213 p.R4810K variant by using a sample collected from the subject; and

when the subject has the RNF213 p.R4810K variant, determining that a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0008] An embodiment of the present invention relates to

a kit for determining, based on the presence or absence of an RNF213 p.R4810K variant, whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high, the kit comprising:

a pair of PCR primers for amplifying a base sequence including a gene variation corresponding to the RNF213 p.R4810K variant; and

a first probe capable of binding to a wild-type base sequence corresponding to the RNF213 p.R4810K variant and a second probe capable of binding to a variant base sequence corresponding to the RNF213 p.R4810K variant, wherein

the first probe is labeled with a fluorescent dye, and the second probe is labeled with a different fluorescent dye.

[0009] An embodiment of the present invention relates to

a genetic marker consisting of an RNF213 p.R4810K variant for determining whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

ADVANTAGEOUS EFFECTS OF INVENTION

[0010] The present invention makes it possible to predict the risk of reocclusion of a blood vessel after endovascular therapy.

BRIEF DESCRIPTION OF DRAWINGS

[0011] Fig. 1 is a flowchart of case study conducted in Examples.

Fig. 2 is a graph showing rates of instant and early reocclusion between variant carriers and noncarriers among large-vessel occlusion patients.

Fig. 3 is a graph showing rates of instant and early reocclusion between variant carriers and noncarriers among intracranial atherosclerotic disease-related large-vessel occlusion patients.

Fig. 4 shows a case of a 59-year-old man with the RNF213 p.R4810K variant who developed sudden left hemiplegia (case 3 in Table 3). (A) Diffusion-weighted magnetic resonance (MR) imaging, showing several acute ischemic lesions in the cortical perfusion territory of the right middle cerebral artery (MCA). (B) MR angiography, showing the right MCA M1 occlusion. (C) Right internal carotid angiography, showing the right proximal MCA M1 occlusion. (D) Right internal carotid angiography, showing successful reperfusion but remaining severe stenosis

after mechanical thrombectomy with delayed peripheral perfusion. (E) Final angiography after angioplasty and intracranial stenting. (F) Early reocclusion at postoperative day 7 with sudden left hemiplegia.

DESCRIPTION OF EMBODIMENTS

5 [0012] [Method for Predicting Reocclusion of Intracranial Cerebral Blood Vessel]

A method for predicting reocclusion of an intracranial cerebral blood vessel according to an embodiment of the present invention comprises:

detecting whether or not a subject has an RNF213 p.R4810K variant by using a sample collected from the subject; and

10 when the subject has the RNF213 p.R4810K variant, determining that a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0013] The method for predicting reocclusion of an intracranial cerebral blood vessel according to the present invention makes it easy to predict whether or not the risk of reocclusion of a blood vessel after endovascular therapy is high. The method for predicting reocclusion of an intracranial cerebral blood vessel according to the present invention makes it possible to identify a patient who has a high risk of reocclusion after endovascular therapy. This, in turn, makes it possible to conduct treatment and/or examination aiming at preventing reocclusion, preferentially or at a high frequency for a patient with a high risk of reocclusion. The present method can be regarded as a method for assisting prediction of a risk of blood vessel reocclusion. The present method does not include a step of performing determination by a doctor, and does not fall under the category of a method of diagnosing a human.

20 [0014] The p.R4810K variant (c.14576G>A) of the ring finger protein 213 gene (RNF213) is a founder variant for moyamoya disease (occlusion of the circle of Willis)¹⁾ in East Asians¹⁻⁴⁾. The RNF213 p.R4810K variant is a single nucleotide variant (SNV) of the nucleic acid sequence as set forth in SEQ ID NO: 2 at 73097 G>A. In healthy populations, this RNF213 SNV is found in 2.5% of Japanese, 2.7% of Korean, and 0.9% of Chinese people, but it is absent in Caucasoid (European)

people^{3,5,6}). A recent study including 46958 Japanese subjects (17752 cases of cerebral infarction and 29206 controls) demonstrated that the RNF213 p.R4810K variant was a risk factor for ischemic stroke (cerebral infarction) in Japanese people; the variant was found in 5.2% of patients with noncardioembolic stroke and 2.1% of healthy controls⁷).

[0015] SEQ ID NO: 2 is a partial nucleotide sequence of human chromosome 17 DNA including the RNF213 gene (also called the myserin gene: moyamoya steno-occlusive disease-associated AAA+ and RING finger protein) and the neighboring gene [FLJ3520, NPTX1, CARD14, and Raptor (KIAA1303)], and corresponds to nucleotides 43560001 to 43795000 of Contig #NT010783.15 registered in NCBI. In the expression "G>A", the major allele base (G in this case) precedes the symbol ">" and the minor allele base (A in this case) follows the symbol.

[0016] The RNF213 gene encodes a protein containing dual AAA+ ATPase domains and an E3 ligase domain, which has an important role in regulating vascular remodeling and angiogenesis^{3,8}). In recent studies, the overexpression of RNF213 p.R4810K, but not the overexpression of wild-type RNF213, inhibited angiogenic activity and the proliferation of human endothelial cells^{8,9}). In addition, the accumulation of filamin A, a substrate of RNF213, led to accelerated vascular remodeling caused by media-to-intima migration and the proliferation of vascular smooth muscle cells^{10,11}). Such vascular cellular changes may cause vascular negative remodeling, a notable feature of intracranial major artery stenosis associated with the RNF213 p.R4810K variant¹²⁻¹⁴).

[0017] (Step of Detecting Whether or Not Subject Has RNF213 p.R4810K Variant Using Sample Collected from Subject)

The presence or absence of the RNF213 p.R4810K variant can be detected using genomic DNA, RNA, or protein. The RNF213 p.R4810K variant as the detection target may be a homozygote of the variant or may be a heterozygote of the variant and the wild type (GA type).

[0018] When a nucleic acid is used for detection of the variant, a SNV in which the

14576th base of the RNF213 gene is adenine (A) in the variant (as compared to the wild type where the base is guanine (G)) may be detected. The method for SNV detection is not particularly limited as long as it is well known in the art, and, for example, it can be a PCR-RFLP (restriction fragment length polymorphism) method, a PCR-SSCP (single strand conformation polymorphism) method, a PCR-SSO (specific sequence oligonucleotide) method, an ASO (Allele Specific Oligonucleotide) hybridization method, a sequencing method, a next-generation sequencing method, an ARMS (Amplification Refracting Mutation System) method, a denaturing gradient gel electrophoresis method, an RNaseA cleavage method, a DOL (Dye-labeled Oligonucleotide Ligation) method, a TaqMan PCR method, a real-time PCR method, a SNaP Shot method, a mass spectrometry method, a primer extension method, an Invader method, a DNA chip method or a DNA-microarray-based method, a single-base extension method, a Southern blot hybridization method, a dot hybridization method, a pyrosequencing method, an exonuclease cycling assay, and/or the like. The SNV may be detected by using a kit described below.

[0019] For detection of the presence or absence of the RNF213 p.R4810K variant using protein, a variation in which arginine (R) at the 4810th position of the protein (SEQ ID NO: 1) encoded by the RNF213 gene is changed to lysine (K) may be detected. The amino acid variation can be detected by using conformational analysis, mass spectrometry, antigen-antibody reaction, ultracentrifugation, electrophoresis, absorbance measurement, or a combination of these, for example.

