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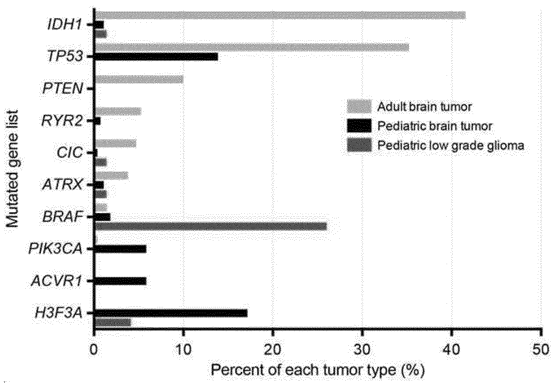
(54) COMPOSITION FOR DIAGNOSIS, PREVENTION, OR TREATMENT OF GANGLIOGLIOMA AND DISEASE RELATED THERETO

(57) The present invention relates to a biomarker of epilepsy, a composition for diagnosing epilepsy, an epilepsy-induced animal, and a composition for preventing or treating epilepsy, and specifically, relates to a composition for diagnosing epilepsy comprising a BRAF mutant protein and a nucleic acid molecule, and an agent capable of detecting the protein or nucleic acid molecule, an epilepsy-induced animal transformed with the BRAF mutant nucleic acid molecule, and a composition for prevention or treatment of epilepsy comprising a BRAF mutant protein activity inhibitor.

【FIG. 1a】



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Description

[TECHNICAL FIELD]

[0001] The present invention relates to a use for diagnosis and treatment of ganglioglioma using a biomarker of ganglioglioma, and specifically, relates to a composition of diagnosing ganglioglioma comprising a BRAF mutant protein and a nucleic acid molecule, and an agent capable of detecting the protein or nucleic acid molecule, a ganglioglioma-induced animal transformed with the BRAF mutant nucleic acid molecule, and a composition for prevention or treatment of ganglioglioma comprising a BRAF mutant protein activity inhibitor.

[BACKGROUND ART]

[0002] Epilepsy is a group of chronic diseases in which an epileptic seizure is repeatedly caused by excessive electricity spasmodically caused by some nerve cells in a short period of time, and is a serious neurological disease accompanying neurobiological, psychological, cognitive and social changes.

[0003] Epilepsy is the third most common neurological disease following Alzheimer and stroke, with about 0.5%~2% of the world's population suffering from epilepsy. In addition, there are 45 new patients per 100,000 people worldwide each year, and in Korea, it is estimated that there are about 300,000 to 400,000 epileptic patients, and it is reported that about 20,000 new epileptic patients occur every year. Furthermore, it is known that 70% of all cases of epilepsy occur in the age of pediatric adolescents, and in particular, the incidence is higher in infancy. The incidence and prevalence are in the form of U-shaped, which are highest in the first year of life, but rapidly decrease, and rapidly increase again in elderly people over 60 years of age, and the prevalence of experiencing a seizure in lifetime is 10 to 15%.

[0004] Among the epilepsy, epilepsy which does not respond to anti-epileptic drugs developed until now is called intractable epilepsy, accounting for about 40% of the total epilepsy.

[0005] As causative diseases of epilepsy, malformations of cortical developments (MCD), ganglioglioma (GG) and hippocampal sclerosis (HS), or Sturge weber syndrome (SWS) and the like are known.

[0006] Ganglioglioma is one of important causes of intractable epilepsy that does not respond to drug treatment, accounting for about 80% of childhood intractable epilepsy resulting from tumor. This is the most common nerve cell tumor of the central nervous system, and is a benign tumor with dysplasia in both nerve cell and glia. Most of them occur in pediatric patients, and repeated epilepsy is accompanied as the main symptom in nearly 80% of them. Surgical resection reduces the number of seizures, but there is a problem that an epileptic seizure is persistent in some patients, and in some cases, surgical treatment is difficult, since the age at onset is low and the location of the tumor is deep. In particular, the molecular genetic causes of ganglioglioma are not known, so it is difficult to develop a new and effective treatment of ganglioglioma.

[0007] Although previous studies have speculated somatic mutations specific to ganglioglioma, there has not yet been a clear causality between these mutations and onset of ganglioglioma, and therefore whether these somatic mutations actually cause epilepsy and the biological mechanism related thereto are not yet known.

[DISCLOSURE]

[TECHNICAL PROBLEM]

[0008] An object of the present invention is to provide a BRAF mutant protein and a nucleic acid molecule as a biomarker for diagnosing epilepsy.

[0009] An additional object of the present invention relates to a composition for diagnosis, a diagnostic kit and a method for diagnosis, of epilepsy comprising an agent capable of detecting a BRAF mutant protein and a nucleic acid molecule.

[0010] Other object of the present invention relates to an epilepsy-induced animal transformed with a BRAF mutant nucleic acid molecule, and a composition for screening and a method for screening, of therapeutic drugs of epilepsy or ganglioglioma using the animal.

[0011] An additional object of the present invention provides a composition for prevention or treatment of epilepsy and ganglioglioma comprising a BRAF mutant protein activity inhibitor.

[TECHNICAL SOLUTION]

[0012] The present inventors have discovered a brain lesion-specific somatic mutation of ganglioglioma using whole exome sequence analysis, for brain tissue samples of ganglioglioma surgery patients, and have prepared an animal model expressing such a brain somatic mutation, and have confirmed that there is an effect of treating epilepsy significantly when an activity inhibitor of BRAF mutant protein is administered into the prepared animal model, thereby completed

the present invention.

[0013] As one aspect to achieve the above object, the present invention relates to a composition for diagnosing ganglioglioma or epilepsy caused by ganglioglioma, comprising an agent capable of detecting a mutant protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1, or

an agent capable of detecting a mutant nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

[0014] As another aspect, the present invention relates to a method for detecting a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1, or a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2, to provide information required for diagnosis of epilepsy.

[0015] As other aspect, the present invention relates to a composition for inducing ganglioglioma or epilepsy caused by ganglioglioma, comprising a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1.

[0016] As other aspect, the present invention relates to a recombinant vector, comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2. As other aspect, the present invention relates to a cell introduced by a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2 is introduced.

[0017] As other aspect, the present invention relates to an animal or an embryo of the animal, in which a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2 is introduced.

[0018] As other aspect, the present invention relates to an animal with an induced ganglioglioma or epilepsy caused by ganglioglioma, wherein the animal is transformed with a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

[0019] As other aspect, the present invention relates to a method for preparing an animal with an induced ganglioglioma or epilepsy caused by ganglioglioma, comprising preparing a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2; and

transforming a mouse with the recombinant vector.

[0020] As other aspect, the present invention relates to a screening method of a therapeutic agent for ganglioglioma or epilepsy caused by ganglioglioma, comprising determining an alleviation of epilepsy after administering a candidate substance for epilepsy treatment into an animal with an induced ganglioglioma or epilepsy caused by ganglioglioma.

[0021] As other aspect, the present invention relates to a composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma comprising an activity inhibitor of a protein comprising an amino acid in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1.

[0022] Hereinafter, the present invention will be described in more detail.

[0023] The present invention relates to a biomarker for diagnosis of intractable epilepsy, for example, intractable epilepsy caused by ganglioglioma (GG), using a brain lesion-specific somatic mutation of ganglioglioma.

[0024] The present invention relates to a biomarker for diagnosing epilepsy and its use. More specifically, the present invention relates to BRAF gene in which sequence mutation occurs or BRAF protein in which amino acid sequence mutation occurs by sequence mutation. In addition, the present invention relates to a composition and a kit for diagnosing epilepsy comprising an agent capable of detecting the gene or the protein. Furthermore, the present invention relates to a method for detecting the gene and protein that are biomarkers for diagnosis epilepsy from a sample of a patient, in order to provide information required for diagnosis of epilepsy.

[0025] Herein, the term "epilepsy" means a chronic disease-causing seizure repeatedly as some of nerve cells cause excessive electricity in a short time. Herein, the epilepsy may include intractable epilepsy, and the epilepsy may be intractable epilepsy caused by ganglioglioma (GG).

[0026] The present inventors have analyzed brain tissue samples after surgery of ganglioglioma (GG) patients accompanying intractable epilepsy having no medicinal effects in conventional antiepileptics among pediatric patients having low grade glioma, based on the correlation that pediatric low grade brain tumor accompanies intractable epilepsy with a high percentage which is different from adult brain tumor, based on the conventional cancer public data analysis, and as a result, the present inventors have confirmed that BRAF V600E brain somatic mutation, in which the valine of 600th amino acid of BRAF protein consisting of the amino acid sequence of SEQ ID NO: 1 is substituted to glutamic acid (hereinafter, BRAF V600E) is specifically present, and the present inventors have confirmed that the BRAF V600E amino acid and a gene encoding the amino acid may be used as a biomarker panel for diagnosing brain tumor-derived

pediatric intractable epilepsy.

[0027] The BRAF V600E mutation was not found in blood of patients, and it was specifically found in the brain tissue samples. In addition, it has been confirmed that the genetic mutation ratio is 30 to 70% in the ganglioglioma patient group, and the ratio of the mutant gene to the normal allele present in each patient is 5% to 35% (Table 3).

[0028] The tumor used in the experiment is a benign tumor and because the copy number of mutant cells is small, as a result of confirmation by whole exome sequence analysis, any other mutations other than BRAF V600E mutation was not found. In other words, it has been confirmed that only the BRAF V600E single mutation is related to ganglioglioma, and thus it has been confirmed that BRAF V600E has a very strong correlation with phenotypes shown in ganglioglioma patients (Example 2).

[0029] The intractable epilepsy of the present invention may be caused by ganglioglioma, and specifically, ganglioglioma may be brain somatic mutation-related ganglioglioma, and preferably, the brain somatic mutation may be brain somatic BRAF V600E mutation, but not limited thereto.

[0030] Herein, the term "brain somatic mutation" means that mutation of a sequence occurs in one or more positions in a wildtype gene. For example, it may be an amino acid mutation of BRAF genes or protein corresponding to these genes.

[0031] The ganglioglioma or epilepsy caused by ganglioglioma may not be caused by activation of the mTOR signaling pathway, and the ganglioglioma or epilepsy caused by ganglioglioma may be caused by expression of the mutant protein or the mutant nucleic acid molecule in nerve cells, not glia, and the ganglioglioma or epilepsy caused by ganglioglioma may be caused by expression of the mutant protein or the mutant nucleic acid molecule in nerve cells at an embryo development stage, but not limited thereto.

[0032] The embryo development stage may mean a time to a gastrula stage, and specifically, it may mean a time before organ is formed.

[0033] The BRAF gene or protein has been known as a protein kinase playing a role in delivering signals of cell division from a cell membrane to a nucleus, and it functions to deliver MAP kinase signals by phosphorylating MEK kinase and ERK kinase. The amino acid sequence of human BRAF is described in SEQ ID NO: 1, and the NCBI accession number of SEQ ID NO: 1 is NP_004324.2, and the sequence encoding SEQ ID NO: 1 is described in SEQ ID NO: 2, and the NCBI accession number of SEQ ID NO: 2 is NM_004333.4. The amino acid sequence of mouse (Mus musculus) BRAF corresponding to the human is described in SEQ ID NO: 3, and the NCBI accession number of SEQ ID NO: 3 is NP_647455.3, and the sequence encoding SEQ ID NO: 3 is described in SEQ ID NO: 4, and the NCBI accession number of SEQ ID NO: 2 is NM_139294.5.

[0034] Herein, a preferable example of the brain somatic mutation means a mutation of the amino acid sequence of SEQ ID NO: 1 which is a wildtype human BRAF gene. For example, it means a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1 (BRAF V600E). As another example, herein, the brain somatic mutation may be a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2 which is a sequence encoding wildtype BRAF protein.

[0035] The mutation of mouse BRAF corresponding to human BRAF may mean a protein consisting of an amino acid sequence comprising a mutation in which the 637th valine is substituted to glutamic acid in the amino acid sequence of SEQ ID NO: 3 (BRAF), and may be a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which the 1910th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 4.

[0036] In one example of the present invention, in order to produce mice expressing the mutation of BRAF, a protein consisting of an amino acid sequence comprising a mutation in which the 637th valine is substituted to glutamic acid in the amino acid sequence of SEQ ID NO: 3 (BRAF V600E), or a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which the 1910th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 4 was used.

[0037] However, mouse BRAF V637E is traditionally represented by BRAF V600E as same as human BRAF V600E, so in the present description, for mice expressing the mouse BRAF V637E mutation, it is described as BRAF V600E.

[0038] In addition, the mutant protein may comprise an additional mutation within a range which does not modify the activity of the molecule totally. The exchange of an amino acid in a protein and a peptide which does not modify the activity of a molecule totally has been known in the art (H. Neurath, R. L. Hill, The Proteins, Academic Press, New York, 1979). Occasionally, the BRAF mutant protein may be under modification by phosphorylation, sulfation, acrylation, glycosylation, methylation, farnesylation and the like.

[0039] The present invention relates to a composition for diagnosing ganglioglioma or epilepsy caused by ganglioglioma, comprising an agent capable of detecting a mutant protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1, or an agent capable of detecting a mutant nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

[0040] Herein, the term "diagnosis" means confirming presence or features of pathological conditions. On the purpose of the present invention, diagnosis is to confirm occurrence or possibility of occurrence of ganglioglioma or epilepsy

caused by ganglioglioma.

[0041] In one example of the present invention, in order to accurately reflect the tumor-derived epilepsy patient model on an animal model, ganglioglioma known as the biggest cause in children was selected as a suitable model disease among epilepsy-related tumors, and brain tissue and blood samples were collected after surgery, and the BRAF V600E mutation was specifically confirmed in the brain tissue samples (Examples 1 and 2), and thereby it was confirmed that the BRAF V600E mutation is usable as a marker for diagnosis of epilepsy, and preferably it is usable as a marker for diagnosis of ganglioglioma or epilepsy caused by ganglioglioma.

[0042] Herein, "agent capable of detecting a gene" means a substance which can be used for detecting the BRAF mutant gene in samples of patients. As one specific example, it may be a primer, a probe, an antisense oligonucleotide and the like, which are complementary to a gene consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2. The primer, probe or antisense oligonucleotide is preferable to specifically bind to a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2, and not specifically bind to a sequence of other nucleic acid substances.

[0043] Then, complementary binding means that an antisense oligonucleotide is sufficiently complementary to be selectively hybridized to a BRAF mutant gene target, under a certain hybridization or annealing condition, preferably a physiological condition, and it has a meaning of including both substantially complementary and perfectly complementary ones, and preferably, it means perfectly complementary one.

[0044] As one example, the agent used for detecting the BRAF mutant gene biomarker of the present invention may be an antisense nucleotide.

[0045] The term "antisense nucleotide" means a molecule based on a nucleic acid which can form a dimer with the BRAF mutant gene as it has a sequence complementary to the targeted BRAF mutant gene, and it may be used for detecting the BRAF mutant gene biomarker of the present invention.

[0046] As other example, the agent used for detecting the BRAF mutant gene biomarker of the present invention is a primer pair or probe, and because the sequence of the BRAF mutant gene is discovered in the present description, those skilled in the art may design a primer or probe which specifically amplifies a certain region of this gene based on the sequence.

[0047] The term "primer" is a nucleic acid sequence having a free 3' hydroxyl group and it means a nucleic acid sequence of 7 to 50, which can form a base pair with a complementary template and function as a starting point for template strand copy. The primer is commonly synthesized, but naturally produced nucleic acids may be used. The sequence of the primer is not necessary to be accurately same as the sequence of the template, and it is enough to be hybridized with the template because it is sufficiently complementary. Preferably, the primer of the present invention may be a primer which can amplify the BRAF mutant gene.

[0048] As other example, the agent used for detecting the BRAF mutant gene biomarker of the present invention may a probe. The term "probe" means a nucleic acid fraction of RNA or DNA and the like corresponding to short several bases to long hundreds of bases, and the presence or absence of specific mRNA can be confirmed as it is labeled. The probe may be prepared in a form of oligonucleotide probe, single stranded DNA probe, double stranded DNA probe, RNA probe and the like.

[0049] In the present invention, hybridization is conducted using a probe complementary to the BRAF genetic mutation, thereby diagnosing through hybridization. Selection of a proper probe and hybridization conditions may be modified based on those known in the art.

[0050] Herein, "agent capable of detecting protein" means a substance which can be used for detecting BRAF mutant protein in samples of patients. Preferably, it may be a certain compound or synthetic substance targeting the BRAF mutant protein. As one specific example, it may be an antibody or aptamer specific to the protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1. Preferably, the antibody may be a monoclonal antibody or polyclonal antibody. The term "antibody" means a specific protein molecule indicated for an antigenic site as the term known in the art. On the purpose of the present invention, the antibody means an antibody which specifically binds to the BRAF mutant protein that is the marker of the present invention, and this antibody may be prepared by common methods from the obtained BRAF mutant protein, after the BRAF mutant gene is cloned into an expression vector according to common methods and BRAF mutant protein which is encoded by the BRAF mutant gene is obtained. It includes a partial peptide which can be produced from the BRAF mutant protein, and the partial peptide of the present invention includes at least 7 amino acids, preferably 9 amino acids, more preferably 12 or more amino acids. The form of the antibody of the present invention is not particularly limited, and the antibody of the present invention includes a polyclonal antibody, a monoclonal antibody or parts having antigen-binding thereof, and all immunoglobulin antibodies are included. Moreover, the antibody of the present invention includes a special antibody such as a humanized antibody.

[0051] The antibody used for detection of the biomarker for diagnosing epilepsy of the present invention includes not only a complete form having 2 of whole length of light chains and 2 of whole length of heavy chains, but also functional

fractions of the antibody molecule. The functional fractions of the antibody molecule mean fractions retaining at least a function of binding to an antigen and there are Fab, F(ab'), F(ab') 2 and Fv and the like.

[0052] In addition, the composition for diagnosing epilepsy comprising an agent capable of detecting BRAF mutant gene or BRAF mutant protein of the present invention may be provided as materialized in a form of kit.