[0020] The sample may be derived from any tissue, cell, or body fluid from which genomic DNA, protein, or the like can be collected. Examples of the sample include hair, nail, skin, mucosa, blood, saliva, lacrimal fluid, urine, stool, sweat, lymph fluid, breast milk, amniotic fluid, and liquid from body cavities (including ascitic fluid, pleural fluid, and cerebrospinal fluid). The blood may be whole blood, or may be plasma or serum. From the viewpoint of minimal invasiveness at the time of collection, the sample is preferably blood or saliva. The sample may be diluted with physiological saline, buffer, and/or the like, and may be an extract or a disruption

product of tissue or cells. The sample may be cryopreserved. The sample collection may be carried out by a known method that is suitable for the type of the sample.

[0021] The subject may be a human, and may be either a human without cerebral infarction or a human with cerebral infarction. The human is not particularly limited in its race, but preferably an East Asian (Eastern Asian/Mongoloid). An East Asian means a person who is from Japan, Korea, China, Taiwan, or Mongolia. The East Asian may be a Japanese person, a Korean person, or a Chinese person. The race refers to a group of people that can be differentiated as a specific subgroup of Homo sapiens. Based on genetic information and/or the like, there are five major races in the world, which are Africans (Negroid), Caucasoid (whites), Oceanians (Australoid), Eastern Asians (Mongoloid), and Native Americans, for example.

[0022] (Step of Determining That Possibility of Reocclusion of Intracranial Cerebral Blood Vessel to Occur after Endovascular Therapy for Occlusion of Intracranial Cerebral Blood Vessel is High When Subject Has RNF213 p.R4810K Variant)

In the present invention, when the patient has the RNF213 p.R4810K variant, it is determined that reocclusion is likely to occur after endovascular therapy.

Reocclusion after endovascular therapy is likely to occur in the following manner:

endovascular therapy can damage vascular endothelial cells, and this damage can facilitate thrombogenesis mechanism to cause reocclusion with a thrombus. As the occlusion of an intracranial cerebral blood vessel before the reocclusion (the occlusion that received endovascular therapy), cardiogenic cerebral infarction and atherosclerotic cerebral infarction may be mentioned. Atherosclerosis refers to a condition where cholesterol, fat, inflammatory cells, and the like are deposited in the lumen of a cerebral blood vessel to form a plaque to make the lumen smaller, and when a thrombus adheres to the plaque or when the plaque ruptures, cerebral infarction can occur. In the case where the occlusion of an intracranial cerebral blood vessel before reocclusion is an occlusion caused by atherosclerosis, the blood vessel tends to be damaged at the time of endovascular therapy and thereby reocclusion is likely to occur. In the case where the patient has the RNF213 p.R4810K variant and the cause of the cerebral infarction is

atherosclerosis, it is predicted that the risk of reocclusion of the blood vessel after endovascular therapy is relatively high. In the case where the patient has the RNF213 p.R4810K variant, it is predicted that restenosis is likely to occur after endovascular therapy. The present invention may be a method for predicting restenosis of an intracranial cerebral blood vessel.

[0023] The endovascular therapy may be a method to catch and collect a thrombus using a stent (a metal mesh) mounted on the tip of a catheter, a method to aspirate and collect a thrombus with an aspiration pump connected to a catheter, a method to expand the blood vessel with a balloon, and/or a method to leave a stent inside the blood vessel. As the endovascular therapy, a therapy that is suitable for the type of the cerebral infarction as well as the size and position of the thrombus, or a combination of these, is selected. The endovascular therapy may also be performed for cerebrovascular stenosis.

[0024] The method for predicting reocclusion of an intracranial cerebral blood vessel may further include selecting a tool to use in the endovascular therapy based on the possibility of reocclusion of the blood vessel. As described above, when a burden is applied to a blood vessel, reocclusion of the blood vessel tends to occur. It is predicted that the burden applied to a blood vessel is relatively small when it is applied by an aspiration-based thrombus-collection method and/or a stent-based thrombus-collection method, and it is relatively great when applied by a balloon-based blood vessel-expanding method and/or a stent-leaving method. When it is determined that reocclusion is highly likely to occur, it is possible to select a treatment method and a tool that apply a relatively small burden to the blood vessel, to lower the risk of reocclusion. As the tool, a drug-eluting stent (DES) may be selected.

[0025] Based on the possibility of reocclusion of the blood vessel, it is also possible to adjust the dose, dosage regimen, administration period, administration timing, and the like of the drug which is to be administered. Examples of the drug include an antiplatelet drug, an antithrombin drug, an anticoagulant drug, dextran, and the like. The drug may be either a drug for oral administration or a drug for injection.

[0026] The step to determining that the possibility of reocclusion of the blood vessel is high may include determining that the possibility of reocclusion of the intracranial cerebral blood vessel to occur at a timing earlier than normal after the endovascular therapy is high. The timing earlier than normal after the endovascular therapy may be from confirmation of successful reperfusion by the endovascular therapy to during the endovascular therapy (instant reocclusion), or may be within 2 weeks after confirmation of successful reperfusion by the endovascular therapy (early reocclusion).

[0027] The intracranial cerebral blood vessel treated by the endovascular therapy may be a cerebral large vessel, and examples of a cerebral large vessel include an intracranial internal carotid artery, a middle cerebral artery, an anterior cerebral artery, a vertebral artery, a basilar artery, and the like. The risk of reocclusion after endovascular therapy tends to be high particularly in the intracranial internal carotid artery and the middle cerebral artery.

[0028] [Kit]

A kit according to an embodiment of the present invention is a kit for determining, based on the presence or absence of an RNF213 p.R4810K variant, whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high, the kit comprising:

a pair of PCR primers for amplifying a base sequence including a gene variation corresponding to the RNF213 p.R4810K variant; and

a first probe capable of binding to a wild-type base sequence corresponding to the RNF213 p.R4810K variant and a second probe capable of binding to a variant base sequence corresponding to the RNF213 p.R4810K variant, wherein

the first probe is labeled with a fluorescent dye, and the second probe is labeled with a different fluorescent dye.

The kit may be a kit for determining whether or not a possibility of restenosis of a blood vessel that received endovascular therapy is high.

[0029] Use of the kit which has the pair of primers as well as a set of the first probe and

the second probe enables rapid and highly-specific detection of the RNF213 p.R4810K variant in a sample collected from a subject. Based on whether or not the subject has the RNF213 p.R4810K variant, it is possible to predict whether or not the risk of reocclusion or restenosis of a blood vessel after endovascular therapy is high. The present kit is capable of providing information that is usable for predicting the risk of reocclusion or restenosis of a blood vessel after endovascular therapy. The present kit is capable of identifying the p.R4810K variant (c.14576G>A) in the RNF213 gene of the nucleic acid, preferably genomic DNA, in the sample collected from the subject. The RNF213 p.R4810K variant thus detected may be a homozygote of the variant or a heterozygote of the variant and the wild type.

[0030] The pair of PCR primers is capable of amplifying a nucleic acid fragment that includes the target RNF213 p.R4810K variant SNV site through polymerase chain reaction (PCR) which uses, as a template, genomic DNA in a specimen collected from the subject. The site to be amplified for wild-type detection is the same as the site to be amplified for variant detection. The length of the nucleic acid fragment to be amplified is preferably 80 to 100 bases that contain the RNF213 p.R4810K variant SNV site. As the pair of PCR primers of this type, an oligonucleotide (a forward primer and a reverse primer) that hybridizes under stringent conditions with a region consisting of the base sequence as set forth in SEQ ID NO: 7 is preferable. The stringent conditions herein refer to conditions where the binding between the template DNA and the primer is specific in PCR annealing, which is a step where the primer binds to the template DNA. T_m of the primer may be set at 58 to 60°C, for example. The base length of the PCR primer is preferably from 15 to 25 bases. From the viewpoint of specificity, the kit according to the present invention preferably comprises a pair of PCR primers that includes a primer having the base sequence as set forth in SEQ ID NO: 3 and a primer having the base sequence as set forth in SEQ ID NO: 4.

5'-TTCCAGAACGTCCAGCAAGT-3' (forward; SEQ ID NO: 3)

5'-ACAGTCCTGGTCCTGTCAGA-3' (reverse; SEQ ID NO: 4)

[0031] The following sequence is the base sequence of a region of the RNF213 gene

that contains the RNF213 p.R4810K variant SNV. As long as it contains the SNV site, any base sequence from the following sequence can be amplified.

5'-

5 CCCAATAACATTTTTTTAGGTAAATAAAAATTGTTACTGGGTGGTCTTCCCTT
CTCCAGGAAGCAGAGCTGAGGCTGGTAAAGTTCCTGCCTGAGATTTTGGCC
TTGCAAAGGGATCTAGTGAAGCAGTTCCAGAACGTCCAGCAAGTTGAATAC
AGCTCCATCAGAGGCTTCCTCAGCAAGCACAGCTCAGGTGTGGCTCTGCTC
TGACAGGACCAGGACTGTCCCGCATTTGGCGGTTCGAAAGGATCACTGCAT
AGGGGAACAGGGTGGGGCGGAGGGGAGGAGGCGCTGATGGGTGCTCTATA
10 GCCTAAGCCCTTACCATGCGGTGAAGGGTGCTTGAACCCCAAAA-3' (SEQ ID
NO: 7)

[0032] The probe is used in real-time PCR that is based on the FRET principle; the sequence of the probe is designed so as to be suitable for the method, and a reporter fluorescent dye and a quencher are selected. The first probe binds to a nucleic acid
15 fragment in which the SNV site at the 14576th position of the RNF213 gene is the wild-type base (G). The second probe binds to a variant nucleic acid fragment in which the SNV site at the 14576th position of the RNF213 gene has a substitution of G>A. Preferably, the first probe hybridizes under stringent conditions with a nucleic acid fragment that has a wild-type base sequence produced by PCR amplification using
20 the wild-type RNF213 gene as a template, but does not hybridize under stringent conditions with a nucleic acid fragment that contains the RNF213 p.R4810K variant SNV site produced by PCR amplification. On the other hand, the second probe hybridizes under stringent conditions with a nucleic acid fragment that contains the RNF213 p.R4810K variant SNV site produced by PCR amplification, but does not
25 hybridize under stringent conditions with a nucleic acid fragment that has a wild-type base sequence produced by PCR amplification using the wild-type RNF213 gene as a template. The stringent conditions refer to conditions where a specific hybrid is formed between the PCR-amplified nucleic acid fragment and the probe at the time of annealing but no non-specific hybrid is formed. It is preferable to design the sequence

of the probe so that it targets substantially the center of the PCR product amplified by the pair of primers.

[0033] The base length of the probe is preferably from 10 to 25 bases, more preferably from 15 to 20 bases. It can be designed so that the probe has a T_m from 68 to 70°C, for example. From the viewpoint of specificity, the kit according to the present invention preferably includes a set of a first probe having the base sequence as set forth in SEQ ID NO: 5 and a second probe having the base sequence as set forth in SEQ ID NO: 6.

First probe: 5'-CTCCATCAGAGGCTTCCT-3' (SEQ ID NO: 5)

Second probe: 5'-CTCCATCAAAGGCTTCCT-3' (SEQ ID NO: 6)

[0034] Each of the above-mentioned sequences is merely for illustration purposes, and the base sequence may be partially modified as long as specific hybridization can occur without any problem. "Partial modification" herein means that the bases have partial deletion, substitution, insertion, and/or addition. The number of modified bases is from 1 to 3, or from 1 to 2, or 1, for example.

[0035] The probe may be, but not limited to, a hydrolysis probe, a molecular beacon, and/or a cycling probe. An example of the hydrolysis probe is an oligonucleotide modified with a fluorescent dye at its 5' end and with a quencher substance at its 3' end. The hydrolysis probe specifically hybridizes with the template DNA at the time of PCR annealing but, due to the presence of the quencher on the probe, fluorescence emission upon excitation light irradiation is inhibited. During the extension reaction step of PCR, the hydrolysis probe hybridized with the template DNA is hydrolyzed by the 5'→3' exonuclease activity of Taq DNA polymerase, which frees the fluorescent dye from the probe, and thereby the quencher no longer inhibits fluorescence emission, resulting in fluorescence emission. By measuring the intensity of this fluorescence, it is possible to measure the amount of the amplification product thus produced. The fluorescent dye may be, but not limited to, FAM (6-carboxyfluorescein), ROX (6-carboxy-X-rhodamine), Cy3 and Cy5 (Cyanine-based dye), HEX (4,7,2',4',5',7'-hexachloro-6-carboxyfluorescein), VIC, TET (tetrachlorofluorescein), NED, CAL

Fluor Orange 560 (CFO), CAL Fluor Gold 540, CAL Fluor Red 590, CAL Fluor Red 610, CAL Fluor Red 635, Quasar 570, Quasar 670, Quasar 705, T (JOE), and/or the like. The quencher may be, but not limited to, TAMRA (tetramethylrhodamine; registered trademark), BHQ (Black Hole Quencher; registered trademark)-1, BHQ-2, 5 BHQ-3, Pulsar 650, Spacer C3, MGB-Eclipse (registered trademark), DABCYL, and/or the like.

[0036] The first probe is labeled with a fluorescent dye, and the second probe is labeled with a different fluorescent dye. The combination of the different fluorescent dyes is not particularly limited as long as they have different fluorescence properties and they 10 do not interfere with one another at the time of fluorescence measurement. Examples of the combination of the fluorescent dye for the first probe and the fluorescent dye for the second probe include FAM and HEX (either of which may be the fluorescent dye for the first probe; the same applies to the other combinations), FAM and CFO, FAM and VIC, FAM and TET, HEX and Joe, HEX and TET, and the like.

[0037] The kit according to the present invention may further comprise a PCR buffer containing a surfactant and proteinase K. The surfactant is capable of lysing cells, biological tissue, and the like. As the surfactant, an anionic surfactant, a cationic surfactant, an amphoteric surfactant, or a nonionic surfactant can be selected. The surfactant is preferably an anionic surfactant, more preferably sodium dodecyl sulfate. 15 In the case where the surfactant is sodium dodecyl sulfate, the concentration is preferably from 0.1 to 0.5% (w/v) when mixed with the sample. Proteinase K has the action of inactivating DNase and RNase. The concentration of the proteinase K is preferably from 100 to 300 $\mu\text{g/mL}$ when mixed with the sample. The PCR buffer may further include KCl, MgCl_2 , and dNTP mix (deoxyribonucleotide 5'-triphosphate; a 20 mixture of dATP, dGTP, dCTP, and dTTP). The PCR buffer is not particularly limited, but it is preferably Tris buffer. The concentrations of dNTP, MgCl_2 , KCl, and the buffer can be determined as appropriate by a person skilled in the art, and, for example, the concentration of MgCl_2 is 1.5 mM, the concentration of KCl is 35 mM, the concentration of dNTP is 200 μM , and the concentration of the Tris buffer is 10 25

mM. The PCR buffer may include a substance that binds to a PCR-inhibiting substance, which is a negatively-charged substance derived from a living thing and adsorbable on DNA polymerase (such as a certain type of sugar and dye, for example) and/or a positively-charged substance derived from a living thing and adsorbable on DNA (such as a certain type of protein, for example), to neutralize the PCR-inhibiting action of the negatively-charged substance and the positively-charged substance. As the PCR buffer of this type, a gene amplification reagent named either Ampdirect or Ampdirect Plus (each of which is registered trademark; Shimadzu Corporation) can be used.