[0053] The kit of the present invention can detect the biomarkers for diagnosing epilepsy, BRAF mutant gene or BRAF mutant protein. The kit of the present invention may comprise not only a primer, a probe or an antisense oligonucleotide for detecting the BRAF mutant gene or BRAF mutant protein, or an antibody recognizing the BRAF mutant protein selectively, but also one kind or more kinds of other constituent composition, solution or device appropriate for analysis.

[0054] As one specific example, in the present invention, the kit for detecting the BRAF mutant gene may be a kit for diagnosing epilepsy comprising essential elements necessary to perform a DNA chip. The DNA chip kit may comprise a substrate in which a gene or cDNA corresponding to its fragment is attached with a probe, and a reagent, an agent, or enzyme for producing a fluorescent labeled probe and the like. In addition, the substrate may comprise a quantitative control gene or cDNA corresponding to its fraction. Moreover, the kit for detecting the BRAF mutant gene may be a kit comprising essential elements necessary to perform PCR. The PCR kit may comprise a test tube or other appropriate container, a reaction buffer solution (various pH and magnesium concentrations), deoxynucleotides (dNTPs), an enzyme such as Taq-polymerase, DNase, RNAse inhibitor, DEPC-water, sterile water and the like, in addition to each primer pair specific to the BRAF mutant gene. Furthermore, it may comprise a primer pair specific to a gene used as a quantitative control group.

[0055] As other specific example, in the present invention, the kit for detecting the BRAF mutant protein may comprise a substrate, a proper buffer solution, a coloring enzyme or a secondary antibody labelled with a fluorescent substance, a coloring substrate and the like, for immunological detection of an antibody. As the substrate, nitrocellulose film, a 96 well plate synthesized by polyvinyl resin, a 96 well plate synthesized by polystyrene, and a slide glass made of glass and the like may be used, and as the coloring enzyme, peroxidase and alkaline phosphatase may be used, and as the fluorescent substance, FITC, RITC and the like may be used, and as the coloring substrate solution, ABTS (2,2'-azino-bis(3-ethylbenzothiazolne-6-sulfonic acid)) or OPD (o-phenylene diamine), TMB (tetramethyl benzidine) may be used.

[0056] The present invention relates to a method for detecting a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1, or a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2, to provide information required for diagnosing epilepsy.

[0057] The method for detecting a nucleic acid molecule may comprise amplifying a nucleic acid from a sample of a patient, and determining the sequence of the amplified nucleic acid.

[0058] The sample may be a brain tissue sample of a patient, but not limited thereto.

[0059] As one aspect to conduct the present invention, in order to provide information required for diagnosing epilepsy, a method for detecting the BRAF mutant gene or BRAF mutant protein from a sample of a patient is provided.

[0060] More specifically, it may be performed as the method for detecting the BRAF mutant gene or BRAF mutant protein, and separation of genome DNA or total protein from a sample of a patient may be performed using a known process.

[0061] Herein, the term "sample of a patient" includes samples such as tissue and cells capable of detecting the BRAF mutant gene or BRAF mutant protein. Preferably, it may be brain tissue, but not limited thereto.

[0062] Preferably, the method for detecting the BRAF mutant gene from a sample of a patient may be performed by the method comprising amplifying a nucleic acid from a sample of a patient, and determining the sequence of the amplified nucleic acid.

[0063] Specifically, the amplifying a nucleic acid, may be performed by polymerase chain reaction (PCR), multiplex PCR, touchdown PCR, hot start PCR, nested PCR, booster PCR, real-time PCR, differential display PCR (DD-PCR), rapid amplification of cDNA ends (RACE), inverse polymerase chain reaction, vectorette PCR, TAIL-PCR (thermal asymmetric interlaced PCR), ligase chain reaction, repair chain reaction, transcription-mediated amplification, self-retaining sequence replication or selective amplification reaction of a target sequence.

[0064] In addition, the determining the sequence of the amplified nucleic acid, may be performed by Sanger sequencing, Maxam-Gilbert sequencing, Shotgun sequencing, pyrosequencing, hybridization by microarray, allele specific PCR, dynamic allele-specific hybridization (DASH), PCR extension analysis, TaqMan method, automatic sequence analysis or next generation sequencing. The next generation sequencing may be conducted using a sequence analysis system widely used in the art, and for example, 454 GS FLX of Roche Company, Genome Analyzer of Illumina Company, SOLid Platform of Applied Biosystems Company and the like may be used.

[0065] As other example, the method for detecting the BRAF mutant protein from a sample of a patient includes western blot, ELISA, radioimmunoassay, radioimmunodiffusion, Ouchterlony immunodiffusion method, Rocket immunoelectrophoresis, immunohistological staining, immunoprecipitation assay, complement fixation assay, FACS, protein chip and the like, but not limited thereto. Through the analysis methods, an antigen-antibody complex between the BRAF

mutant protein and an antibody against it can be confirmed, and the antigen-antibody complex between the BRAF mutant protein and an antibody against it is determined, thereby diagnosing epilepsy.

[0066] Herein, "antigen-antibody complex" means a combination of the BRAF mutant protein and an antibody specific thereto, and formation of the antigen-antibody complex can be measured through signals of a detection label. This detection label may be selected from the group consisting of enzyme, fluorescent materials, ligands, luminous materials, microparticles, redox molecules and radioactive isotopes, but not limited thereto.

[0067] As one specific example, the measurement of the antigen-antibody complex between the BRAF mutant protein and an antibody against it is using ELISA method. In addition, preferably, it is to use a protein chip in which one or more antibodies against the BRAF mutant protein are arranged on the designated locations on a substrate and are fixed with a high density. The method for analyzing a sample using a protein chip can confirm occurrence of epilepsy by separating a protein from a sample, hybridizing the separated protein with a protein chip to form an antigen-antibody complex, and reading it to confirm presence of the protein.

[0068] Preferably, the method for detecting the BRAF mutant protein from a sample of a patient may be conducted by the method comprising separating the whole protein from a sample of a patient; and analyzing the amino acid sequence of the separated protein and comparing it to a reference sequence. In addition, preferably, it may be conducted by the method for measuring activation of the BRAF protein increased by BRAF genetic mutation. When the BRAF mutant gene or BRAF mutant protein is detected, through the above detecting methods, epilepsy may be diagnosed.

[0069] The present invention relates to a BRAF V600E recombinant vector, comprising a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

[0070] In one example of the present invention, a gene in which the 1910th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 4 was amplified using the known site-directed mutagenesis method, and this was linked to the Cre recombinase-dependent loxP sequence, and then conditional mutant transgenic mice expressing the Cre-dependent BRAF V600E protein were prepared with a homologous recombination method by adding it to a mouse embryo, and at the 14th day of pregnancy of the mouse obtained by mating the prepared mouse and tdTomato mouse, the uterine horn was exposed and a Cre recombinase was injected into lateral ventricles of each embryo (introduction of gene in uterus), and thereby an epilepsy-induced mouse expressing the BRAF V600E mutation by the Cre recombinase was produced.

[0071] The mouse may express BRAF V637E protein in which the 637th valine is substituted to glutamic acid in the amino acid sequence of SEQ ID NO: 3, or the mouse may express a gene in which the 1910th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 4.

[0072] However, mouse BRAF V637E is traditionally represented by BRAF V600E as same as human BRAF V600E, so in the present description, the mouse BRAF V637E mutation is described as BRAF V600E.

[0073] The prepared recombinant vector may be a vector which can be expressed in mammals or rodents.

[0074] The present invention relates to a composition for inducing ganglioglioma or epilepsy caused by ganglioglioma, comprising a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1.

[0075] The composition for inducing ganglioglioma or epilepsy caused by ganglioglioma is characterized by comprising a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1, which is a mutant protein specifically found in ganglioglioma or epilepsy caused by ganglioglioma. The protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1 may be encoded by a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

[0076] Herein, the term "inducing" means inducing a change from a normal condition to a pathological condition. On the purpose of the present invention, inducing is a change from a condition without epilepsy to a condition with epilepsy. Specifically, epilepsy may be induced by injecting a composition comprising a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1. In addition, epilepsy may be induced by injecting a composition comprising a gene consisting of a sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2, but not limited thereto, epilepsy may be induced by injecting the protein of SEQ ID NO: 1 in which the amino acid sequence is mutated or the gene of SEQ ID NO: 2 in which the gene sequence is mutated.

[0077] The ganglioglioma or epilepsy caused by ganglioglioma may not occur by activation of the mTOR signaling pathway, and the ganglioglioma or epilepsy caused by ganglioglioma may occur by expression of the mutant protein or the mutant nucleic acid molecule in nerve cells not glia, and the ganglioglioma or epilepsy caused by ganglioglioma may occur by expression of the mutant protein or the mutant nucleic acid molecule in nerve cells at an embryo development stage, but not limited thereto.

[0078] The embryo development stage may mean a time to a gastrula stage, and specifically, it may mean a time

before organ is formed.

[0079] In one example of the present invention, it was confirmed that the gangliogliomaor epilepsy caused by gangliogliomawas independent on the BRAF V600E mutation originated from glia, and the BRAF V600E mutation occurring in nerve cells played an important role in an epilepsy occurrence mechanism, and it was confirmed that when the BRAF V600E mutation occurred only in glia, glia proliferation ability and benign tumors increased. Through the above result, it could be seen that the gangliogliomaor epilepsy caused by gangliogliomaof the present invention occurred by the BRAF V600E mutation occurring in nerve cells, but the BRAF V600E occurring in glia did not induce the gangliogliomanor epilepsy caused by gangliogliomaof the present invention, but induced proliferation of cells (Example 6).

[0080] In one example of the present invention, it was confirmed that the gangliogliomaor epilepsy caused by gangliogliomawas not induced by the BRAF V600E mutation expressed in adulthood, but it was induced by the BRAF V600E mutation expressed at the embryo development stage before the organogenesis stage (Example 5).

[0081] In addition, in one example of the present invention, it was confirmed that the gangliogliomaor epilepsy caused by gangliogliomawas not induced by the mTOR signaling pathway (Example 9).

[0082] The present invention may be a recombinant vector, comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

[0083] The present invention relates to a cell in which a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2 is introduced. The cell may be a brain cell.

[0084] The present invention relates to an embryo in which a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2 is introduced.

[0085] The embryo may be a mammal except for a human or a rodent, and the embryo may be an embryo at a brain formation or development stage.

[0086] The present invention relates to an animal except for a human, which is transformed with a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2 and thereby gangliogliomaor epilepsy caused by gangliogliomais induced in.

[0087] The present invention relates to a transgenic animal transformed by the recombinant vector.

[0088] Herein, the term "transgenic animal" means an animal in which modification of traits is induced so that the BRAF V600E protein activity is increased in cells compared to a normal cell, and transformation may be induced by flowing in the vector expressing the BRAF protein in which the amino acid sequence is mutated in cells. The transgenic animal in which epilepsy occurs may be effectively used as an epilepsy animal model.

[0089] The animal may be a mammal except for a human or a rodent, and it may be one in which gangliogliomaor epilepsy caused by gangliogliomais induced.

[0090] The present invention relates to a method for producing an animal with an induced gangliogliomaor epilepsy caused by ganglioglioma, comprising preparing a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2; and transforming a mouse with the recombinant vector.

[0091] The method for introducing the recombinase vector is not particularly limited. For example, through methods such as transformation, transfection or transduction and the like, the vector may be inserted into a cell. The vector inserted into a cell may produce the BRAF protein in which the amino acid sequence is mutated by consistent gene expression in the cell.

[0092] The recombinant vector may be introduced into brain of an embryo in a period of formation of a cerebral cortical layer in the embryonic period, but not limited thereto.

[0093] In one specific example of the present invention, a conditional mutant transgenic mouse capable of expressing a gene in which the 1910th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 4 in a Cre-dependent manner was prepared, and a plasmid having a Cre recombinase was introduced in uterus of the mouse to prepare an epilepsy-induced mouse animal model.

[0094] The present invention relates to an epilepsy-induced animal which is transformed with a recombinant vector comprising a sequence encoding an amino acid in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1. The animal may be a mammal except for a human or a rodent.

[0095] Herein, "animal model" or "disease model" means an animal to be a model which can be an object of study capable of investigating causes of a disease and confirming pathological conditions, as it has a specific disease similar to a disease of a human. The animal to be used as the animal model may have a predicted effect same as a human and may be easily produced and has reproducibility. In addition, it should be progressed as same or similar to causes of a human disease. Thus, an animal which is a mammal vertebrate like a human, and has similar internal structure, immune system, body temperature and the like with human, and suffers from a disease such as high blood pressure, cancer, immunodeficiency, and the like, is appropriate. Such an animal may be preferably a mammal such as horse, ship, pig, goat, camel, antelope, dog, rabbit, mouse, rat, guinea pig, hamster, and the like, and more specifically, it may

be a rodent such as mouse, rat, guinea pig, hamster and the like.

[0096] In one example of the present invention, it was confirmed that BRAF V600E had a very strong correlation to phenotypes shown in ganglioglioma patients, and based on this, it was confirmed whether phenotypes of epilepsy were shown in the animal model when a normal BRAF gene was substituted to the BRAF V600E mutation (Example 4).

[0097] In one example of the present invention, before confirming whether phenotypes of epilepsy was shown in the BRAF gene substitution model, patient tissues were stained with a marker specific to nerve cells and a marker specific to glia, respectively, and only the cells in a specific tissue form were separated using a method of laser capture microscope anatomy to confirm presence or absence of the BRAF V600E mutation, thereby confirming that the BRAF V600E mutation was originated from both nerve cell and glia-based cell lines (FIG. 2a and FIG. 2b). Through the above result, it could be inferred that the BRAF V600E mutation occurred in a common ancestor of both nerve cells and glia (Example 3).

[0098] In one example of the present invention, as a result of conducting Video-Electroencephalography (video-EEG) surveillance from the 3rd week after birth, after an embryo obtained by electroporation of a plasmid having a Cre recombinase into the lateral ventricle of embryonic mice at 14th day (E14), a spontaneous seizure accompanying an epilepsy wave was confirmed in mice in which a plasmid in which a mutant gene with the sequence mutation of the present invention was inserted (FIG. 3b and 3c). Furthermore, as a result of cutting brain of the animal model mice and measuring the activity of tissue using a multichannel electrode analysis device, it was confirmed that synchronized burst firing (FIG. 3c) was shown in which spontaneous activity waves and temporally short period of high amplitude energy are simultaneously emitted from several channels, which are distinctive to epilepsy in the brain tissue having the BRAF V600E mutation, different from the control group (Example 4).

[0099] In one example of the present invention, it was confirmed that in case of GFP positive cells of cerebral region in which the BRAF V600E mutation was induced by electroporation, dysplasia of nerve cells distinctively shown in ganglioglioma was accompanied, and the size of cells became bigger (FIG. 5a), and the shape of cells were dented (FIG. 5b), and the shape of cells and arrangement of branches of nerve cells were arranged in any direction different to normal nerve cells (FIG. 5c).

[0100] In addition, in one example of the present invention, in order to trace daughter cells of neural progenitor cells having the BRAF V600E mutation, as a result of tracing the daughter cells by mating with a conditional floxed tdTomato mouse and preparing a plasmid gene having a Cre recombinase using a method of gene introduction (FIG. 6a), it was confirmed that in the neural progenitor cells having the BRAF V600E mutation, glia-related astrocytes or oligodendrocytes were significantly increased compared to normal brain tissue (FIG. 6b) (Example 5).

[0101] Through the above results, it could be seen that mice produced by the method of the present method were appropriate as an animal model in which ganglioglioma was induced, as dysplasia of nerve cells and numerical increment of glia that were features of ganglioglioma were observed.

[0102] The epilepsy animal model of the present invention may be effectively used in study on gene functions, and study of molecular mechanisms of epilepsy and investigation of novel antiepileptic drugs, and the like.

[0103] The present invention relates to a screening method of therapeutic agent for epilepsy comprising determining epilepsy reduction after administering therapeutic candidates for epilepsy into an animal with an induced ganglioglioma or epilepsy caused by ganglioglioma.

[0104] After administering therapeutic candidates for epilepsy into the epilepsy-induced animal, a substance reducing epileptic symptoms indirectly or directly may be selected as a therapeutic agent for epilepsy. In other words, under the absence of therapeutic candidates for epilepsy, epileptic symptoms are measured, and under the presence of therapeutic candidates for epilepsy, epileptic symptoms are measured, and both are compared, and then a substance reducing epileptic symptoms in case of presence of therapeutic candidates for epilepsy than symptoms in case of absence of therapeutic candidates for epilepsy may be predicted as a therapeutic agent for epilepsy.

[0105] The present invention relates to a composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma comprising an activity inhibitor of a protein comprising an amino acid in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1.

[0106] A specific example of the activity inhibitor of a protein comprising an amino acid in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1 may include one or more selected from the group consisting of Vemurafenib or its salt and Dabrafenib or its salt.

[0107] The inhibitor may be Vemurafenib or Dabrafenib, and the Vemurafenib is also called PLX4032, PLX4720, or Zelboraf.

[0108] In one example of the present invention, it was confirmed that when the BRAF V600E mutation was introduced to cells, the BRAF protein was hyperactivated, and therefore intractable epilepsy could be induced, and it was confirmed that there were effects of prevention, improvement or treatment of intractable epilepsy caused by ganglioglioma (GG), and prevention, improvement or treatment of ganglioglioma (GG) that was a causative disease of intractable epilepsy, when the activity inhibitor of the BRAF V600E protein was administered, by reduction of seizures (Example 8).

[0109] The present invention is to provide a composition, a kit or a method for prevention, improvement or treatment of intractable epilepsy and prevention, improvement or treatment of ganglioglioma (GG) that is a causative disease of

intractable epilepsy. Preferably, the intractable epilepsy relates to a use for prevention, treatment and/or improvement related to brain somatic mutation-related intractable epilepsy.

[0110] The ganglioglioma or epilepsy caused by ganglioglioma may not occur by activation of the mTOR signaling pathway, and the ganglioglioma or epilepsy caused by ganglioglioma may occur by expression of the mutant protein or the mutant nucleic acid molecule in nerve cells, not glia, and the ganglioglioma or epilepsy caused by ganglioglioma may occur by expression of the mutant protein or the mutant nucleic acid molecule in nerve cells at an embryo development stage, but not limited thereto.