[0038] The kit according to the present invention can be used in a rapid genotype determination system that is capable of conducting easy, simple, and rapid genetic testing in clinical settings. Examples of the rapid genotype determination system include "GTS-7000" manufactured by Shimadzu Corporation.

[0039] An embodiment of the present invention is

a use of:

a pair of PCR primers for amplifying a base sequence including a gene variation corresponding to an RNF213 p.R4810K variant; and

a first probe capable of binding to a wild-type base sequence corresponding to the RNF213 p.R4810K variant and a second probe capable of binding to a variant base sequence corresponding to the RNF213 p.R4810K variant,

in the production of the above-mentioned kit for determining, based on the presence or absence of the RNF213 p.R4810K variant, whether or not a possibility of reocclusion or restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0040] A description will be given of the method for detecting the RNF213 p.R4810K variant by using the present kit. An example of the method for detecting the RNF213 p.R4810K variant includes:

(1) a step of mixing a sample collected from a subject with a PCR buffer containing a surfactant and proteinase K;

(2) a step of mixing the mixed liquid obtained from the step (1), with DNA polymerase, a pair of PCR primers for amplifying a base sequence including a gene variation corresponding to the RNF213 p.R4810K variant, and a set of a first probe and a second probe, to perform PCR; and

5 (3) a step of detecting the PCR product.

[0041] The sample collected from the subject may be the sample described in [Method for Predicting Reocclusion of Intracranial Cerebral Blood Vessel]. Preferably, the sample includes genomic DNA of the subject. The amount of the sample depends on the amount of genomic DNA thus contained, and when the sample is saliva or
10 peripheral blood, the amount may be approximately from 0.1 μ L to 10 μ L, or from 0.5 μ L to 2 μ L.

[0042] (1) As a result of mixing the sample collected from the subject with the PCR buffer containing a surfactant and proteinase K, the sample is lysed and the nucleic acid becomes free.

15 [0043] (2) When the mixed liquid of the sample and the PCR buffer is mixed with DNA polymerase as well as the pair of PCR primers for amplifying a base sequence including a gene variation corresponding to the RNF213 p.R4810K variant, PCR can be initiated. The DNA polymerase is not particularly limited, and may be a heat-resistant DNA polymerase derived from thermophilic bacteria; and Taq, Tth, KOD,
20 Pfu, and a variant thereof can be used. For avoiding non-specific amplification, a hot start DNA polymerase may be used. Examples of the hot start DNA polymerase include BIOTAQ (registered trademark) Hot Start DNA Polymerase. Examples of the hot start DNA polymerase include a DNA polymerase to which an anti-DNA polymerase antibody is bound, and a DNA polymerase in which the active site of the
25 enzyme has thermosensitive chemical modification. The conditions for PCR (the temperature, the time, and the number of cycles) can be easily determined by a person skilled in the art. In the method using the present kit, detection of the PCR product can be performed by real-time measurement (real-time PCR).

[0044] (3) In the real-time measurement of the PCR product, a fluorescence filter

suitable for the fluorescent dye is used to monitor the PCR product amplification curve. When the fluorescence intensity increases along with the increase of the number of PCR cycles, it is determined that the presence of the target DNA in the sample is positive, and when the fluorescence intensity does not increase during the PCR, it is determined that the presence of the target DNA is negative. In the PCR product detection method, by comparing the increase of the fluorescence intensity attributable to the first probe with that of the fluorescence intensity attributable to the second probe, it is possible to detect the RNF213 p.R4810K variant. In the case where the RNF213 p.R4810K variant is a homozygote, an increase of the fluorescence intensity attributable to the second probe is observed, while an increase of the fluorescence intensity attributable to the first probe is not observed. In the case where the RNF213 p.R4810K variant is a heterozygote of the variant and the wild type, an increase of the fluorescence intensity attributable to the first probe and that of the second probe are both observed. In the case of the wild-type RNF213 gene (homozygote), an increase of the fluorescence intensity attributable to the first probe is observed alone. When an increase of the fluorescence intensity attributable to the second probe is observed, it is determined that the RNF213 p.R4810K variant is detected.

[0045] Another detection method can be one that involves determining the presence of the RNF213 p.R4810K variant based on the number of PCR cycles (the Cq value) at which the PCR product amplification curve crosses a certain threshold value line (Threshold Line). Normally, when the Cq value is small, it indicates that the amount of template DNA is great, and when the Cq value is great, it indicates that the amount of template DNA is small. When a certain Cq value is given when the second probe is used, it is determined that the RNF213 p.R4810K variant is detected. When the RNF213 p.R4810K variant is a heterozygote of the variant and the wild type, a certain Cq value is given regardless of which one of the fluorescently-labeled probes is used, but the values are not necessarily the same as one another. In the case of the wild-type RNF213 gene (homozygote), a certain Cq value is given only when the first probe is used. When the PCR product amplification curve does not cross the certain

threshold value line, in other words, when no Cq value is given, it indicates that there is no base sequence to which the fluorescently-labeled probe thus used is bound.

[0046] [Marker]

An embodiment of the present invention is
5 a genetic marker consisting of an RNF213 p.R4810K variant for determining whether or not a possibility of reocclusion or restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

The present genetic marker allows for making determination that when the
10 subject has the RNF213 p.R4810K variant, a possibility of reocclusion or restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high. The present genetic marker is capable of providing information that is usable for predicting the risk of reocclusion of a blood vessel after endovascular therapy.

15 [0047] An embodiment of the present invention is a use of an RNF213 p.R4810K variant for determining whether or not a possibility of reocclusion or restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0048] [Prediction Method]

20 An embodiment of the present invention is a method of predicting reocclusion or restenosis of an intracranial cerebral blood vessel, the method comprising detecting whether or not a subject has an RNF213 p.R4810K variant by using a sample collected from the subject.

When the subject has the RNF213 p.R4810K variant, prediction can be made
25 that the possibility of reocclusion or restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high. As the method for detecting whether or not the subject has the RNF213 p.R4810K variant, the method described in the above section [Method for Predicting Reocclusion of Intracranial Cerebral Blood Vessel] may be used.

[Examples]

[0049] In the following, a more detailed description will be given of the present invention by way of examples, but these are not intended to limit the scope of the present invention.

5 [0050] Mechanical thrombectomy (MT) is a standard treatment for acute ischemic stroke (AIS) caused by occlusion of the internal carotid artery (ICA) and M1 (M1 segment) of the middle cerebral artery (MCA)¹⁵⁾. However, about 15 to 25% of large-vessel occlusion (LVO) cases in East Asians for whom MT was performed were reportedly caused by intracranial atherosclerotic disease (ICAD)^{16,17)}. Recent studies
10 have suggested that reocclusion is more likely to occur after endovascular therapy (EVT) in intracranial atherosclerotic disease-related large-vessel occlusion (ICAD-related LVO)¹⁸⁻²⁰⁾, because the stenotic segment harbors unstable plaques susceptible to rupture, and thrombectomy aggravates vascular endothelial damage and platelet aggregation^{21,22)}. The RNF213 p.R4810K variant may be involved in ICAD-related
15 LVO in Asian patients undergoing MT. Negative remodeling in patients with ICAD with the RNF213 p.R4810K variant may lead to a different frequency of reocclusion after EVT procedures, including balloon angioplasty, compared with conventional ICAD-related LVO. Differences in the impact of EVT on outcomes in RNF213 p.R4810K variant carrier and noncarrier patients with anterior-circulation intracranial
20 LVO have not been investigated. In this study, we investigated the impact of the RNF213 p.R4810K variant on the clinical outcome of EVT in patients with anterior-circulation intracranial LVO.

[0051] [Methods]

25 Anonymized data that support the findings of this study are available from the inventors upon reasonable request and after permission from the corresponding ethics committees.

[0052] (Study Population)

All patients with AIS admitted to National Cerebral and Cardiovascular Center (NCVC) within 7 days from symptom onset or the last-known-well date were

registered in the NCVC Stroke Registry²³⁻²⁵). In the current study, the patients enrolled in this registry from January 2011 to March 2021 were retrospectively reviewed, and patients who met the following criteria were included: (1) occlusion of the intracranial internal carotid artery or M1 of the middle cerebral artery; (2) underwent EVT; and (3) provided written informed consent for participation in the present study and the RNF213 p.R4810K variant genotyping examination. Clinical and radiological data were prospectively collected in a database and retrieved for retrospective analysis. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the NCVC. The NCVC Stroke Registry is registered with ClinicalTrials.gov (NCT02251665). [0053] (RNF213 Genotyping Examination)

Genotyping of the RNF213 p.R4810K variant was performed using a fully automated gene analysis system (GTS-7000; Shimadzu Corporation; or LightCycler 96; Roche) after EVT. These systems allow the direct detection of SNVs from 1 µL of whole blood using real-time PCR²⁶). The primer sequences used here were as follows:

5'-TTCCAGAACGTCCAGCAAGT-3' (forward; SEQ ID NO: 3)

5'-ACAGTCCTGGTCCTGTCAGA-3' (reverse; SEQ ID NO: 4).

The probe sets were as follows:

5'-CTCCATCAGAGGCTTCCT-3' (SEQ ID NO: 5); and

5'-CTCCATCAAAGGCTTCCT-3' (SEQ ID NO: 6).

In 22 patients, the genotyping results obtained by real-time PCR were confirmed by DNA sequencing. As a result of the RNF213 p.R4810K variant analysis, the GA variant for p.R4810K was used to define variant carriers and the wild-type homozygote GG genotype was used to define variant noncarriers.

[0054] (EVT)

All endovascular procedures were performed in accordance with the American Heart Association/American Stroke Association guidelines¹⁵) or the Japanese Guidelines for Neuroendovascular Mechanical Thrombectomy²⁷). All patients underwent EVT under local anesthesia, and conscious sedation was added when

required. In the present study, a balloon guide catheter was used and navigated to the extracranial internal carotid artery as far as possible. EVT techniques included stent retriever thrombectomy, contact aspiration, intracranial balloon angioplasty, intracranial stenting, or some combination thereof. For reperfusion grading, the modified Thrombolysis In Cerebral Infarction (mTICI) scale was used²⁸⁾. When ICAD-related LVO was diagnosed as described below, dual-antiplatelet medication with aspirin and clopidogrel was administered during the perioperative period.

[0055] (Clinical Data Collection)

The following clinical data were collected: age, sex, prestroke modified Rankin Scale (mRS) score, systolic blood pressure on admission, baseline National Institutes of Health Stroke Scale (NIHSS) score, current smoking, atrial fibrillation, vascular risk factors (hypertension, diabetes, and dyslipidemia), and medical history (stroke or transient ischemic attack before index stroke). The extent of ischemic change in the MCA territory was graded using the Alberta Stroke Program Early Computed Tomography Score (ASPECTS) on diffusion-weighted magnetic resonance (MR) imaging or noncontrast computed tomography (CT)^{29,30)}. Occlusion sites were determined using digital subtraction angiography on admission. Intravenous thrombolysis was performed using alteplase at 0.6 mg/kg (the dose approved in Japan)³¹⁾. Time metrics included time from onset confirmation to thrombolysis and time from onset confirmation to groin puncture. The causes of acute LVO were classified into 3 groups (ICAD related, embolism, and arterial dissection) by board-certified stroke neurologists. ICAD-related LVO was defined as acute intracranial artery occlusion with any evidence of $\geq 50\%$ residual stenosis at the target arterial lesion subsequent to EVT, as detected on postprocedural angiography and follow-up angiography, and no evidence of any definite proximal sources of embolism^{18,20,32,33)}. Stenosis of the intracranial artery was measured according to a standardized method³⁴⁾. LVO caused by embolism was defined as an acute intracranial artery occlusion with evidence of any definite proximal sources of embolism, accompanied by atrial fibrillation, cerebral embolic stroke of undetermined source, or the like³⁵⁾.

Antithrombotic therapy at discharge included antiplatelet drugs (aspirin, clopidogrel, and cilostazol) and anticoagulant drugs (direct oral anticoagulants [dabigatran, rivaroxaban, apixaban, and edoxaban] and warfarin).

[0056] (Outcomes)

5 Procedural outcomes were instant reocclusion, final mTICI $\geq 2b$ reperfusion, and early reocclusion. Instant reocclusion was defined as reocclusion during the procedure, and early reocclusion was defined as reocclusion detected on MR angiography within 2 weeks after confirmation of successful reperfusion following the procedure^{19,20}. Clinical outcomes were achievement of an mRS score of 0 to 2 at 90
10 days, subarachnoid hemorrhage, parenchymal hematoma, and symptomatic intracranial hemorrhage (ICH). ICH was assessed by noncontrast CT or gradient-echo MR imaging within 36 hours after onset. Parenchymal hematoma was defined as hemorrhage in the infarcted area with space-occupying effects, as described by the European Cooperative Acute Stroke Study³⁶. The definition of symptomatic ICH was
15 defined as any ICH with a ≥ 4 -point increase in the NIHSS score from baseline³⁷.

[0057] (Statistical Analysis)

 Data were summarized as the median (interquartile range [IQR]) for continuous variables and as frequencies and percentages for categorical variables. Statistical differences between variant carriers and variant noncarriers were assessed using the
20 Mann-Whitney U-test or 2-sided Fisher exact test. For procedural outcomes, age-adjusted multivariate logistic regression models were conducted to determine an association with the RNF213 p.R4810K variant, where variant noncarriers were used as a reference³⁸. Odds ratios (ORs) with 95% confidence intervals (CIs) were
25 calculated. Because the RNF213 p.R4810K variant is associated with intracranial internal carotid artery or middle cerebral artery M1 stenosis/occlusion caused by ICAD^{8,38-40}, clinical data and outcomes were compared between variant carriers and variant noncarriers only in cases with ICAD-related LVO (secondary analysis; Fig. 1). A $P < 0.05$ was considered statistically significant. All analyses were performed using the Stata/IC statistical package, version 15.1 (Stata Corp LP).