[0111] The embryo development stage may mean a time to a gastrula stage, and specifically, it may mean a time before organ is formed.

[0112] The composition comprising the inhibitor may be characterized by reducing seizures occurring by ganglioglioma or epilepsy caused by ganglioglioma.

[0113] Herein, the inhibitor may be Vemurafenib, or Dabrafenib, and the inhibitor may include all derivatives or analogues of the compound and pharmaceutically acceptable salts or hydrates.

[0114] The pharmaceutically acceptable salts or hydrates may be salts or hydrates induced from an inorganic acid or organic acid, and as one example, salts may be hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, acetic acid, glycolic acid, lactic acid, pyruvic acid, malonic acid, succinic acid, glutaric acid, fumaric acid, malic acid, mandelic acid, tartaric acid, citric acid, ascorbic acid, palmitic acid, maleic acid, hydroxymaleic acid, benzoic acid, hydroxybenzoic acid, phenylacetic acid, cinnamic acid, salicylic acid, methanesulfonic acid, benzenesulfonic acid, toluenesulfonic acid, but not limited thereto. The hydrates may mean that Vemurafenib formed by binding to water molecules.

[0115] Herein, "treat" may used as a meaning including all of alleviation or improvement of symptoms, reduction of range of disease, delay or alleviation of disease progress, improvement, alleviation or stabilization of disease conditions, partial or complete recovery, survival extension, and any other beneficial treatment outcomes and the like.

[0116] The symptoms related to the ganglioglioma or epilepsy caused by ganglioglioma are shown as nerve cells fail to move to a proper region of brain in the brain development process, and spontaneous seizures, behavior seizures, brainwave seizures and abnormal generation of nerve cells in cerebrum and the like may be exemplified.

[0117] Thus, the treatment in the present invention may mean reducing the number of spontaneous seizures, behavior seizures or brainwave seizures significantly, and reducing abnormal activity or nerve cells or abnormal signals in cerebrum, by administering a BRAF V600E protein activity inhibitor, for example, Vemurafenib, for patients of ganglioglioma or epilepsy caused by ganglioglioma.

[0118] Depending on use aspects and use methods of the pharmaceutical composition of the present invention, an effective dose of the BRAF protein activity inhibitor may be used by adjusting properly according to the choice of those skilled in the art.

[0119] As one example, the pharmaceutical composition may comprise the BRAF protein activity inhibitor in an amount of 0.1 to 10 % by weight, more preferably 0.5 to 5 % by weight, based on the total weight of the total composition.

[0120] The BRAF protein activity inhibitor may be comprised in the pharmaceutical composition alone, or a pharmacologically acceptable additive other than it may be further comprised. The pharmaceutically acceptable additive is commonly used in formulation, and includes lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia gum, calcium phosphate, alginate, gelatin, calcium silicate, microcrystal cellulose, polyvinylpyrrolidone, cellulose, water, syrup, methyl cellulose, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, and mineral oil and the like, and in addition, a pharmaceutically acceptable excipient includes a lubricant, a sweetener, a flavoring agent, an emulsifier, a suspension, a preservative and the like, but not limited thereto. In other words, the pharmaceutically acceptable additive which can be added in the pharmaceutical composition of the present invention may be composed by selection of those skilled in the art depending on use purposes without difficulty, and its addition amount may be selected within a range which does not damage the object and effect of the present invention.

[0121] The preferable dose for patients of the pharmaceutical composition of the present invention is different depending on condition and body weight of patients, degree of disease, drug form, administration route and period, but it may be appropriately selected by those skilled in the art. However, for a preferable effect, the extract of the present invention is preferably administered in an amount of 1 mg/kg to 1000 mg/kg, preferably 50 mg/kg to 500 mg/kg, more preferably 150 mg/kg to 300 mg/kg a day. The administration may be carried out once or several times a day. Thus, the dose does not limit the scope of the present invention in any way.

[0122] The composition of the present invention may be administered in various routes into mammal animals such as rat, mouse, stock, human and the like. All the methods of administration may be predicted, and for example, it may be administered by oral, intrarectal or intravenous, intramuscular, subcutaneous or intracerebroventricular injection.

[0123] The present invention relates to a food composition for prevention or improvement of ganglioglioma or epilepsy caused by ganglioglioma, comprising a BRAF protein activity inhibitor, for example, Vemurafenib or its salt, as other aspect.

[0124] The food composition may be used together with common components of other food compositions, and it may be used properly according to common methods. The BRAF protein activity inhibitor may be properly determined de-

pending on the use purpose (prevention, health or therapeutic treatment). In general, it may be added in an amount of 0.01 to 10 parts by weight, preferably, 0.05 to 1 part by weight, based on the raw material of active ingredients when preparing the food composition. However, in case of long-term consumption intended for health and hygiene or health control, the amount may be less than the above range.

[0125] The food composition may be contained in health food on a purpose for prevention or improvement of ganglioglioma or epilepsy caused by ganglioglioma, and there is no particular limitation on its kind. Examples of food in which the above substance can be added may include meat, sausage, bread, chocolate, candies, snack, crackers, pizza, ramen, other noodles, gum, dairy products including ice cream, various soup, beverage, tea, drink, alcohol beverage and vitamin complexes and the like, and all the functional foods in the common meaning may be included. Besides the above, the food composition of the present invention may further comprise a food acceptable additive. The percentage of this additive is not greatly important, but it is general to select it within the range of 0.01 to 0.1 part by weight per 100 parts by weight of the composition of the present invention.

[ADVANTAGEOUS EFFECTS]

[0126] The present invention relates to a biomarker of epilepsy, a composition for diagnosing epilepsy, an epilepsy-induced animal, and a composition for preventing or treating epilepsy, and specifically, relates to a composition for diagnosing epilepsy comprising a BRAF mutant protein and a nucleic acid molecule, and an agent capable of detecting the protein or nucleic acid molecule, an epilepsy-induced animal transformed with the BRAF mutant nucleic acid molecule, and a composition for prevention or treatment of epilepsy comprising a BRAF mutant protein activity inhibitor, and the composition for preventing or treating epilepsy comprising a BRAF mutant protein activity inhibitor can be used to prevent or treat ganglioglioma, or epilepsy caused therefrom.

[BRIEF DESCRIPTION OF DRAWINGS]

[0127]

FIG. 1a is a picture of confirming that BRAF V600E is significantly present in pediatric low grade glioma with statistical significance.

FIG. 1b is a picture of confirming the magnetic resonance imaging opinion of the head of the ganglioglioma patient (GG221) (left) and the histopathological opinion confirming the corresponding lesion in the arrow during surgery (right).

FIG. 1c is a picture of confirming the magnetic resonance imaging opinion of the head before surgery of ganglioglioma patients (GG57, GG163, GG221, GG231, GG249, and GG381).

FIG. 2a is a schematized picture of the experimental method for presence or absence of BRAF V600E mutation in a nerve cell and glia using the laser capture microscope anatomical method.

FIG. 2b is a picture of confirming the presence or absence of BRAF V600E mutation in a nerve cell (left) and glia (right) using the laser capture microscope anatomical method.

FIG. 3a is a picture of confirming that the mouse produced according to one example of the present invention expresses a BRAF V600E mutant gene.

FIG. 3b is a picture of confirming that a spontaneous seizure accompanying epileptic spikes in the mouse in which the plasmid, in which the BRAF V600E mutant gene is inserted, is injected according to one example of the present invention.

FIGs. 3c, d, e are pictures of confirming pathological features specific to epilepsy in the brain tissue obtained from the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 4 is a picture of confirming that synchronized burst firing is shown in which spontaneous activity waves and temporally short period of high amplitude energy are simultaneously emitted from several channels, in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 5 is a picture of confirming that dysplasia of the nerve cell characteristically shown in ganglioglioma is accompanied, in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 6a is picture showing a process of mating conditional floxed tdTomato mice for tracing daughter cells of neural progenitor cells having BRAF V600E mutation.

FIG. 6b is a picture of confirming that glia-related astrocytes or oligodendrocytes are significantly increased compared to normal brain tissue in the neural progenitor cells having BRAF V600E mutation produced according to one example of the present invention.

FIG. 6c is a picture of magnifying the photograph of tissue of FIG. 6b at high magnification and retaking it.

FIG. 6d is a picture of confirming that CD34 expression is increased by immunohistochemical staining in the actual

patient tissue.

FIG. 7 is a picture of confirming that the expression of CD34 is increased in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 8 is a picture showing the immunofluorescent staining opinion (left) and immunohistochemical staining opinion (right) which show the increase of the CD34 marker in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 9 is a picture of observing abnormal lamination of the cortex in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 10 is a picture of confirming that when a mouse model is produced by the method of one example of the present invention, the brain tissue having BRAF V600E mutation has the changed distribution in the upper and lower parts different from normal brain tissue and shows cortical dysplasia. In the picture at the bottom of FIG. 10, this observation was magnified and it was confirmed that there was no change in the amount of the total nerve cells.

FIG. 11 is a picture of confirming that patterns of behavior of epilepsy are not shown, but the number of glia-based cells is increased, in the mouse expressing BRAF V600E mutation produced according to one example of the present invention only in glia.

FIG. 12 is a picture showing the method for producing a BRAF V600E mutant mouse which is inducible using tamoxifen in an adult mouse.

FIG. 13 is a picture showing the method for producing a BRAF V600E mutant mouse which is inducible using a virus in an adult mouse.

FIG. 14 is a picture of confirming the abnormal cytological aspect in the case of inducing BRAF V600E mutation in an adult mouse.,

FIG. 15 is a picture of confirming reduction of seizures through chronic intracerebroventricular injection (cICV) of the BRAF V600E-specific inhibitor in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention using measurement of interictal spikes through video monitoring brainwave analysis. POD (post operation day) means days after surgery, and ictal seizure means an epileptic seizure.

FIG. 16 is a picture of confirming reduction of seizures through chronic intracerebroventricular injection of the BRAF V600E-specific inhibitor in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention using measurement of interictal spikes and electrographic seizure spikes through video monitoring brainwave analysis.

FIG. 17 is a picture of confirming that there was no reduction of seizures in the video monitoring brainwave analysis through chronic intracerebroventricular injection (cICV) of the BRAF V600E-specific inhibitor and oral administration (PO, per oral) in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

FIG. 18 is a picture of confirming the activation of the mTOR signaling pathway in the mouse expressing the BRAF V600E mutant protein produced according to one example of the present invention.

[DETAILED DESCRIPTION OF THE EMBODIMENTS]

[0128] Hereinafter, the present invention will be described in more detail by the following examples. However, these examples are intended to illustrate the present invention only, but the scope of the present invention is not limited by these examples.

Example 1. Genome range profiling through genome public data analysis

[0129] Pediatric low grade glioma has much different clinical aspects from brain tumor of general adults, and particularly, it is known that the ratio of accompanying epilepsy is high in this patient group. To confirm why epilepsy is caused with high frequency in pediatric low grade glioma, by analyzing genome public data such as TCGA (The Cancer Genome Atlas) and PeCan (Pediatric Cancer Genomic Data Portal), mutant gene types distinguished in adult and pediatric brain tumors were classified.

[0130] As a result, as shown in FIG. 1, mutations of genes such as IDH1 and TP53 were present remarkably many in the brain tumor of adults, but BRAF V600E mutation was present significantly many in pediatric low grade glioma with statistical significance.

[Table 1]

	Adult brain tumor	Pediatric brain tumor	Pediatric low grade glioma
Number of patients (n)	283	274	73

(continued)

Gene type	Number of mutations (percentage%)		
IDH1	215(75.97)	3(1.09)	1(1.37)
TP53	128(45.23)	38(13.87)	0(0)
H3F3A	0(0)	47(17.15)	3(4.11)
CIC	26(9.19)	1(0.36)	1(1.37)
BRAF	2(0.71)	5(1.82)	19(26.03)
ATRX	16(5.65)	3(1.09)	1(1.37)
PIK3CA	2(0.71)	16(5.84)	0(0)
SMARCA4	8(2.83)	9(3.28)	0(0)
ACVR1	0(0)	16(5.84)	0(0)
NOTCH 1	14(4.95)	2(0.73)	0(0)
Total	411	140	25

Example 2. Confirmation of BRAF V600E mutation using whole exome sequencing

2-1. Confirmation of BARF V600E candidates by whole exome sequencing in 5 patients

[0131] To accurately reflect a tumor-derived epilepsy patient model to an animal model, ganglioglioma known as the biggest cause causing epilepsy-related tumor in children was selected, and to sort gene mutations present in the ganglioglioma, brain tissue and blood samples of 5 ganglioglioma patients (named GG29, GG30, GG221, GG231, and GG249) were collected.

[0132] In the brain tissue sample of ganglioglioma patients, the whole exome sequencing (deep whole exome sequencing, read depth 630-672) was performed, and the candidate mutation found both algorithms of Strelka and Mutect of the whole exome sequencing simultaneously, BRAF V600E was sorted.

[0133] Specifically, a sequencing library was constructed using Agilent library preparation protocols (Agilent Human All Exon 50 Mb kit) provided by the manufacturer for acquisition of data of whole exome sequencing. Sequencing was conducted for the constructed sequencing library using Hiseq2000 (Illumina), and to increase the accuracy of analysis, sequencing was performed with 5 times depth (~500x) of the general sequencing depth.

[0134] The data after the sequencing were converted into a file in a form to be analyzed using Broad Institute best practice pipeline (https://www.broadinstitute.org/gatk/) (bam file) and were stored.

[Table 2]

Sample	Total yield (bp)	Total depth of target site (X)	Average depth of target site (X)
GG221-Blood	44,917,301,962	630.4	293.35
GG221-Brain	39,837,168,006	559.1	296.33
GG29-Blood	47,943,121,572	579.8	327.14
GG29-Brain	43,394,449,010	596.3	328.11
GG30-Blood	43,443,678,228	609.0	329.51
GG30-Brain	41,317,104,442	609.7	341.22
GG231-Blood	45,607,318,610	630.7	351.96
GG231-Brain	42,488,301,048	640.0	352.19
GG249-Blood	44,944,663,064	669.9	360.64
GG249-Brain	47,736,544,858	672.8	361.99

[0135] As a result, as shown in Table 2, it could be confirmed that the BRAF V600E mutation was present commonly

in 3 patients of 5 ganglioglioma patients, and mutations of other genes except for BRAF V600E mutation were not observed. The BRAF V600E mutation confirmed in ganglioglioma is a mutation in which the 1799 thymine of human gene BRAF gene of SEQ ID NO: 4 is substituted to adenine, and this is a mutation in which valine (V) at position 600 of human BRAF protein of SEQ ID NO: 3 is substituted to glutamic acid (E).

2-2. Confirmation of BRAF V600E mutation in expanded patient group

[0136] To confirm whether BRAF V600E confirmed as present in 5 ganglioglioma patients in Example 2-1 was present in other patient group, by expanding the patient group, gene mutations present in 12 patients in total (including 5 patients confirmed in Example 2-1 (GG29, GG30, GG221, GG231, and GG249)) were investigated (Table 3).

[Table 3]

Patient code	Age at surgery (year)	Main opinion	Period of occurrence of epilepsy (year)	Gender	Pathological opinion	Radiologic opinion	Sequencing method	BRAF V600E mutation allele percentage (%)
GG29	14	Recurrent seizure	3	Male	Ganglioglioma in white matter	In the preoperative image, a 1 cm round lesion accompanying edema was located in supramarginal gyrus	Whole	-
GG30	15	Tonic intermittent spasmodic seizure	4	Male	Low grade glioma accompanying multiple calcification close to ganglioglioma	In the preoperative image, cystic lesion with increased size in thalamus	Whole exome sequencing	-
GG54	16	Recurrent seizure	10	Female	Low grade glioma close to ganglioglioma	Low grade cortex tumor close to ganglioglioma in left parahippocampal gyrus, fusiform gyrus and onsil	Target panel sequencing	-
GG57	14	Long-term intractable epilepsy	1	Male	Low grade glioma close to ganglioglioma	1.4cm lump ii mesial temporal lobe	Target panel sequencing	18.50
GG163	6	Long-term intractable epilepsy	0.5	Male	Low grade glioma close to ganglioglioma	Cystic lesion increased at T2 in posteromedial temporal lobe	Target panel sequencing	28.87
GG221	6	Long-term intractable epilepsy	0.5	Female	Low grade glioma close to ganglioglioma	1 cm Cystic lesion in left temporal lobe	Whole exome sequencing	15.44
GG231	7	Long-term intractable epilepsy	2	Male	Low grade glioma close to ganglioglioma	1.5cm T2 increased lesion in right uncus	Whole exome sequencing	17.86
GG249	5	Long-term intractable epilepsy	1	Female	Low grade glioma close to ganglioglioma	T2 increased lesion in right temporalis posterior	Whole exome sequencing	19.05
GG263	2	Recurrent seizure	1	Male	Low grade glioma close to ganglioglioma	T2 increased signal in left frontal lobe	Target panel sequencing	-

(continued)

Patient code	Age at surgery (year)	Main opinion	Period of occurrence of epilepsy (year)	Gender	Pathological opinion	Radiologic opinion	Sequencing method	BRAF V600E mutation allele percentage (%)
GG351	5	Seizure	1	Male	Tumor-related intractable epilepsy close to ganglioglioma	-	Target panel sequencing	-
GG356	9	Seizure	2	Male	Tumor-related intractable epilepsy close to ganglioglioma	-	Target panel sequencing	-
GG381	7	Long-term intractable epilepsy	1	Male	Tumor-related intractable epilepsy close to ganglioglioma	2.8cm cystic lesion in regions including uncus, parahippocampal gyrus and inferotemporal lobe gyrus	Target panel sequencing	7.41

[0137] The BRAF V600E mutation was not found in blood of patients, but was found specifically in the brain tissue sample.

[0138] As a result, as could be seen in Table 3, it could be confirmed that the BRAF V600E mutation was present in 6 patients in 12 ganglioglioma patients in total (Genetic variation ratio was 50%), and it could be seen that the ratio of the BRAF V600E mutation allele to the BRAF normal allele present in each patient was about 7 to 30%.