[0058] [Results]

(Patient Characteristics)

Among the 341 patients who underwent EVT for acute intracranial ICA/MCA M1 occlusion, 64 were excluded because of a lack of RNF213 R4810K genotyping data (Fig. 1). When compared with patients without RNF213 p.R4810K genotyping data, patients who underwent genotyping were younger (76 versus 80 years; $P=0.02$) and more frequently had dyslipidemia (45.7% versus 31.2%; $P=0.04$). Among the analyzed data for 277 patients (128 women [46.2%]; median age, 76 years [IQR, 69-82 years]; median NIHSS score, 11 [IQR 8-17]), the RNF213 p.R4810K variant was found in 10 patients (3.6%). Variant carriers were younger (67 versus 76 years; $P<0.01$), more likely to be currently smoking (80.0% versus 43.4%; $P=0.05$), less likely to have atrial fibrillation (30.0% versus 63.4%; $P=0.05$), and had a higher baseline systolic blood pressure (172 versus 151 mmHg; $P=0.02$) compared with variant noncarriers (Table 1). Variant carriers had a higher frequency of ICAD-related LVO and a lower frequency of embolic LVO compared with variant noncarriers ($P<0.01$ for both). Antiplatelet drugs were more frequently administered at discharge to variant carriers than to variant noncarriers ($P<0.01$ for both aspirin and clopidogrel).

[0059] (RNF213 p.R4810K Variant Carrier Versus Noncarriers)

The EVT details and outcomes between variant carriers and variant noncarriers are shown in Table 2. Angioplasty was more frequently performed (40.0% versus 12.0%; $P<0.01$), the median number of angioplasties was doubled (4 versus 2; $P=0.27$), and intracranial stenting was more frequently performed (20.0% versus 2.2%; $P=0.03$) in variant carriers compared with variant noncarriers. The time from groin puncture to the end of the procedure was longer for variant carriers compared with variant noncarriers (177 versus 72 minutes; $P=0.08$). Variant carriers had a higher rate of instant reocclusion (40.0% versus 5.6%; $P<0.01$; age-adjusted OR, 9.83; 95% CI 2.47-39.22) (Table 2 and Fig. 2), but the rates of final mTICI $\geq 2b$ reperfusion were 100.0% in carriers and 81.8% in noncarriers ($P=0.22$; age-adjusted OR, 3.91; 95% CI 0.64-23.99). Early reocclusion was more frequent in variant carriers compared with variant

noncarriers (60.0% versus 0.4%; $P<0.01$; age-adjusted OR, 346.4; 95% CI 33.21-3612) (Table 2 and Fig. 2). The median (IQR) number of days from the end of the procedure to early reocclusion was 4 (2-9) days. Repeated EVT for early reocclusion was performed in 4 variant carriers and 1 variant noncarrier. Achievement of an mRS score of 0 to 2 at 90 days was seen in 60.0% of variant carriers and 51.7% of variant noncarriers ($P=0.75$). The rates of subarachnoid hemorrhage, parenchymal hematoma, and symptomatic ICH were not different between variant carriers and variant noncarriers (Table 2). A summary of patients with the RNF213 p.R4810K variant is provided in Table 3, and a representative patient with the RNF213 p.R4810K variant is shown in Figure 4.

[0060] (Within-Group Analysis of Patients with ICAD-Related LVO)

Similar analyses focusing on patients diagnosed with ICAD-related LVO ($n=30$) were conducted (secondary analysis; Fig. 1). Variant carriers ($n=7$) were younger compared with variant noncarriers ($n=23$) (60 versus 73 years; $P=0.04$). No significant differences in the rates of angioplasty, total number of angioplasty procedures, and intracranial stenting were seen between variant carriers and variant noncarriers. Time from groin puncture to end of the procedure was longer in the variant carriers (190 versus 142 minutes; $P=0.09$). Instant reocclusion rates were not significantly different between variant carriers (57.1%) and variant noncarriers (34.8%) ($P=0.39$; age-adjusted OR, 2.38; 95% CI 0.39-14.47) (Table 2 and Fig. 3). The rates for final mTICI $\geq 2b$ reperfusion were comparable between variant carriers (100.0%) and variant noncarriers (91.3%) ($P=1.00$). Variant carriers had a higher frequency of early reocclusion compared with variant noncarriers (71.4% versus 4.3%; $P<0.01$; adjusted OR, 47.6; 95% CI 3.53-674.0) (Table 2 and Fig. 3). The median (IQR) number of days from the end of the procedure to early reocclusion was 2 (2-6) days. No significant intergroup differences were seen in terms of clinical outcomes.

[0061] [Discussion]

By analyzing our cohort of patients with anterior-circulation LVO who underwent EVT ($n=277$), the present study showed that RNF213 p.R4810K variant

carriers had higher rates of instant reocclusion during EVT (40.0% versus 5.6%) and early reocclusion within 2 weeks after EVT (60.0% versus 0.4%) compared with variant noncarriers. In RNF213 p.R4810K variant carriers, ICAD caused 70.0% of cases of acute LVO compared with 8.6% in variant noncarriers. Early reocclusion

5 was also more frequent in variant carriers than in variant noncarriers, even when the analysis was restricted to patients with ICAD-related LVO. Such differences in the occurrence of instant and early reocclusion between variant carriers and variant noncarriers might be associated with the vascular pathology of ICAD specifically attributable to the RNF213 p.R4810K variant¹²⁻¹⁴).

10 [0062] No studies have reported the outcomes of EVT in association with specific gene variants, and the RNF213 gene variant is unique to the East Asian population. The frequency of the RNF213 p.R4810K variant was 3.6% in the study conducted by the inventors of the present invention, which is similar to the 4.2% to 5.2% frequency reported in studies of patients with ischemic stroke^{7,41}). However, in contrast to this,

15 the rate of the RNF213 p.R4810K variant was clearly higher (23.3%) when the sample population was limited to patients with ICAD-related LVO, suggesting the usefulness of examining this variant when ICAD-related LVO is suspected. The RNF213 p.R4810K variant can now be identified as early as 1 hour after blood drawing using specific primer and probe sets. Because the present invention suggests more frequent

20 instant and early reocclusion in variant carriers compared with variant noncarriers, rapid genotyping of the gene variant may aid the selection of EVT procedures and postprocedural patient care. Furthermore, the genotyping results may be utilized for risk assessment for EVT in patients with acute anterior-circulation LVO stroke⁴²).

[0063] [Table 1]

Table 1. Data of variant carriers and non-carriers.