2-3. Patient sample collection and genome DNA extraction

[0139] For 12 patients of intractable epilepsy caused by ganglioglioma (GG) used in Example 2-2, with the consent of all the patients, their brain tissue (1~2g), saliva (1~2 mL), blood (about 5 mL), frozen tissue and formalin fixed paraffin-embedded brain tissue were obtained (Severance Hospital Pediatric Neurosurgery and Pediatric Neurology). Using the following kits with the manufacturers' protocols, the genome DNA of brain tissue, blood, saliva, frozen and formalin fixed paraffin-embedded brain tissue of patients was separated:
brain tissue: Qiamp mini DNA kit (Qiagen, USA), blood: Flexigene DNA kit (Qiagen, USA), saliva: prepIT2P purification kit (DNAgenotek, USA), frozen tissue and formalin fixed paraffin-embedded brain tissue: Qiamp mini FFPE DNA kit (Qiagen, USA).

2-4. Ganglioglioma-specific gene mutation sequencing

[0140] To further confirm whether the BRAF V600E mutation was present in ganglioglioma patients, for the rest 7 patients except for 5 patients confirmed in Example 2-1 among 12 patients confirmed in Example 2-2, hybrid capture sequencing was performed by the following method so that the lead depth was 100-17,700. For the hybrid capture sequencing, a BRAF gene-specific probe was produced using SureDesign online tools (Agilent Technologies). Using Agilent library preparation protocols provided by the manufacturer, a sequencing library was constructed. For the constructed sequencing library, using Hiseq2500 (illumina), the sequencing was performed so that the central lead depth was 500x. The data after the sequencing were made into a file in a form that could be analyzed using Broad Institute best practice pipeline (https://www.broadinstitute.org/gatk/).

[0141] To find a brain tissue-specific de novo somatic mutation, the somatic mutation found in both algorithms of Strelka and Mutect among gene sequencing results of blood and brain tissue simultaneously was selected. In addition, only genetic mutations satisfying screening criteria of 100 or more depth and 3 or more mutated calls (30 or more mapping quality) among genetic mutations all found in the hybrid capture sequencing result were selected as a disease-related gene candidate.

[0142] To definitely eliminate errors caused in the sequencing process, only the cases of 1% or more genetic variation rate were considered as positive, and only the cases that all variations were shown when the hybrid capture exome sequencing was performed in the genome DNA of blood and brain tissue were considered as positive, to select these variations as genuine mutations.

[0143] As a result, as shown in the following Table 4, the BRAF V600E mutation was not found in the saliva and blood (control group) of genetic variation positive patients (1% or more genetic variation rate) (negative). However, the BRAF V600E mutation was repeatedly detected in 3 patients (GG221, GG231, GG249), and the percentage of BRAF V600E mutation allele to BRAF normal allele was 7% to 30%.

[Table 4]

Patient	Saliva (Presence or absence of BRAF V600E mutation)	Blood (Presence or absence of BRAF V600E mutation)	BRAF V600E mutation allele percentage in brain tissue (%)
GG221	-	-	15.44
GG231	-	-	17.86
GG249	-	-	19.05

Example 3. Confirmation of cell-specific presence of BRAF V600E mutation using laser capture cell lamination

[0144] A prototype of an appropriate model living organism should be prepared to use for patient treatment, but before preparing the prototype of the model organism, it was required to confirm whether the BRAF V600E mutation was present accurately in a nerve cell, or whether it was present in a glia-based cell line, and therefore the following experiment was conducted.

[0145] To confirm whether the BRAF V600E mutation was present accurately in a nerve cell, or whether it was present

in a glia-based cell line, after staining tissue of GG221 and GG231 patients in which the BRAF V600E mutation was confirmed with a marker specific to a nerve cell and a marker specific to glia, respectively, using an immunofluorescent staining method, only a specific tissue type of cells were separated using a method called laser capture microscope anatomy to confirm presence or absence of BRAF V600E.

[0146] The surgical tissue block in which BRAF V600E mutation was confirmed among ganglioglioma patient tissue used in Example 2 (GG221, GG231 patient tissue) was cultured in newly prepared 4% (w/v) paraformaldehyde under phosphate-buffered (PB) (fixation), 20% (w/v) buffered sucrose (cryoprotect), and 7.5% (w/v) gelatin under 10% (w/v) sucrose/PB overnight to make a gelatin-embedded tissue block, and it was stored at -80°C.

[0147] The prepared gelatin-embedded tissue block was rapidly soaked in 2-methylbutanes at - 50°C, and the gelatin embedded tissue block was rapidly frozen, and a cryostat-cut section having a thickness of 10 um was made using a cryostat microtome (Leica) under the circumstance of -20°C or below, and the cryostat-cut section was put on a glass slide, and it was blocked with PBS-GT (0.2% (w/v) gelatin and 0.2% (v/v) Triton X-100 in PBS) at a room temperature for one hour, and it was stained with the following antibodies: mouse monoclonal anti-NeuN (1:200, #MAB377, Millipore) and rabbit polyclonal anti-oligodendrocyte Lineage Transcription Factor 2 (Olig2) (1:500, AB9610, Millipore). After 3 times of PBS cleaning, the tissue slide was stained with the following secondary antibodies: Alexa Fluor 555-conjugated goat antibody to mouse (1:200 dilution; A21422, Invitrogen) or Alexa Fluor 488-conjugated goat antibody to rabbit (1:200 dilution; A11008, Invitrogen). DAPI comprised in a mounting solution (P36931, Life technology) was used for nuclear staining. A fluorescent image was obtained using Leica DMI3000 B inverted microscope. Approximately 50 cells only that were NeuN positive and Olig2 negative, or in contrast, NeuN negative and Olig2 positive were detached using PALM MicroBeam (Carl Zeiss) microscope among stained tissue, and only the genomic DNA was extracted with QIAamp DNA Micro Kit, and then PCR was performed using primers of Table 5, and then the amplified PCR products were purified using MEGAquick spin total fragment purification kit (Intron, Korea), and then Sanger sequencing was performed using BioDye Terminator and automatic sequencer system (Applied Biosystems).

[Table 5]

Name	Primer	SEQ ID NO
BRAF_LCM_F	5'-TGCTTGCTCTGATAGGAAAATG -3'	5
BRAF_LCM_R	5'-AGCCTCAATTCTTACCA TCCAC -3'	6

[0148] As a result, as could be confirmed in FIG. 2b, it could be confirmed that the BRAF V600E mutation (BRAF Chr7: 140453136 for c.1799T>A mutation) was present in two different kinds of cells that were a nerve cell (left in FIG. 2b) and glia (right in FIG. 2b).

[0149] As shown in FIG. 2b, the presence of the same mutation in two different cells, a nerve cell and glia, suggested that the mutation occurred in the common ancestral cells of both nerve cell and glia.

Example 4. Confirmation of prototypes of epilepsy patients in mice expressing BRAF V600E mutation

4-1. Confirmation of spontaneous seizures in animal model mice

[0150] Conditional floxed BRAF V600E mice capable of expressing BRAF V600E mutation shown in epilepsy patients confirmed in Examples 1 to 3 were produced, and the mice were mated with conditional floxed tdTomato mice to produce a timed pregnant of 14 days of embryo, and then a plasmid gene having a Cre recombinase was introduced in uterus by electroporation, and thereby an animal model similar to conditions of ganglioglioma patients.

[0151] Specifically, in one example of the present invention, using the known site-directed mutagenesis method, a gene in which the 1910th thymine was substituted to adenine in the sequence disclosed in SEQ ID NO: 4 was amplified, and a product in which this was added to a Cre-dependent LoxP sequence was injected into a mouse embryo, thereby producing a transformed mouse which was substituted by a Cre-dependent condition mutation, and at the 14th day of pregnancy of a mouse obtained by mating the produced mouse and tdTomato mouse (E14), the uterine horn was exposed, and 2 ug/ml of Fast Green (F7252, Sigma, USA) combined to a 2 to 3 ug of plasmid having a Cre recombinase was injected to lateral ventricles of each embryo using a pulled glass capillary. The plasmid having a Cre recombinase (pCAG-Cre-IRES2-GFP, addgene, #26646) was subjected to electroporation by discharging 50V to the head of embryo with an ECM830 eletroporator (BTX-harvard apparatus) which was an electric pulse 5 times of 100 ms at an interval of 900 ms.

[0152] Genotyping PCR was conducted using primers of Table 6 and i-Taq TM DNA Polymerase kit (Intron, #25021), by detaching the brain tissue of the mouse embryo electroporated with the plasmid having a Cre recombinase.

[Table 6]

Name	Primer	SEQ ID NO
LSL-Braf^{V600E} Fwd	5'-CCCAGGCTCTTTATGAGAA-3'	7
LSL-Braf^{WT} Rev	5'-AGTCAATCATCCACAGAGACCT-3'	8
LSL-Braf^{V600E} Rev	5'-GCTTGGCTGGACGTAAACTC-3'	9

[0153] As a result, as shown in FIG. 3a, it could be confirmed that the produced mice expressed the BRAF V637E mutant gene (It is mouse BRAF V637E mutation, but it is commonly referred to as BRAF V600E as same as human BRAF V600E, so hereinafter it is described as BRAF V600E).

[0154] In order to confirm that the epilepsy wave actually occurred electrophysiologically in the brain of the produced model mice, after a mouse embryo which was electroporated with the plasmid having a Cre recombinase was born, a video-electroencephalography (video-EEG) test was performed from the 3rd week after birth. To analyze the seizure wave with a signal, video electroencephalography surveillance was conducted from the 3rd week after birth. After separating the embryo with its mother, whether a tonic-clonic seizure was started was confirmed by video surveillance for 12 hours a day. Then, the video-electroencephalography for the mice exhibiting a seizure was conducted for 6 hours a day for 2 days or more, thereby investigating the spontaneous seizure exhibiting a seizure wave.

[0155] To measure the frequency of the interictal spike and nonspastic brainwave seizure, video electroencephalography data filmed for about 10 to 12 hours were used, and from these data, data of 1 minute were extracted at an interval of 1 hour and were analyzed.

[0156] The frequency of the interictal spike and nonspastic brainwave seizure was measured by an observer who did not know the genotype of mice. The interictal spike showed a wave in an epilepsy shape of 200 ms or less at a certain interval and it was defined as the case having an amplitude of 2 times or more than the background brainwave, and the nonspastic brainwave seizure showed at least 2 or more of connected spike-wave (1~4 Hz) with an amplitude of 2 times or more than the background brainwave and it was defined as the case observed in all 4 electrodes.

[0157] Specifically, after the mice were weaned (>3 weeks), occurrence of a seizure was confirmed only by video monitoring, and then surgery to implant an electrode to measure electroencephalography was progressed. The electrodes were located on an epidural layer, and A total of 5 electrodes were implanted by implanting 2 in the frontal region (AP+2.8mm, ML ± 1.5 mm), 2 in the temporal region (AP-2.4mm, ML ± 2.4 mm), and 1 in the cerebellum region, based on the bregma. After the recovery period of 4 days, measurement of electroencephalography for 2~5 days (6 hours a day) per mouse was performed at 6 PM to 2 AM. The electroencephalography signal was amplified by an amplifier (GRASS model 9 EEG/Polysomnograph, GRASS technologies, USA), and the signal was analyzed using pCLAMP program (Molecular Devices, USA) or RHD2000 amplifier, board (Intan technoloties, USA) and MATLAB EEGLAB (http://sccn.ucsd.edu/eeglab).

[0158] As a result, as shown in FIG. 3b, 90% or more of mice expressing BRAF V600E mutation showed the spontaneous seizure with the epilepsy wave, and the epilepsy wave showed a high frequency of high amplitude, a spike-wave of high amplitude, and a high frequency of low amplitude. It could be confirmed that the interictal spike was also shown in mice expressing the BRAF V600E mutant gene. The mice showing such a spontaneous seizure showed a systemic tonic-clonic seizure consisting of a tonic phase, a clonic phase and a post-ictal phase, and this was similar to symptoms occurred in ganglioglioma patients. In addition, it was confirmed that the tonic brainwave of the mice showed low-voltage and high-frequency synchronized multifrequency, and the brainwave of the clonic phase showed a constant form of high-voltage, and the post-ictal phase showed the synchronized damped amplitude. On the other hand, the mice expressing the wildtype BRAF protein did not show a spontaneous seizure or an epilepsy wave.

[0159] Thus, based on the above result, it could be seen that a spontaneous seizure with an epilepsy wave occurred in mice in which a plasmid, in which the BRAF V600E mutant gene was inserted, was injected.

4-2. Confirmation of hyperactivity of nerve cells in animal model mice

[0160] In order to analyze the epilepsy wave shown in the model mice produced in Example 4-1 electrophysiologically, the experiment was performed by the following method.

[0161] Specifically, for the model mice produced in Example 4-1, mouse brain cortex slices were obtained by vibratome and then the spontaneous action potential was measured in artificial cerebrospinal fluid. The composition of the artificial cerebrospinal fluid was as follows: in mM, 124 NaCl, 26 NaHCO3, 3 KCl, 1.25 KH2PO4, 2 CaCl2, 1 MgSO4, and 10 D-glucose. The mouse brain cortex slices were put on a brain multichannel electrode recorder (MED64 probe, #P515A, Panasonic Alpha-Med Sciences), and were supported using a slice anchor kit (SHD-22CKIT, Warner Instruments), and

then the artificial cerebrospinal fluid was flowed at a rate of 2 mL/minute for 15 minutes and the epilepsy wave occurring under the condition of 37°C/5% CO2 was measured. Spikes were detected using Mobius software (Alpha Med Scientific).

[0162] The result was shown in FIG. 3c and FIG. 4.

[0163] According to the left in FIG. 3c, in case of inducing BRAF V600E mutation, an epilepsy seizure occurred in mice close to 90%, and according to the middle in FIG. 3c, in case of mice causing a seizure, they showed an epilepsy prototype from 6 weeks to 7 weeks on average in FIG. 3c.

[0164] According to the right in FIG. 3c, the timing of individual seizures of BRAF V600E mutation type of mice could be confirmed.

[0165] According to FIG. 4, it was confirmed that synchronized burst firing was shown, in which a spontaneous activity wave and a short period of high amplitude energy were simultaneously emitted on several channels, which was distinctive of epilepsy in brain tissue having BRAF V600E mutation, different from the control group.

[0166] Thus, through the above result, it could be confirmed that synchronized burst firing was shown, in which a spontaneous activity wave and a short period of high amplitude energy were simultaneously emitted on several channels, which was distinctive of epilepsy in brain tissue obtained from mice expressing the BRAF V600E mutant protein, different from the brain tissue obtained in mice expressing a wildtype BRAF protein that was the control group. Through this, it could be seen that nerve cells were hyperactivated in the model mice produced in Example 4-1.

Example 5: Confirmation of epilepsy-related tumor specificity of mice expressing BRAF V600E mutation

5-1. Immunofluorescent staining of BRAF V600E mutation animal model mice

[0167] For the brain tissue of the animal model mice produced in Example 4-1, the experiment was performed by the method as Example 3 to prepare gelatin-embedded tissue block, and it was stored at -80°C.

[0168] By the method as Example 3, the gelatin-embedded tissue block was produced as cryostat-cut sections, and they were put on a glass slide, and were stained with the following antibodies: mouse monoclonal anti-NeuN (1:200, #MAB377, Millipore), rabbit polyclonal anti-glial fibrillary acidic protein (GFAP) (1:500, #z0334, DAKO), rabbit polyclonal anti-oligodendrocyte Lineage Transcription Factor 2 (Olig2) (1:500, AB9610, Millipore), rabbit monoclonal anti-SIOO beta (1:500, ab52642, Abcam), rabbit monoclonal anti-CD34 (1:500, ab81289, Abcam), mouse monoclonal anti-glutamate decarboxylase 67 (GAD67) (1:500, #MAB5406, Millipore), mouse monoclonal anti-parvalbumin (PV) (1:500, #MAB1572, Millipore), rabbit polyclonal anti-vesicular glutamate transporter 1 (VGLUT1) (1:500, #135 303, Synaptic Systems), rabbit polyclonal anti-vesicular GABA transporter (VGAT) (1:500, #135 002, Synaptic Systems), mouse monoclonal anti-GFP (1:500, #Ab1218, Abcam), rabbit polyclonal anti-GFP (1:500, #Ab290, Abcam), rabbit polyclonal anti-REST (1:200, IHC-00141, Bethyl Laboratories) and rabbit polyclonal anti-CUXl (1:400, SC13024, Santa cruz). After three times of PBS washing, the tissue slide was stained with secondary antibodies as follows: Alexa 488-conjugated goat anti-rabbit IgG (1:500, #A11008, Invitrogen) or Alexa 555-conjugated goat anti-rabbit IgG (1:500, #21428, Invitrogen). DAPI comprised in a mounting solution (P36931, Life technology) was used for nuclear staining. Images were obtained using Leica DMI3000 B inverted microscope or Zeiss LSM780 confocal microscope. The number of cells which were positive to NeuN was measured using 10x objective lens; 4 to 5 fields were obtained per one subject in the neuron-rich region, and 100 or more cells were recorded per region. The number of DAPI-positive cells represents the total number of cells. The size of neuron cells was measured using an automated counting protocol of ImageJ software (http://rsbweb.nih.gov/ij/) in NeuN positive cells. The circularity and aspect ratio of nerve cells and the angle to the cortex side of dendrites were referenced to values calculated automatically by ImageJ.

5-2. Confirmation of nerve cell dysplasia through quantitative analysis of immunofluorescent stained tissue

[0169] The result of immunofluorescent staining by the method as Example 5-1 was shown in FIG. 5.

[0170] As a result, as could be seen in FIG. 5, it could be confirmed that in case of GFP positive cells of cerebral region in which BRAF V600E mutation was induced by electroporation by the method as Example 4, dysplasia of nerve cells distinctively shown in ganglioglioma was accompanied (A in FIG. 5), and it could be confirmed that in case of the GFP positive cells, the size of cells became bigger and the shape of cells were dented (B in FIG. 5), and the shape of cells and arrangement of branches of nerve cells were arranged in any direction different to normal nerve cells (C in FIG. 5).

5-3. Confirmation of increases of glia proliferation through quantitative analysis of immunofluorescent stained tissue

[0171] In order to trace daughter cells differentiated from neural progenitor cells having BRAF V600E mutation in animal model mice produced using the method as Example 4-1, mice were obtained by mating transformed mice substituted with a gene expressing BRAD V600E protein dependent to a Cre recombinase with mice capable of producing tdTomato fluorescent protein using the method as Example 4-1, and a Cre recombinase was introduced by electroporation

in uterus for the mice, and daughter cells differentiated from neural progenitor cells having BRAF V600E mutation derived from the mice were traced by tdTomato fluorescent staining.

[0172] As a result, as could be seen in FIG. 6b, in the daughter cells differentiated from neural progenitor cells having BRAF V600E mutation, glia-based related astroglia cells (left picture in FIG. 6b) or oligodendroglia (right picture in FIG. 6b) were significantly increased compared to the brain tissue of mice expressing a BRAF normal gene.

[0173] To sum up the results of Examples 5-2 and 5-3, it could be seen that the BRAF V600E mutation produced in Example 4 was an animal model which reflected a ganglioglioma disease accompanied with dysplasia of nerve cells and numerical increment of glia well.

5-4. Confirmation of CD34 marker through quantitative analysis of immunofluorescent stained tissue

[0174] It was known that ganglioglioma had a characteristic of increased expression of CD34 pathologically in tumor tissue in the past, and therefore the expression of CD34 marker was confirmed by performing immunofluorescent staining by the method as Example 5-1 in the animal model mouse tissue produced by the method as Example 4-1.

[0175] As a result, as shown in FIG. 7, it could be confirmed that the expression of CD34 increased 2.2 times even in animal model mouse tissue produced by the method as Example 4-1, different from the control group.

[0176] In addition, in order to further confirm characteristics of ganglioglioma, the animal model mouse tissue produced by the method as Example 4-1 was stained using hematoxylin-eosin and DAB immunohistochemical staining used when diagnosing ganglioglioma pathologically.

[0177] Specifically, formalin (Sigma, # HT501128) was injected through heart of the animal model mice produced by the method as Example 4-1 and was fixed, and then paraffin was penetrated into a paraffin embedding device (Leica TP1020) over one day to obtain formalin-fixed paraffin-embedded mouse brain tissue. The formalin-fixed paraffin-embedded mouse brain tissue was cut in a thickness of 4um using microtome (Leica RM 2135), and then general hematoxylin-eosin staining was conducted. For immunohistochemical staining of CD34, at first, antigen collection for slides was performed using sodium citrate, and they were hydrated using a gradual concentration of alcohol, and then the intrinsic peroxidase activity was removed using 3% (w/w) hydrogen peroxide solution (H_2O_2) (Sigma aldrich, H6520). Then, the tissue was blocked using a blocking solution, PBS-GT solution (0.2% (w/v) gelatin and 0.2% (v/v) Triton X-100 under PBS), and then it was stored at a room temperature using an anti-CD34 primary antibody (1 :500, ab81289, Abcam). After three times of PBS washing and staining with a secondary antibody, antigen-binding sites in tissue were visualized using 3,3'-Diaminobenzidine substrate solution (DAB, Vector Laboratories). To exclude a bias by the image intensity, normal and BRAF V600E mutant mouse tissue was reacted for the same time, and background staining was performed with hematoxylin.

[0178] As a result, as shown in the right picture of FIG. 8, it could be confirmed that the expression of CD34 which was not stained in the normal tissue was remarkable in the area where the mutation occurred in the BRAF V600E mutant mouse brain tissue.

[0179] To sum up the results of FIG. 7 to FIG. 9, it could be seen that the expression of CD34 was significantly increased in the mouse brain tissue having BRAF V600E somatic genome mutation, compared to the mouse brain tissue having a normal gene, and such a pattern was distinctively shown along with nerve cells and tissue in which the mutation was induced by electroporation in uterus particularly.

5-5. Confirmation of cortical dyslamination through quantitative analysis of immunofluorescent stained tissue

[0180] Since ganglioglioma has been known to accompany local cortical dysplasia or have many cases of observed cortical dyslamination as a part of malformation of cortical development in tumor tissue, in order to confirm whether the similar aspect was shown in the animal model mice produced by the method as Example 4-1, the animal model mouse tissue produced by the method as Example 401 was fixed and prepared by the method as Example 5-4, and then the layer of cortex was classified by the method as Example 5-1, and thereby staining was performed by Cux1 marker which could be confirmed.

[0181] As a result, as shown in FIG. 9, it could be confirmed that the Cux1 marker was intensively observed in the bottom part as well as the top part of cortex in the mouse brain tissue having BRAF V600E somatic genome mutation, different from the mouse brain tissue having a normal gene. In particular, it was confirmed that the distribution of nerve cells which were positive to Cux1 had negative correlation statistically significantly in mice having BRAF V600E mutation different from the normal group, and thereby it could be seen that cortical dyslamination of mouse brain tissue having BRAF V600E somatic genome mutation was remarkably shown.

[0182] In addition, when the animal model mouse tissue produced by the method as Example 4-1 was under immunohistofluorescent analysis to confirm the distribution of cells which were positive to NeuN and tdTomato concurrently, as could be confirmed in the top of FIG. 10, it could be confirmed that nerve cells which were positive to NeuN and positive to tdTomato were significantly distributed in the layer of the bottom part of cortex in which cells derived from the

mouse brain tissue having BRAF V600E mutation were not normally distributed ($r = -0.1122$, $p < 0.0001$).

[0183] However, by the bottom of FIG. 10, it could be seen that hyperplasia in glia shown in Example 5-3 was not simultaneously transmitted in nerve cells. In other words, only the malposition in cortex was shown without proliferation of cells in nerve cells.

5-6. Result arrangement "G summary

[0184] Through the results of Examples 5-2, 5-3, 5-4 and 5-5, it could be confirmed that the animal model mouse tissue produced by the method as Example 4-1 reflected opinions of tumors with high frequency of accompanying epilepsy among pediatric low grade glioma including ganglioglioma. The above opinions include dysplasia of nerve cells, glia hyperplasia, abnormality of arrangement of dendrites of nerve cells, CD34-positive opinion and transmission of cortical dyslamination, by BRAF V600E mutation, and the like.

Example 6. Phenotypes according to spatial acquisition of BRAF V600E mutation

[0185] In case of pediatric epilepsy-related tumor and pediatric low grade glioma and the animal model mice suggested in the present invention examined in Examples 3 and 5-2, the BRAF V600E mutation was present in glia, and therefore it is required to confirm which mutation plays an important role in inducing epilepsy among mutations derived from nerve cells or glia.

[0186] For this, a mouse capable of expressing the BRAF V600E mutation found in ganglioglioma patients in a Cre-dependent manner was produced by a similar method to Example 4-1, and a plasmid having a Cre recombinase at the 1th day after the mouse birth was electroporated, and then behavior was monitored for about 90 days.

[0187] Specifically, in the differentiation of cortical nervous system, the differentiation of nerve cells and glia were actively proceeded at E14 and after birth, respectively, and therefore, when a Cre plasmid was injected at the 14th day of pregnancy of the mother mouse who was in pregnancy of an embryo capable of expressing a Cre-dependent gene, the mutation occurred in nerve cells and glia, and when a Cre plasmid was injected at the first day after birth of the mouse, the mutation occurred in glia, so in order to generate BRAF V600E mutation only in glia using the above fact, mice were produced as Example 4-1, but a Cre plasmid was injected at the first day after birth of the mouse capable of expressing a Cre-dependent gene.

[0188] As a result, as shown in B of FIG. 11, it could be confirmed that a seizure did not occur at all in the mouse produced so as to generate BRAF V600E mutation only in glia (GFAP, and OLIG2 were glia-confirming markers).

[0189] In addition, in order to confirm whether glia hyperplasia was caused in the mouse produced so as to generate BRAF V600E mutation only in glia, the experiment was performed as Example 5-3, and as a result, as shown in C of FIG. 11, it could be confirmed that glia hyperplasia was significantly shown continuously.

[0190] Thus, to sum up the results, it could be seen that the development of epilepsy by BRAF V600E somatic genome mutation was independent of the mutation originated from glia and BRAF V600E mutant generated in nerve cells played an important role in an epilepsy pathogenic mechanism, and in addition, it could be seen that proliferation of a benign tumor in which BRAF V600E mutation occurred only in glia increased.

Example 7: Phenotypes according to temporal acquisition of BRAF V600E mutation

[0191] Since epilepsy-related tumor was found with a high ratio in children, whether there was a phenotypic difference between acquisition of BRAF V600E mutation of childhood and acquisition of the mutation in adult mice in the mouse model of the present invention such as epidemiological information of patients was confirmed by the following method.

7-1. BRAF V600E mutation inducible using tamoxifen in adult mice

[0192] In order to change time of occurrence of BRAF V600E mutation in the animal model mouse, the following method was used.

[0193] Timed pregnant of conditional floxed BRAF V600E mice was made by the method as Example 4-1, and a plasmid gene having a tamoxifen inducible Cre recombinase was introduced in uterus, and then from the 30th day after birth, tamoxifen was intraperitoneally injected to induce the BRAF V600E somatic genome mutation in model mouse brain appropriately. Tamoxifen was treated from the 30th day after birth, and behavior of mice was monitored for about 30 days.

[0194] Specifically, for introduction of genome in uterus, the uterine horn was exposed of timed pregnant 14th day (E14), and 2 ug/ml of Fast Green (F7252, Sigma, USA) combined to a 2 to 3 ug of plasmid having a Cre recombinase was injected to lateral ventricles of each embryo using a pulled glass capillary. The plasmid was subjected to electroporation by discharging 50V to the head of embryo with an ECM830 eletroporator (BTX-harvard apparatus) which was

an electric pulse 5 times of 100 ms at an interval of 900 ms. In addition, tamoxifen (Sigma, T5648) was shaded in corn oil (Sigma, C8267) at a concentration of 10mg/ml at 37°C for 1 day to store, and 100 ug/g tamoxifen was intraperitoneally injected for both normal mice and mutant mice, and it was treated once a day for 5 consecutive days, and after the withdrawal period for immediately next one week, it was treated again for 5 consecutive days. After treatment of tamoxifen, behavior observation for mice was started, and behavior was compared using a mouse treated with only corn oil without tamoxifen or a normal mouse treated with tamoxifen as a control group. A schematic flow of the above experimental procedure was shown in FIG. 12.

[0195] As a result, as could be confirmed in the right graph in FIG. 12, in case that the BRAF V600E mutation was induced in nerve cells of adult mice of after 30 days after birth, mice did not exhibit icta seizures.

7-2. BRAF V600E mutation adult mice using a virus

[0196] In order to change time of occurrence of BRAF V600E mutation in the animal model mice, BRAF V600E mutation adult mice were produced using a virus by the following method.

[0197] Specifically, mice after 30 days after birth were anesthetized, and heads were fixed in a stereotactic surgery device (Stoelting Co., Wood Dale, IL) and a hole having a diameter of about 1 mm was made in a brain bone with a dental drill. 0.5 uL AAV9.CamKII.HI.eGFP-Cre.WPRE.SV40 (Penn Vector Core Philadelphia, PA; titer 6.54 x 1013 GCml-1) was injected into somatosensory cortex (AP: -0.5; ML: -2.0~-2.5; DV: -0.5) using a glass micropipette at a slow rate (1 nL per sec). In 2 weeks, behavior of mice in which a virus was injected was observed and measurement of brainwaves was started. After observation was finished, frozen tissue slides of brain were prepared in a thickness of 20 um with paraformaldehyde by the method as Example 5-1, and they were observed with a microscope, and whether fluorescent reporter protein injected through the virus expressed well was re-confirmed.

[0198] As a result, as could be confirmed in FIG. 13, as same as inducement of BRAF V600E mutation in adult mice using tamoxifen in Example 7-1, a seizure was not shown also in mice in which the mutation was induced using a virus.

7-3. Confirmation of a cytologically abnormal aspect by BRAF V600E mutation induction in adult mice

[0199] After inducing the BRAF V600E somatic genome mutation in adult mice as the method of Examples 7-2 and 7-3, dysplasia of nerve cells was confirmed by the method as Example 5-2.

[0200] As a result, as could be confirmed in FIG. 14, in case of inducing (BRAFWT/LSL-V600E) the BRAF V600E mutation in adult mice, as same as the BRAF V600E mutant mouse produced by the method as Example 4-1, compared to the mouse having a normal BRAF gene (BRAFWT/WT), there was no big difference in the size of cells (A in FIG. 14), circularity and aspect ratio of cells (B in FIG. 14) and the angle to the cortical surface of dendrites (C in FIG. 14) and the like from the mouse having a normal BRAF gene (absence of dysplasia of nerve cells).

[0201] In addition, in case of inducing the BRAF V600E mutation in adult mice using tamoxifen, compared to the mouse having a normal BRAF gene, there was no big difference in the size of cells, the circularity and aspect ratio of cells, and the angle to the cortical surface of dendrites and the like from the mouse having a normal BRAF gene (D in FIG. 14) (absence of dysplasia of nerve cells).

[0202] Through the above result, it could be seen that when the BRAF V600E mutation occurred only in nerve cells at an embryonic development stage, dysplasia of nerve cells was induced and thereby epilepsy was caused.

Example 8. Confirmation of reduction of seizures by injection of a BRAF V600E specific inhibitor

[0203] In order to reduce the frequency of seizures generated in BRAF V600E mutant mice produced by the method as Example 4-1, a BRAF V600E specific inhibitor was treated and its effect was confirmed.

[0204] Specifically, a BRAF V600E mutant protein-specific activity inhibitor (Vemurafenib) was injected into brain tissue of BRAF V600E mutant mice produced by the method as Example 4-1 for a long period. They were generally anesthetized by peritoneal injection of a solution in which Zoletil (0.01 mg/kg) and rompun (0.2 mg/kg) were mixed, and vehicle (50% (v/v) DMSO in DW) or 500 um PLX4032 (Vemurafenib) (V-2800, LC laboratories) was under chronic intracranioventricular injection (cICV) into right ventricle space (AP: -0.6; ML: -1.0; DV: -2.0) in an osmotic pump (ALZET pumps 2004 or 2006, ALZET Osmotic Pumps, Cupertino, CA) with infusion cannula (ALZET brain infusion kit 3). In order to trace brainwaves, after inserting 4 EEG probes (left/right frontal lobes and left temporal lobes, cerebellum as a reference) into the animal model mouse head by the method as Example 4-1, brainwave signals were obtained, and 5~6 weeks later, mice were sacrificed and brain was removed to use for the further research, and then whether the semipermeable membrane in the osmotic pump was reduced was re-confirmed. Drug injection was conducted by 4 weeks ~ 6 weeks, and at that time, the brainwaves of mice were monitored and recorded.

[0205] The result was shown in FIG. 15 to FIG. 17.

[0206] According to FIG. 15, it could be confirmed that icta seizures were reduced about 4 times in case of mice in

which the BRAF V600E specific inhibitor was injected (FIG. 15 B), and it could be electrophysiologically confirmed that seizures were continuously reduced when treating the BRAF V6 00E specific inhibitor for 6 weeks (FIG. 15 C).

[0207] According to FIG. 16, it could be confirmed that the electrographic seizures were reduced 2 times to 3 times (middle in FIG. 16), and the interictal spikes were reduced 1.3 times to 1.5 times.

[0208] In addition, according to FIG. 17, it could be confirmed that the seizure frequency was not reduced in case of acute intracerebroventricular injection (3 times/day, 5 uL injection for 8 minutes) (left in FIG. 17) or oral administration (PO, per oral) (right in FIG. 17) of the BRAF V600E specific inhibitor.

Example 9. Seizure-related mechanism by BRAF V600E mutation

[0209] The abnormal activity of the mTOR signaling pathway has been known to induce an epileptic seizure, and the BRAF V600E mutation has been known to activate the MARK signaling pathway of Ras-Raf-MEK-ERK. On the other hand, the MAPK signaling pathway of Ras-Raf-MEK-ERK known to be activated by the BRAF V600E mutation has been known to activate the mTOR signaling pathway by crosstalk with the mTOR signaling pathway, and it has been reported that the expression of mTOR signaling pathway-related proteins was increased in tissue of ganglioglioma patients. Thus, in order to confirm whether an epilepsy seizure was caused by activation of the mTOR signaling pathway by the BRAF V600E mutation, the following experiment was performed.

[0210] Specifically, for the animal model mice produced by the method as Example 4-1, rapamycin which was an mTOR-specific inhibitor was intraperitoneally injected, and then the change was confirmed. Rapamycin (LC Labs, USA) was diluted by 20 mg/ml in 100% ethanol to prepare a stock solution, and then was stored at 20°C. Before injecting rapamycin to the animal model mice, the rapamycin stock solution was diluted in 5% (w/v) polyethylene glycol 400 and 5% (v/v) Tween80 to prepare a solution of 1 mg/mL rapamycin and 4% (v/v) ethanol. The prepared solution was administered by intraperitoneal injection at a concentration of 1 to 10 mg/kg for 2 weeks (10 mg/kg/d intraperitoneal injection, for 2 weeks).

[0211] As a result, as shown in FIG. 18, despite of administration of rapamycin, the frequency of epilepsy seizures of BRAF V600E animal model mice did not decrease, and the BRAF V600E mutation animal model mouse tissue did not show a big difference compared to the normal mouse tissue in phosphorylation of S6 protein in the mTOR subpath.

Claims

1. A composition for inducing ganglioglioma or epilepsy caused by ganglioglioma,
comprising a protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1.

2. The composition for inducing ganglioglioma or epilepsy caused by ganglioglioma according to claim 1, wherein the protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid is encoded by a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

3. The composition for inducing ganglioglioma or epilepsy caused by ganglioglioma according to claim 1, wherein the ganglioglioma or epilepsy caused by ganglioglioma is not caused by activation of the mTOR signaling pathway.