	<i>RNF213</i> p.R4810K (c.14429G>A)		
	Variant carriers (<i>n</i> = 10)	Variant noncarriers (<i>n</i> = 267)	<i>P</i> value
Women	2 (20.0)	126 (47.2)	0.11
Age at stroke onset, years	67 (59–74)	76 (69–83)	0.01
Prestroke mRS score	0 (0–0)	0 (0–2)	0.31
Baseline systolic blood pressure, mmHg	172 (162–178)	151 (133–170)	0.02
Baseline NIHSS score	16 (12–19)	19 (12–24)	0.40
ASPECTS*	9 (7–9)	8 (6–9)	0.43
Intracranial internal carotid artery	1 (10.0)	96 (36.0)	0.17
M1 segment of the middle cerebral artery	9 (90.0)	171 (64.0)	0.17
Intravenous alteplase	6 (60.0)	130 (48.7)	0.53
Time from last-known-well to thrombolysis, min	96 (74–136)	105 (81–154)	0.62
Time from last-known-well to groin puncture, min	195 (96–240)	202 (128–381)	0.39
Medical history			
Current smoking	8 (80.0)	116 (43.4)	0.05
Atrial fibrillation	3 (30.0)	168 (63.4)	0.05
Hypertension	9 (90.0)	184 (68.9)	0.29
Diabetes mellitus	1 (10.0)	53 (20.0)	0.69
Dyslipidemia	4 (40.0)	122 (45.9)	0.76
Stroke/TIA prior to index stroke	2 (20.0)	54 (20.3)	1.00
Cause of acute LVO			
ICAD-related	7 (70.0)	23 (8.6)	<0.01
Embolism	3 (30.0)	236 (88.4)	<0.01
Arterial dissection	0	8 (3.0)	1.00
Antithrombotic therapy at discharge			
Aspirin	7 (70.0)	55 (20.6)	<0.01
Clopidogrel	6 (60.0)	33 (12.4)	<0.01
Cilostazol	0	4 (1.5)	1.00
Direct oral anticoagulant	1 (10.0)	111 (41.6)	0.06
Warfarin	3 (30.0)	103 (38.6)	0.75

Data are presented as median (interquartile range) or number (percent).

ASPECTS, Alberta Stroke Program Early Computed Tomography Score;

CT, computed tomography;

DWI, diffusion-weighted imaging;

ICAD, intracranial atherosclerotic disease;

LVO, large vessel occlusion;

mRS, modified Rankin Scale;

NIHSS, National Institutes of Health Stroke Scale;

TIA, transient ischemic attack.

* ASPECTS was measured using non-contrast CT (*n* = 2) and DWI (*n* = 275).

[0064] [Table 2]

Table 2. Endovascular therapy and outcomes of variant carriers and non-carriers.

	<i>RNF213</i> p.R4810K (c.14429G>A)		
	Variant carriers (<i>n</i> = 10)	Variant noncarriers (<i>n</i> = 267)	<i>P</i> value
Endovascular technique			
Stent retriever	4 (40.0)	133 (49.8)	0.75
Contact aspiration	4 (40.0)	113 (42.3)	1.00
Angioplasty	4 (40.0)	32 (12.0)	<0.01
Total number of angioplasty procedures	4 (2–5)	2 (2–2)	0.27
Intracranial stenting	2 (20.0)	6 (2.2)	0.03
Time from groin puncture to the end of the procedure, min	177 (47–212)	72 (43–123)	0.08
Procedural outcome			
Instant re-occlusion	4 (40.0)	15 (5.6)	<0.01
Final mTICI $\geq$ 2b reperfusion	10 (100.0)	218 (81.6)	0.22
Early re-occlusion	6 (60.0)	1 (0.4)	<0.01
Clinical outcome			
mRS score of 0–2 at 90 days	6 (60.0)	138 (51.7)	0.75
Subarachnoid hemorrhage	2 (20.0)	38 (14.2)	0.64
Parenchymal hematoma*	1 (10.0)	13 (7.7)	0.61
Symptomatic ICH [†]	0	5 (1.9)	1.00

Data are presented as median (interquartile range) or number (percent).

5 ICH, intracranial hemorrhage;

mRS, modified Rankin Scale;

mTICI, modified Thrombolysis In Cerebral Infarction;

NIHSS, National Institutes of Health Stroke Scale.

* Parenchymal hematoma was defined as blood clots in the infarcted area with space-occupying effects,

10 as described by the European Cooperative Acute Stroke Study.

[†] Any ICH with an $\geq$ 4-point increase in NIHSS score from baseline.

[0065] [Table 3]

Table 3. Summary of LVO patients with the *RNF213* p.R4810K variant.

Case	Age	Sex	Baseline NIHSS score	Occluded vessel	First-line EVT technique	IR	Total number of angioplasties	Final mTICI	Stenting	ER	Number of days to ER	Repeated EVT	Cause of acute LVO	mRS score at 90 days
1	69	Female	10	MCA M1	Angioplasty	No	2	2b	No	Yes	9	No	ICAD-related	6
2	81	Female	34	MCA M1	Angioplasty	No	3	2b	No	Yes	10	No	Embolism	5
3	59	Male	19	MCA M1	Stent retriever	Yes	4	2b	No	Yes	6	Yes	ICAD-related	2
4	76	Male	23	MCA M1	Aspiration	No	0	2c	No	No	--	No	Embolism	2
5	59	Male	18	MCA M1	Aspiration	Yes	1	3	No	Yes	2	Yes	ICAD-related	2
6	67	Male	12	MCA M1	Aspiration	Yes	5	2b	Yes	Yes	1	Yes	ICAD-related	4
7	67	Male	18	Intracranial ICA	Stent retriever	No	0	2b	No	No	--	No	Embolism	3
8	74	Male	13	MCA M1	Stent retriever	No	1	2b	No	No	--	No	ICAD-related	3
9	60	Male	12	MCA M1	Aspiration	No	4	3	Yes	No	--	No	ICAD-related	1
10	52	Male	10	MCA M1	Stent retriever	Yes	14	2c	No	Yes	2	Yes	ICAD-related	0

ER, early re-occlusion;

EVT, endovascular therapy;

ICA, internal carotid artery;

ICAD, intracranial atherosclerotic disease;

IR, instant re-occlusion;

LVO, large vessel occlusion;

MCA, middle cerebral artery;

mRS, modified Rankin Scale;

mTICI, modified Thrombolysis In Cerebral Infarction;

NIHSS, National Institutes of Health Stroke Scale.

[0066] [References]

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[0067] [Aspects]

As will be appreciated by those skilled in the art, the above-described example embodiments and Examples are specific examples of the below aspects. Unless otherwise specified, the terms used in the present specification have the same meaning

as generally understood by a person skilled in the art in such fields as medicine, anatomy, pharmacy, molecular biology, organic chemistry, and the like. When any term defined in the present specification does not have the same meaning as generally understood, the description of the present specification is given priority.

5 [0068] (Item 1)

A method for predicting reocclusion of an intracranial cerebral blood vessel according to an aspect, the method comprising:

detecting whether or not a subject has an RNF213 p.R4810K variant by using a sample collected from the subject; and

10 when the subject has the RNF213 p.R4810K variant, determining that a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0069] The method according to Item 1 makes it easy to predict the risk of reocclusion of a blood vessel after endovascular therapy.

15 [0070] (Item 2)

The method according to Item 1, further comprising selecting a tool to use in the endovascular therapy based on the possibility of reocclusion of the intracranial cerebral blood vessel.

20 [0071] The method according to Item 2 makes it possible to lower the risk of reocclusion.

[0072] (Item 3)

The method according to Item 1 or Item 2, wherein the determining includes determining that a possibility of reocclusion of the intracranial cerebral blood vessel to occur at a timing earlier than normal after the endovascular therapy is high.

25 [0073] (Item 4)

The method according to Item 3, wherein the timing earlier than normal after the endovascular therapy is from confirmation of successful reperfusion by the endovascular therapy to during the endovascular therapy.

[0074] (Item 5)

The method according to Item 3, wherein the timing earlier than normal after the endovascular therapy is within 2 weeks after confirmation of successful reperfusion by the endovascular therapy.

5 [0075] The methods according to Item 3 to Item 5 make it possible to identify a patient with a high risk of instant or early reocclusion.

[0076] (Item 6)

The method according to any one of Item 1 to Item 5, wherein the RNF213 p.R4810K variant is a heterozygote of a variant and a wild type.

[0077] (Item 7)

10 The method according to any one of Item 1 to Item 6, wherein the sample is blood or saliva.

[0078] The method according to Item 7 makes it possible to obtain a sample in a minimally invasive fashion.

[0079] (Item 8)

15 The method according to any one of Item 1 to Item 7, wherein the intracranial cerebral blood vessel is an intracranial internal carotid artery or a middle cerebral artery.

[0080] (Item 9)

20 The method according to any one of Item 1 to Item 8, wherein the occlusion of an intracranial cerebral blood vessel is a blood vessel occlusion caused by atherosclerosis

[0081] When the occlusion of the intracranial cerebral blood vessel is atherosclerosis, determination can be made that the risk of reocclusion of the blood vessel in a patient having the RNF213 p.R4810K variant is relatively high.

25 [0082] (Item 10)

A kit according to an aspect is

a kit for determining, based on the presence or absence of an RNF213 p.R4810K variant, whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial

cerebral blood vessel is high, the kit comprising:

a pair of PCR primers for amplifying a base sequence including a gene variation corresponding to the RNF213 p.R4810K variant; and

5 a first probe capable of binding to a wild-type base sequence corresponding to the RNF213 p.R4810K variant and a second probe capable of binding to a variant base sequence corresponding to the RNF213 p.R4810K variant, wherein

the first probe is labeled with a fluorescent dye, and the second probe is labeled with a different fluorescent dye.

10 [0083] The kit according to Item 10 makes it possible to predict whether or not the risk of reocclusion of a blood vessel after endovascular therapy in a subject is high.

[0084] (Item 11)

The kit according to Item 10, wherein the pair of PCR primers includes a primer having a base sequence as set forth in SEQ ID NO: 3 and a primer having a base sequence as set forth in SEQ ID NO: 4.

15 [0085] The kit according to Item 11 makes it possible to perform PCR with less non-specific amplification.

[0086] (Item 12)

20 The kit according to Item 10 or Item 11, wherein the first probe has a base sequence as set forth in SEQ ID NO: 5, and the second probe has a base sequence as set forth in SEQ ID NO: 6.

[0087] The kit according to Item 12 makes it possible to reduce non-specific detection of PCR products.

[0088] (Item 13)

25 The kit according to any one of Item 10 to Item 12, further comprising a PCR buffer containing a surfactant and proteinase K.

[0089] The kit according to Item 13 makes it possible to perform PCR using genomic DNA as a template, in an easy, simple, and rapid fashion.

[0090] (Item 14)

An invention according to an aspect is a genetic marker consisting of an

RNF213 p.R4810K variant for determining whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0091] The invention according to Item 14 is capable of providing information that is usable for predicting the risk of reocclusion of a blood vessel after endovascular therapy.

[0092] (Item 15)

A method for predicting restenosis of an intracranial cerebral blood vessel according to an aspect, the method comprising:

detecting whether or not a subject has an RNF213 p.R4810K variant by using a sample collected from the subject; and

when the subject has the RNF213 p.R4810K variant, determining that a possibility of restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

[0093] When the patient has the RNF213 p.R4810K variant, it is predicted that restenosis is likely to occur after endovascular therapy. When the risk of restenosis is high, the risk of reocclusion is expected to be high as well.

[0094] (Item 16)

A kit according to an aspect is

a kit for determining, based on the presence or absence of an RNF213 p.R4810K variant, whether or not a possibility of restenosis of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high, the kit comprising:

a pair of PCR primers for amplifying a base sequence including a gene variation corresponding to the RNF213 p.R4810K variant; and

a first probe capable of binding to a wild-type base sequence corresponding to the RNF213 p.R4810K variant and a second probe capable of binding to a variant base sequence corresponding to the RNF213 p.R4810K variant, wherein

the first probe is labeled with a fluorescent dye, and the second probe is labeled

with a different fluorescent dye.

[0095] (Item 17)

5 An invention according to an aspect is a genetic marker consisting of an
RNF213 p.R4810K variant for determining whether or not a possibility of restenosis of
an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion
of the intracranial cerebral blood vessel is high.

CLAIMS

[Claim 1] A method for predicting reocclusion of an intracranial cerebral blood vessel, the method comprising:

5 detecting whether or not a subject has an RNF213 p.R4810K variant by using a sample collected from the subject; and

when the subject has the RNF213 p.R4810K variant, determining that a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.

10

[Claim 2] The method according to claim 1, further comprising selecting a tool to use in the endovascular therapy based on the possibility of reocclusion of the intracranial cerebral blood vessel.

15 [Claim 3] The method according to claim 1 or 2, wherein the determining includes determining that a possibility of reocclusion of the intracranial cerebral blood vessel to occur at a timing earlier than normal after the endovascular therapy is high.

20 [Claim 4] The method according to claim 3, wherein the timing earlier than normal after the endovascular therapy is from confirmation of successful reperfusion by the endovascular therapy to during the endovascular therapy.

25 [Claim 5] The method according to claim 3, wherein the timing earlier than normal after the endovascular therapy is within 2 weeks after confirmation of successful reperfusion by the endovascular therapy.

[Claim 6] The method according to claim 1, wherein the RNF213 p.R4810K variant is a heterozygote of a variant and a wild type.

[Claim 7] The method according to claim 1, wherein the sample is blood or saliva.

5 [Claim 8] The method according to claim 1, wherein the intracranial cerebral blood vessel is an intracranial internal carotid artery or a middle cerebral artery.

[Claim 9] The method according to claim 1, wherein the occlusion of an intracranial cerebral blood vessel is a blood vessel occlusion caused by atherosclerosis.

10 [Claim 10] A kit for determining, based on the presence or absence of an RNF213 p.R4810K variant, whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high, the kit comprising:
a pair of PCR primers for amplifying a base sequence including a gene variation
15 corresponding to the RNF213 p.R4810K variant; and
a first probe capable of binding to a wild-type base sequence corresponding to the RNF213 p.R4810K variant and a second probe capable of binding to a variant base sequence corresponding to the RNF213 p.R4810K variant, wherein
the first probe is labeled with a fluorescent dye, and the second probe is labeled
20 with a different fluorescent dye.

[Claim 11] The kit according to claim 10, wherein the pair of PCR primers includes a primer having a base sequence as set forth in SEQ ID NO: 3 and a primer having a base sequence as set forth in SEQ ID NO: 4.

25

[Claim 12] The kit according to claim 10 or 11, wherein the first probe has a base sequence as set forth in SEQ ID NO: 5, and the second probe has a base sequence as set forth in SEQ ID NO: 6.

[Claim 13] The kit according to claim 10, further comprising a PCR buffer containing a surfactant and proteinase K.

5 [Claim 14] A genetic marker consisting of an RNF213 p.R4810K variant for determining whether or not a possibility of reocclusion of an intracranial cerebral blood vessel to occur after endovascular therapy for occlusion of the intracranial cerebral blood vessel is high.