4. The composition for inducing ganglioglioma or epilepsy caused by ganglioglioma according to claim 1, wherein the ganglioglioma or epilepsy caused by ganglioglioma is caused by expression of the mutant protein or the mutant nucleic acid molecule in a nerve cell.

5. The composition for inducing ganglioglioma or epilepsy caused by ganglioglioma according to claim 1, wherein the ganglioglioma or epilepsy caused by ganglioglioma is caused by expression of the mutant protein or the mutant nucleic acid molecule in a nerve cell of an embryonic development stage.

6. A recombinant vector, comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

7. A cell introduced by a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

8. An embryo of an animal other than a human, introduced by a recombinant vector comprising a nucleic acid molecule

comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

9. An animal other than a human with an induced ganglioglioma or epilepsy caused by ganglioglioma, wherein the animal is transformed with a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

10. The animal other than a human with the induced ganglioglioma or epilepsy caused by ganglioglioma according to claim 9, wherein the animal is a mammal other than a human or a rodent.

11. A method for preparing an animal with an induced ganglioglioma or epilepsy caused by ganglioglioma, comprising preparing a recombinant vector comprising a nucleic acid molecule comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2; and
transforming a mouse with the recombinant vector.

12. The method for preparing an animal with the induced epilepsy according to claim 11, wherein the recombinant vector is introduced to brain of an embryo during the formation of a cortical layer in the embryonic period.

13. A method for screening a therapeutic agent for ganglioglioma or epilepsy caused by ganglioglioma, comprising determining an alleviation of epilepsy after administering a candidate substance for epilepsy treatment to the animal with an induced ganglioglioma or epilepsy caused by ganglioglioma of claim 11.

14. A composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma, comprising an activity inhibitor of a protein comprising an amino acid in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1.

15. The composition for preventing or treating epilepsy according to claim 14, wherein the inhibitor comprises one or more selected from the group consisting of Vemurafenib or its salt, and Dabrafenib or its salt.

16. The composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma epilepsy according to claim 14, wherein the protein is encoded by a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in the nucleotide sequence of SEQ ID NO: 2.

17. The composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma epilepsy according to claim 14, wherein the ganglioglioma or epilepsy caused by ganglioglioma is not caused by activation of the mTOR signaling pathway.

18. The composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma epilepsy according to claim 14, wherein the ganglioglioma or epilepsy caused by ganglioglioma is caused by expression of the mutant protein or the mutant nucleic acid molecule in a nerve cell.

19. The composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma epilepsy according to claim 14, wherein the ganglioglioma or epilepsy caused by ganglioglioma is caused by expression of the mutant protein or the mutant nucleic acid molecule in a nerve cell of an embryonic development stage.

20. The composition for preventing or treating ganglioglioma or epilepsy caused by ganglioglioma epilepsy according to claim 14, wherein the composition is **characterized by** reducing a seizure caused by ganglioglioma or epilepsy caused by ganglioglioma epilepsy.

21. A composition for diagnosing ganglioglioma or epilepsy caused by ganglioglioma epilepsy, comprising an agent capable of detecting
a mutant protein consisting of an amino acid sequence comprising a mutation in which valine at position 600 is substituted to glutamic acid in an amino acid sequence of SEQ ID NO: 1, or
a mutant nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which thymine at position 1799 is substituted to adenine in a nucleotide sequence of SEQ ID NO: 2.

22. The composition for diagnosing epilepsy according to claim 21, wherein the ganglioglioma is caused by a brain somatic mutation.

23. The composition for diagnosing epilepsy according to claim 21, wherein the ganglioglioma or epilepsy caused by ganglioglioma is not caused by activation of the mTOR signaling pathway.

24. The composition for diagnosing epilepsy according to claim 21, wherein the ganglioglioma or epilepsy caused by ganglioglioma is caused by expression of the mutant protein or the mutant nucleic acid molecule in a nerve cell.

25. The composition for diagnosing epilepsy according to claim 21, wherein the ganglioglioma or epilepsy caused by ganglioglioma is caused by expression of the mutant protein or the mutant nucleic acid molecule in a nerve cell of an embryonic development stage.

26. The composition for diagnosing epilepsy according to claim 21, wherein the protein is encoded by a nucleotide sequence comprising a mutation in which the 1799th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 2.

27. The composition for diagnosing epilepsy according to claim 21, wherein the agent capable of detecting the nucleic acid molecule is a primer, a probe or an antisense nucleic acid complementary to the nucleotide sequence.

28. The composition for diagnosing epilepsy according to claim 21, wherein the agent capable of detecting the protein is an antibody or an aptamer specific to the protein.

29. The composition for diagnosing ganglioglioma or epilepsy caused by ganglioglioma epilepsy according to claim 28, wherein the antibody is a monoclonal antibody or a polyclonal antibody.

30. A method for detecting a protein consisting of an amino acid sequence comprising a mutation in which the 600th valine is substituted to glutamic acid in the amino acid sequence of SEQ ID NO: 1, or
a nucleic acid molecule consisting of a nucleotide sequence comprising a mutation in which the 1799th thymine is substituted to adenine in the nucleotide sequence of SEQ ID NO: 2,
from a sample of a patient, to provide information required for diagnosis of epilepsy.

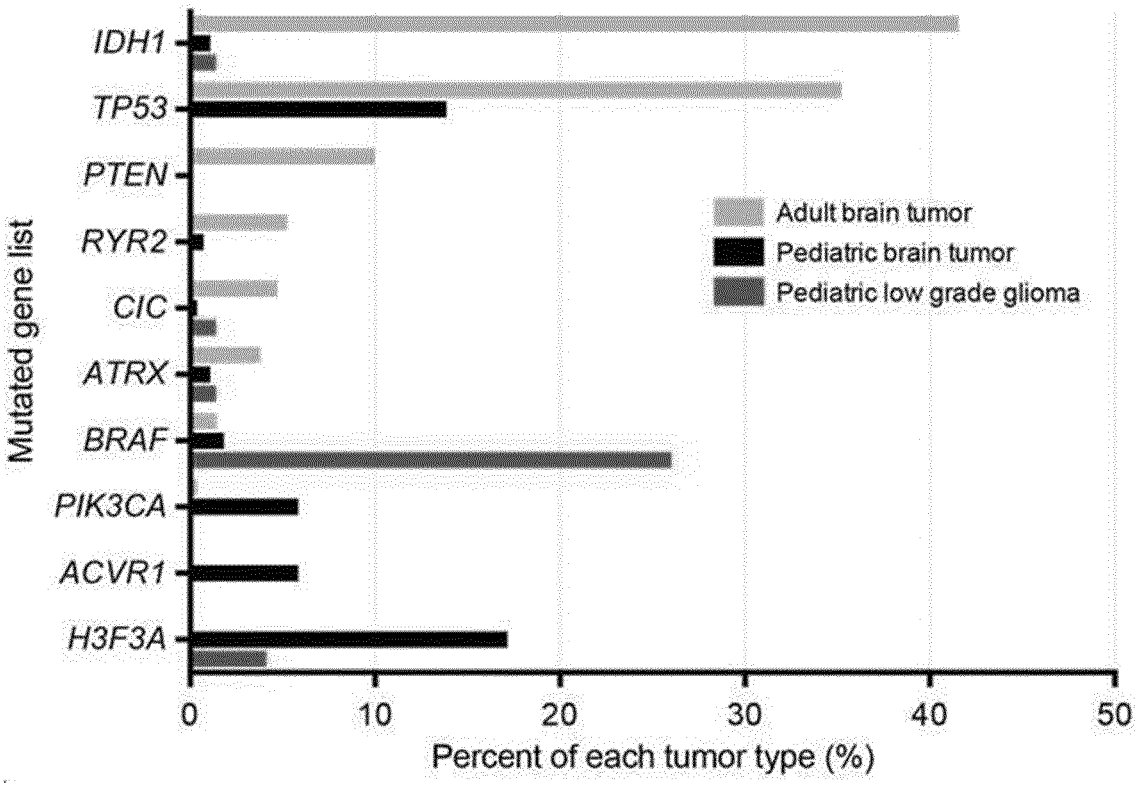
31. The method according to claim 30, wherein the sample is a brain tissue sample of a patient.

32. The method according to claim 30, wherein the method for detecting the nucleic acid molecule comprises
amplifying a nucleic acid from a sample of a patient, and
determining a sequence of the amplified nucleic acid.

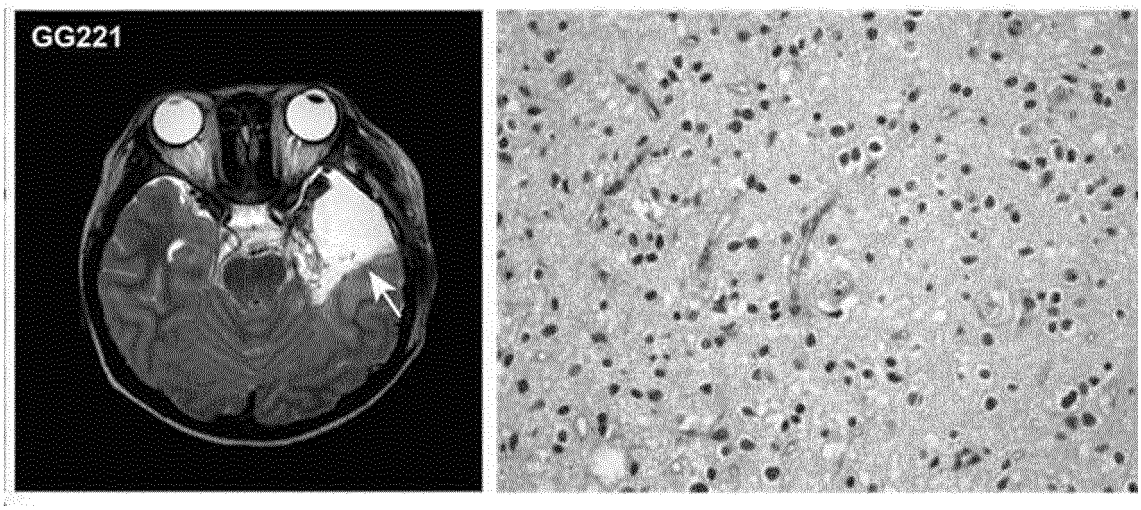
33. The method according to claim 32, wherein the amplifying a nucleic acid is conducted by polymerase chain reaction (PCR), multiplex PCR, touchdown PCR, hot start PCR, nested PCR, booster PCR, real-time PCR, differential display PCR (DD-PCR), rapid amplification of cDNA ends (RACE), inverse polymerase chain reaction, vectorette PCR, TAIL-PCR (thermal asymmetric interlaced PCR), ligase chain reaction, repair chain reaction, transcription-mediated amplification, self-retaining sequence replication or selective amplification reaction of a target sequence.

34. The method according to claim 32, wherein the determining a sequence of the amplified nucleic acid is conducted by next generation sequencing, Sanger sequencing, Maxam-Gilbert sequencing, Shotgun sequencing, pyrosequencing, hybridization by microarray, allele specific PCR, dynamic allele-specific hybridization (DASH), PCR extension analysis, TaqMan method, or automatic sequence analysis.

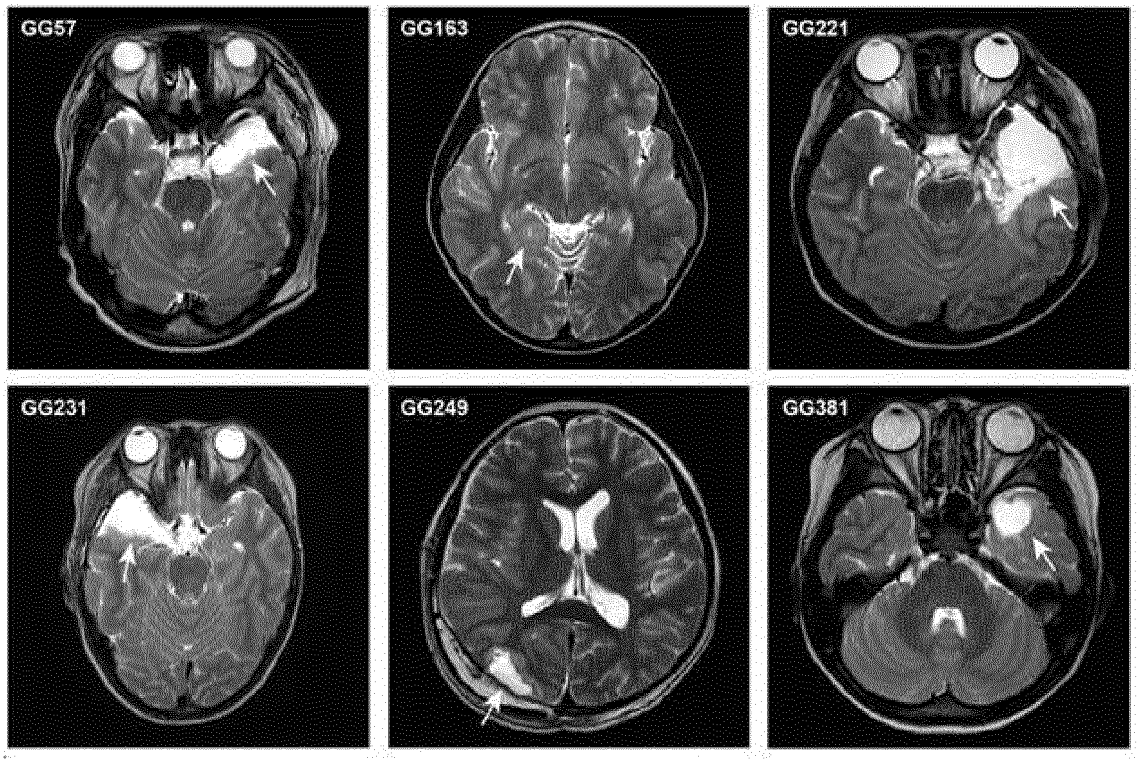
【FIG. 1a】


IDH1
TP53
PTEN
RYR2
CIC
ATRX
BRAF
PIK3CA
ACVR1
H3F3A
Mutated gene list
Adult brain tumor
Pediatric brain tumor
Pediatric low grade glioma
0
10
20
30
40
50
Percent of each tumor type (%)

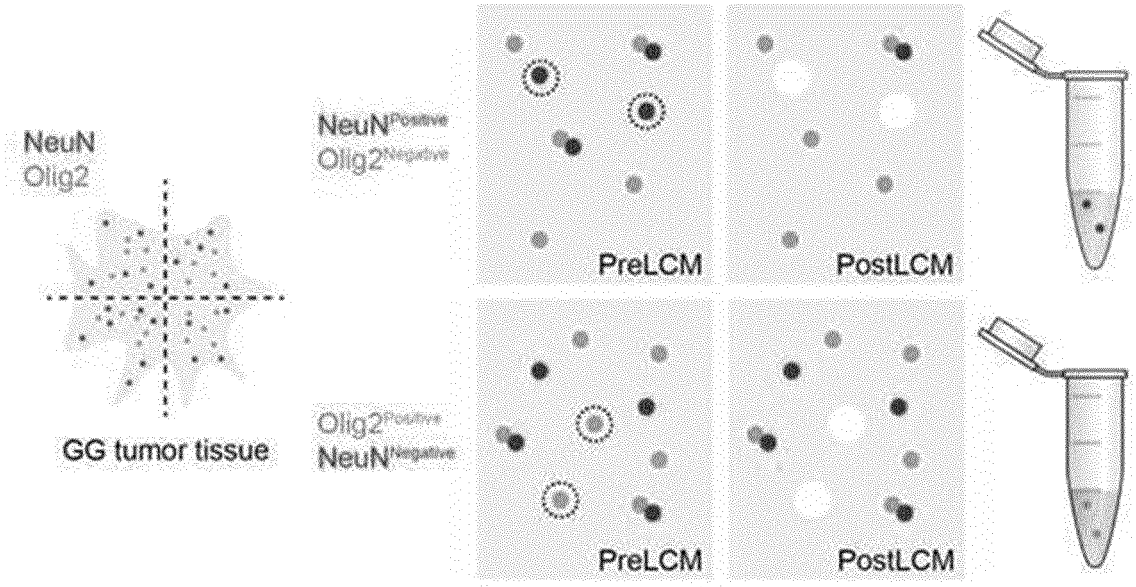
【FIG. 1b】



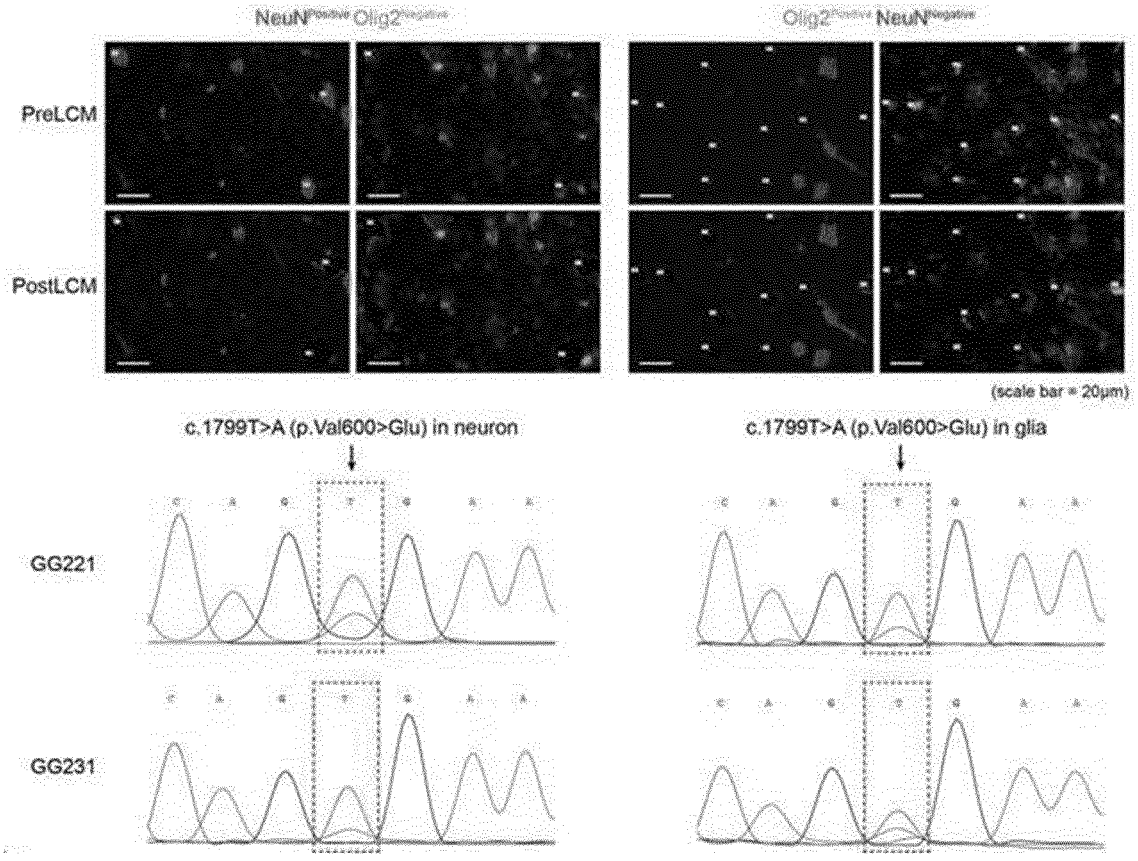
【FIG. 1c】



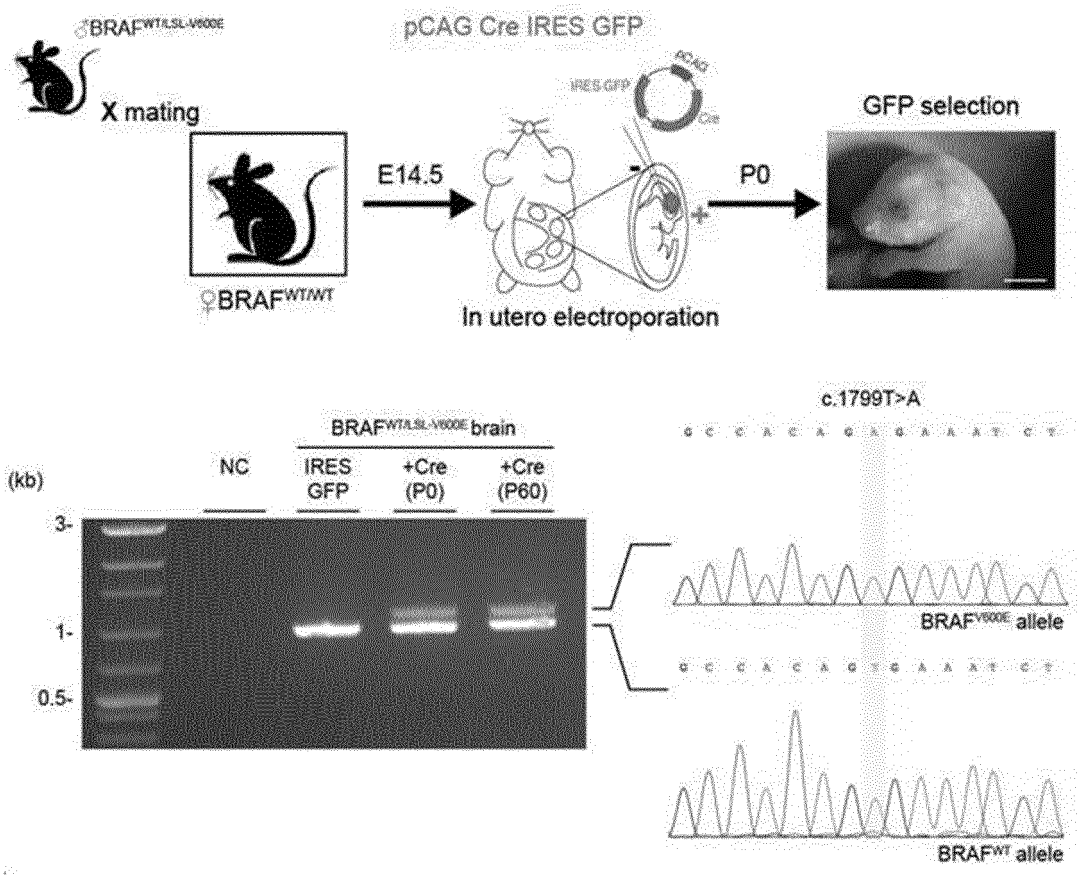
【FIG. 2a】


NeuN
Olig2
GG tumor tissue
NeuN Positive
Olig2 Negative
PreLCM
PostLCM
Olig2 Positive
NeuN Negative
PreLCM
PostLCM

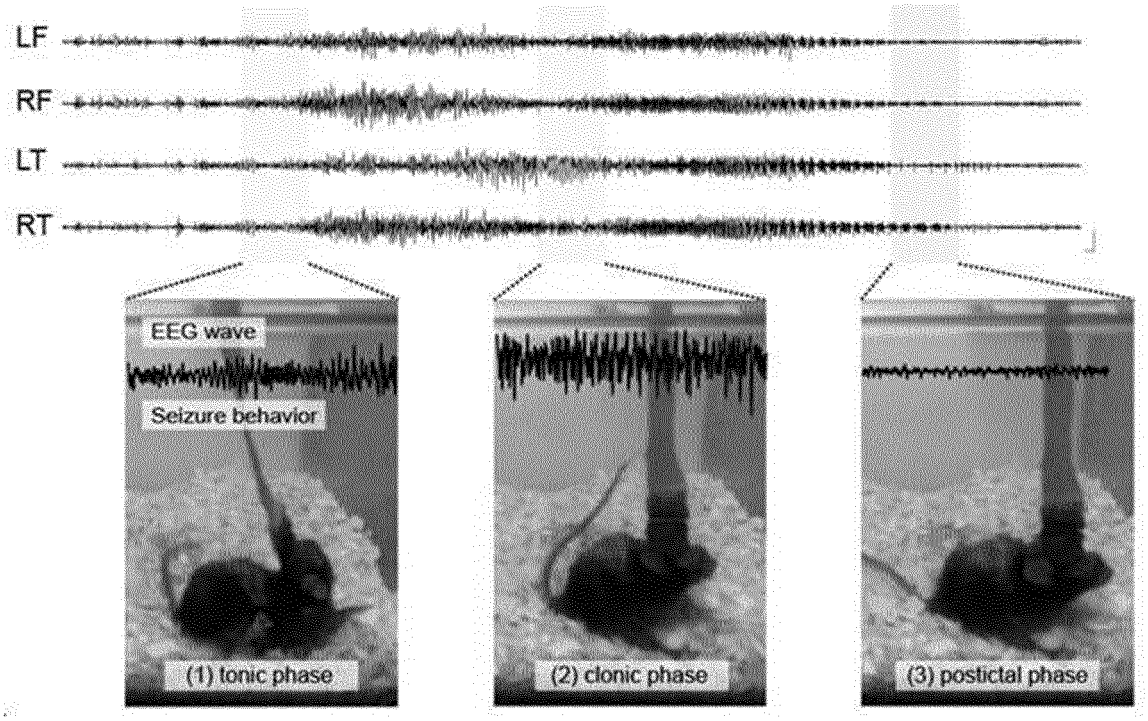
【FIG. 2b】


NeuN Positive Olig2 Negative
Olig2 Positive NeuN Negative
PreLCM
PostLCM
(scale bar = 20μm)
c.1799T>A (p.Val600>Glu) in neuron
c.1799T>A (p.Val600>Glu) in glia
GG221
GG231

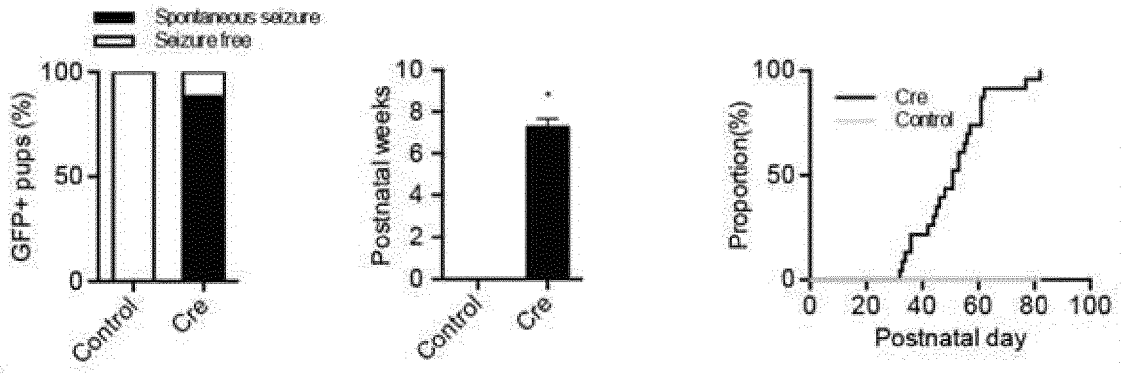
【FIG. 3a】


♂BRAF WT/LSL-V600E
X mating
♀BRAF WT/WT
E14.5
pCAG Cre IRES GFP
In utero electroporation
P0
GFP selection
BRAF WT/LSL-V600E brain
NC
IRES GFP
+Cre (P0)
+Cre (P60)
(kb)
3-
1-
0.5-
c.1799T>A
BRAF V600E allele
BRAF WT allele

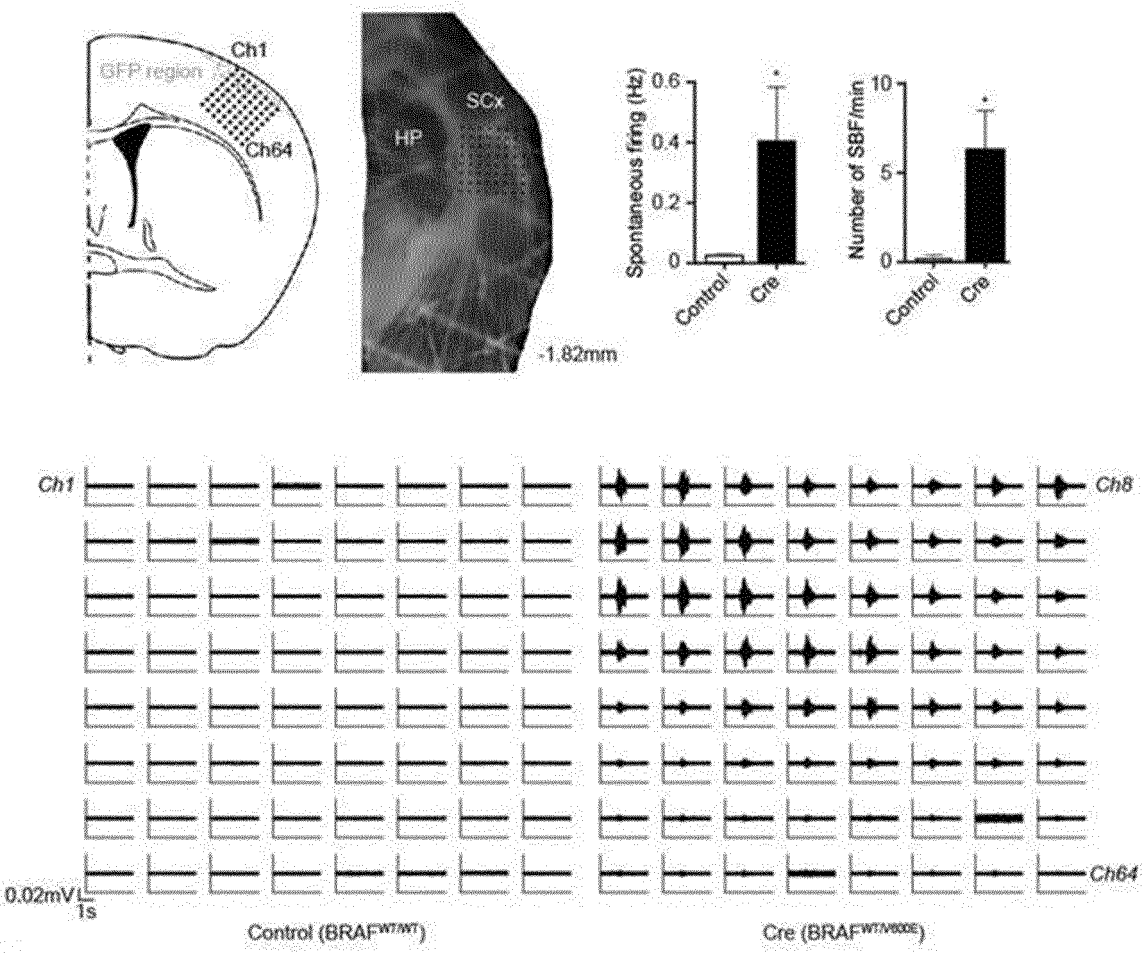
【FIG. 3b】


LF
RF
LT
RT
EEG wave
Seizure behavior
(1) tonic phase
(2) clonic phase
(3) postictal phase

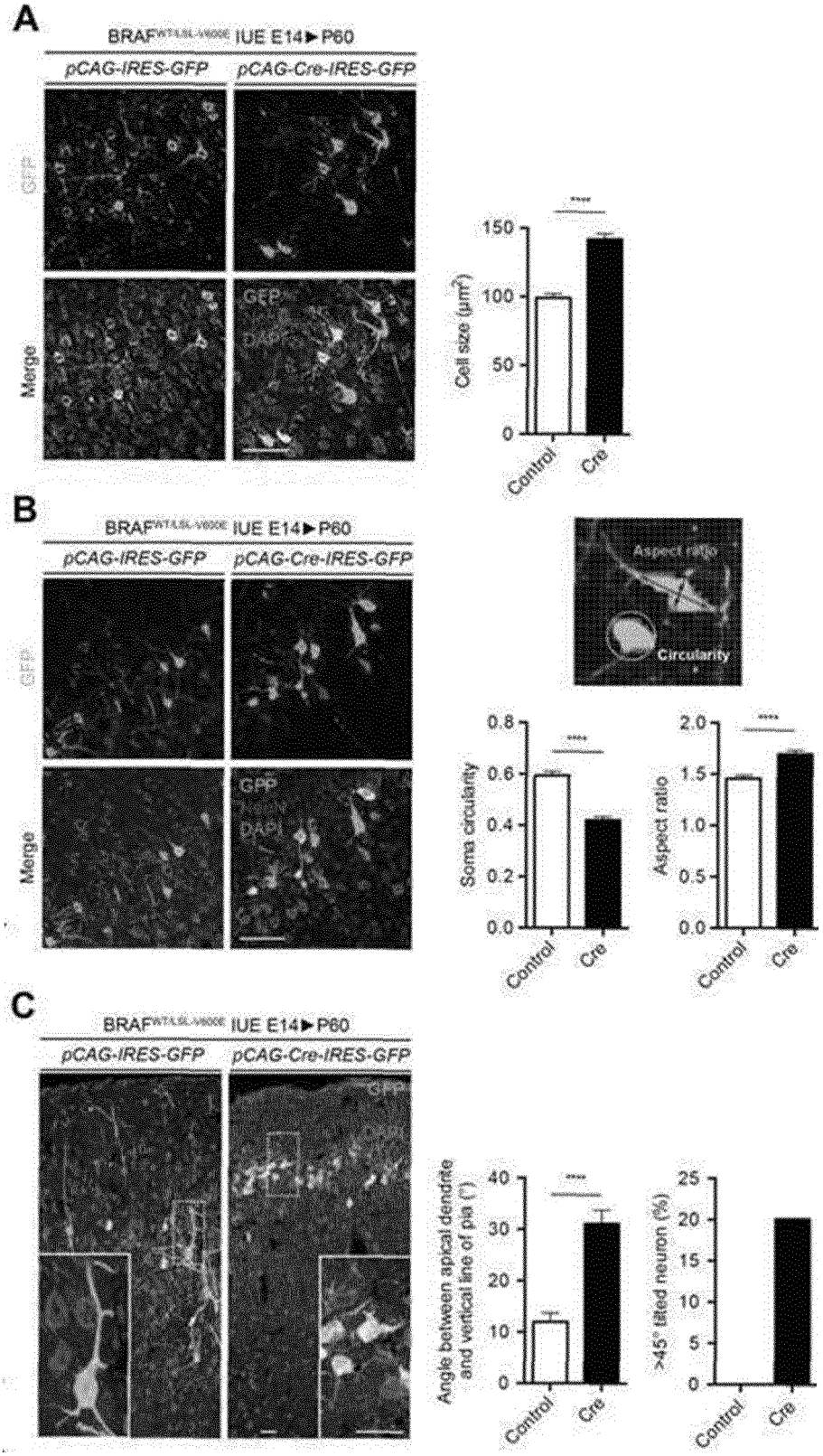
【FIG. 3c】


Spontaneous seizure
Seizure free
GFP+ pups (%)
100
50
0
Control
Cre
Postnatal weeks
10
8
6
4
2
0
Control
Cre
*
Proportion(%)
100
50
0
Cre
Control
0
20
40
60
80
100
Postnatal day

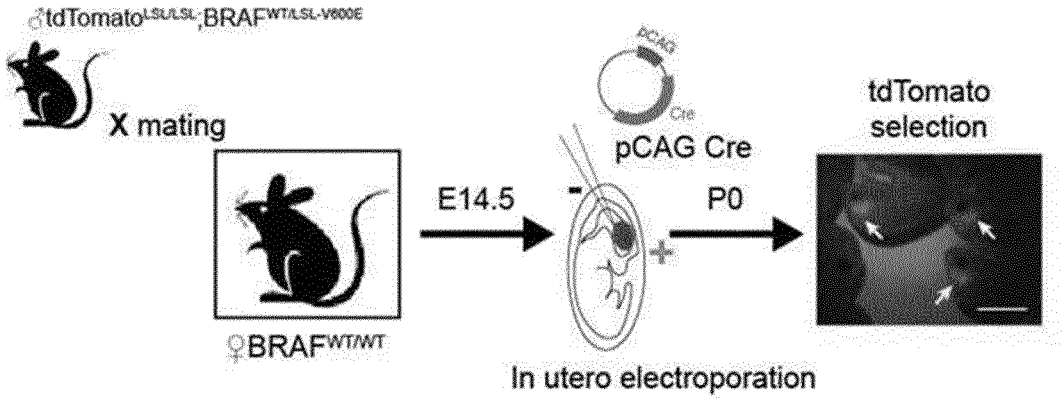
【FIG. 4】


GFP region
Ch1
Ch64
SCx
HP
-1.82mm
Spontaneous firing (Hz)
0.6
0.4
0.2
0
Control
Cre
*
Number of SBF/min
10
5
0
Control
Cre
*
Ch1
Ch8
Ch64
0.02mV
1s
Control (BRAF^WT/WT)
Cre (BRAF^WT/V600E)

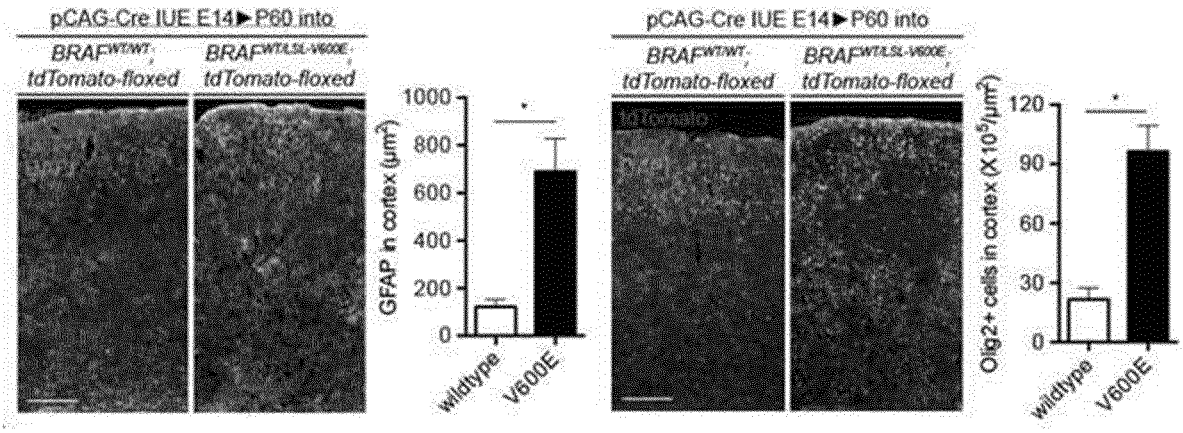
【FIG. 5】


A
BRAF IUE E14►P60
pCAG-IRES-GFP
pCAG-Cre-IRES-GFP
GFP
Merge
DAPI
Cell size (μm²)
150
100
50
0
Control
Cre
B
Aspect ratio
Circularity
Soma circularity
0.8
0.6
0.4
0.2
0.0
2.0
1.5
1.0
0.5
C
Angle between apical dendrite and vertical line of pia (°)
40
30
20
10
>45° tilted neuron (%)
25
15
5

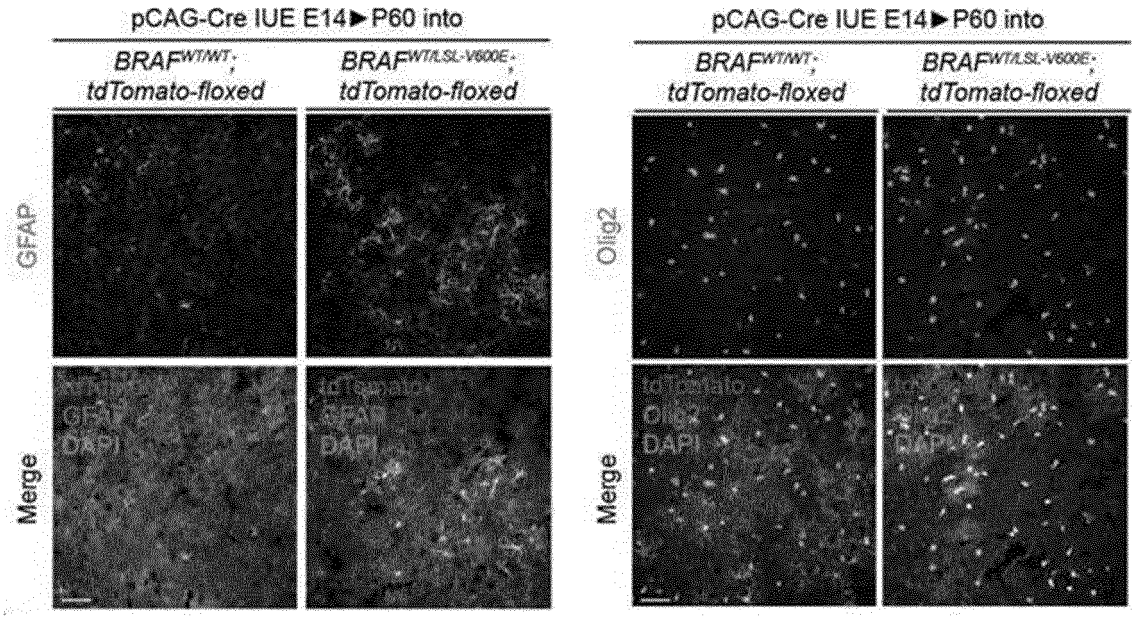
【FIG. 6a】


♂tdTomato$^{LSL/LSL}$;BRAF$^{WT/LSL-V600E}$
X mating
♀BRAF$^{WT/WT}$
E14.5
pCAG
Cre
pCAG Cre
In utero electroporation
P0
tdTomato
selection

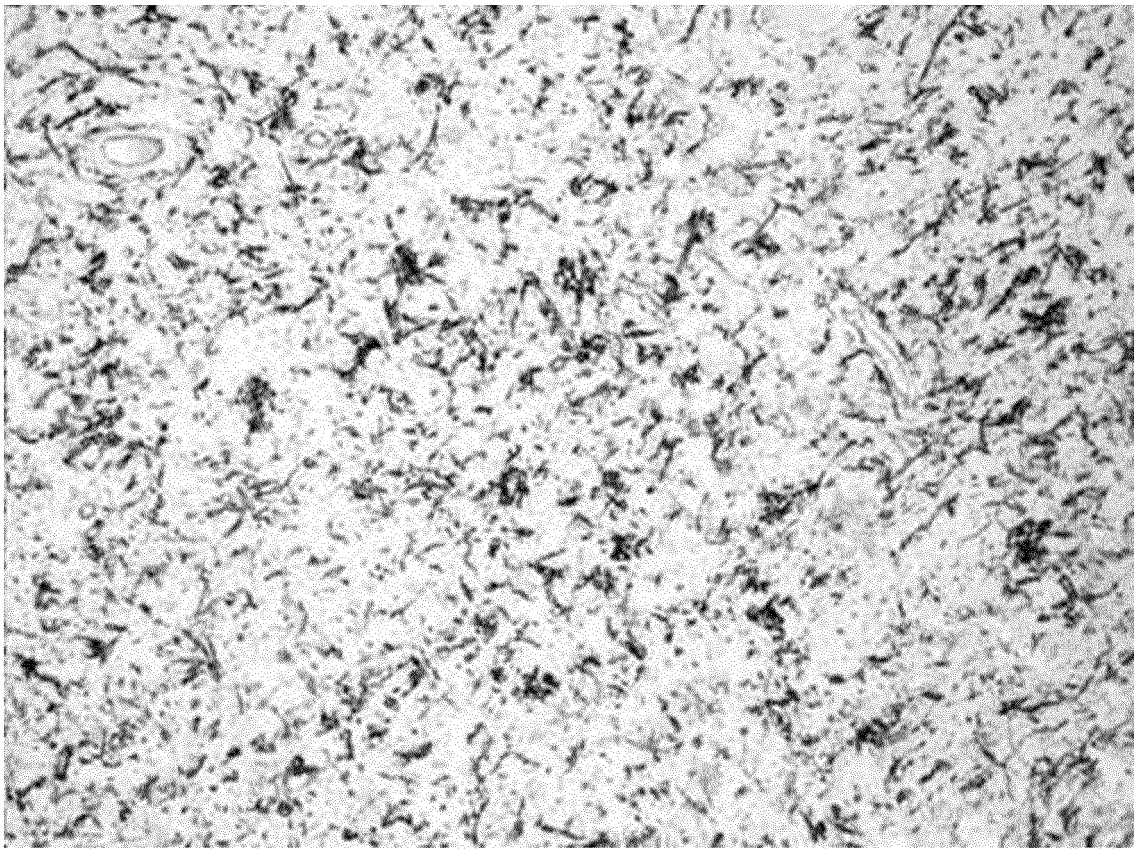
【FIG. 6b】


pCAG-Cre IUE E14►P60 into
BRAF$^{WT/WT}$; tdTomato-floxed
BRAF$^{WT/LSL-V600E}$; tdTomato-floxed
GFAP in cortex (μm²)
1000
800
600
400
200
0
wildtype
V600E
*
pCAG-Cre IUE E14►P60 into
BRAF$^{WT/WT}$; tdTomato-floxed
BRAF$^{WT/LSL-V600E}$; tdTomato-floxed
tdTomato
GFAP
Olig2+ cells in cortex (X10⁻³/μm²)
120
90
60
30
0
wildtype
V600E
*

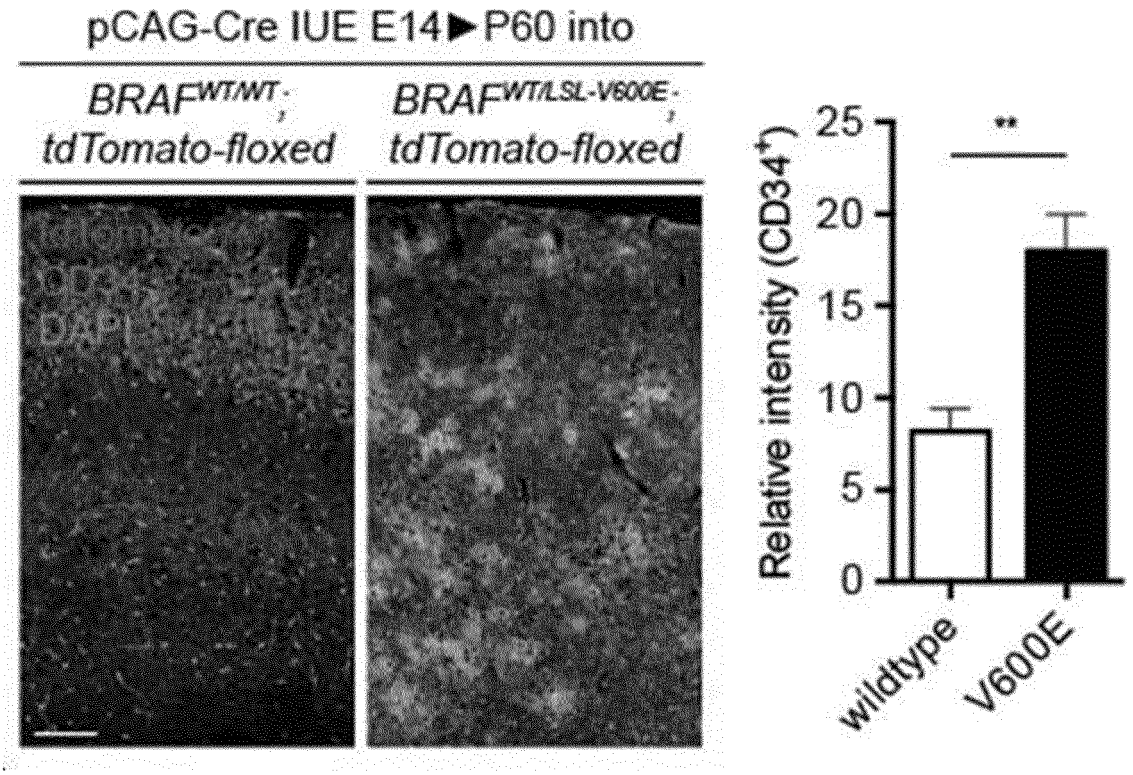
【FIG. 6c】


pCAG-Cre IUE E14▶P60 into
BRAF WT/WT; tdTomato-floxed
BRAF WT/LSL-V600E; tdTomato-floxed
GFAP
Merge
tdTomato GFAP DAPI
pCAG-Cre IUE E14▶P60 into
BRAF WT/WT; tdTomato-floxed
BRAF WT/LSL-V600E; tdTomato-floxed
Olig2
Merge
tdTomato Olig2 DAPI

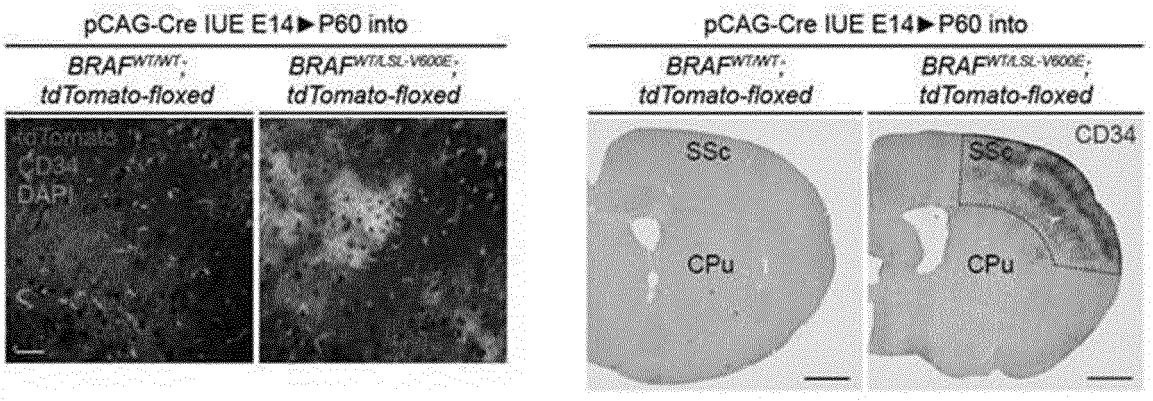
【FIG. 6d】



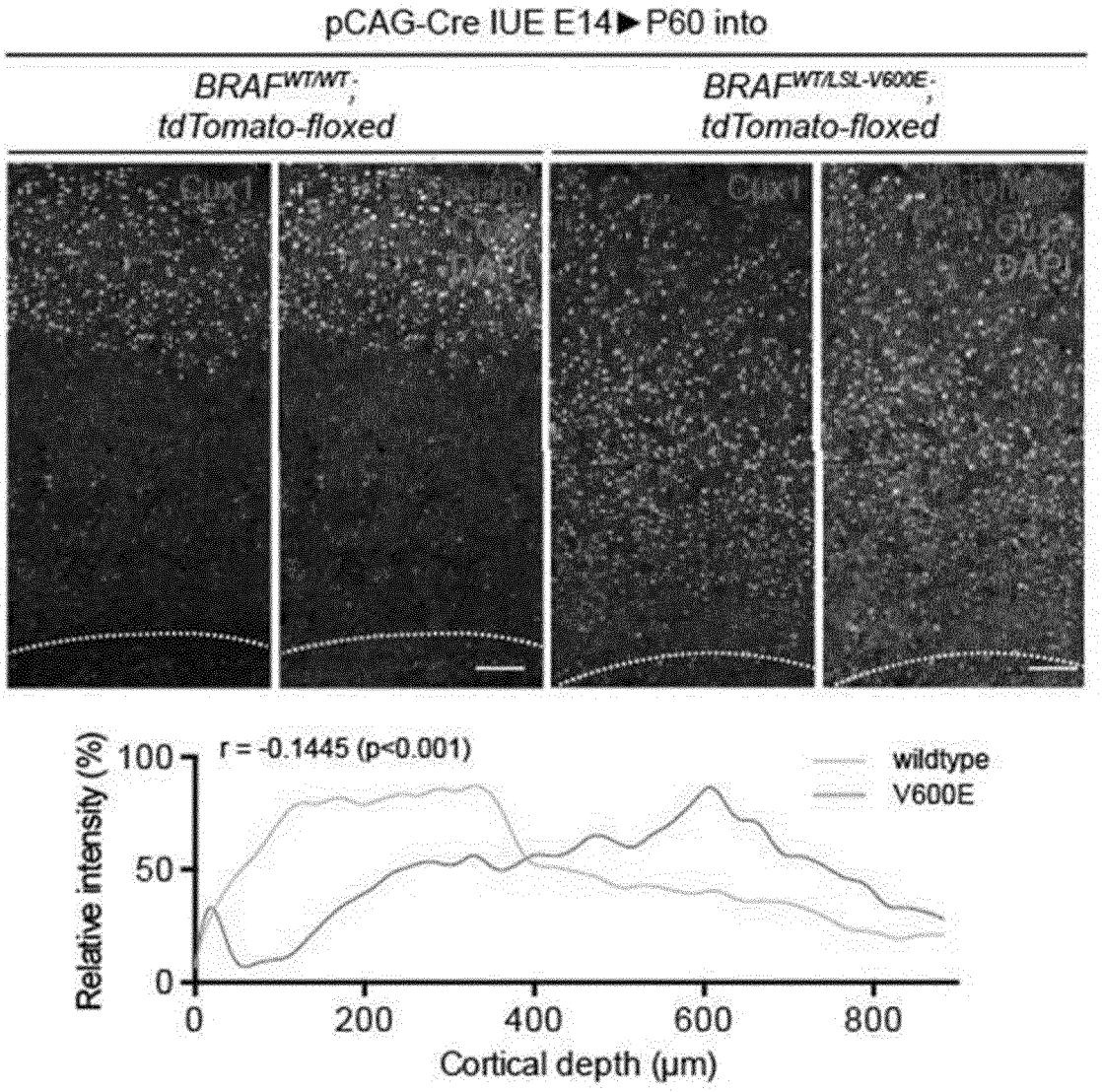
【FIG. 7】


pCAG-Cre IUE E14►P60 into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
tdTomato
CD34
DAPI
Relative intensity (CD34+)
25
20
15
10
5
0
**
wildtype
V600E

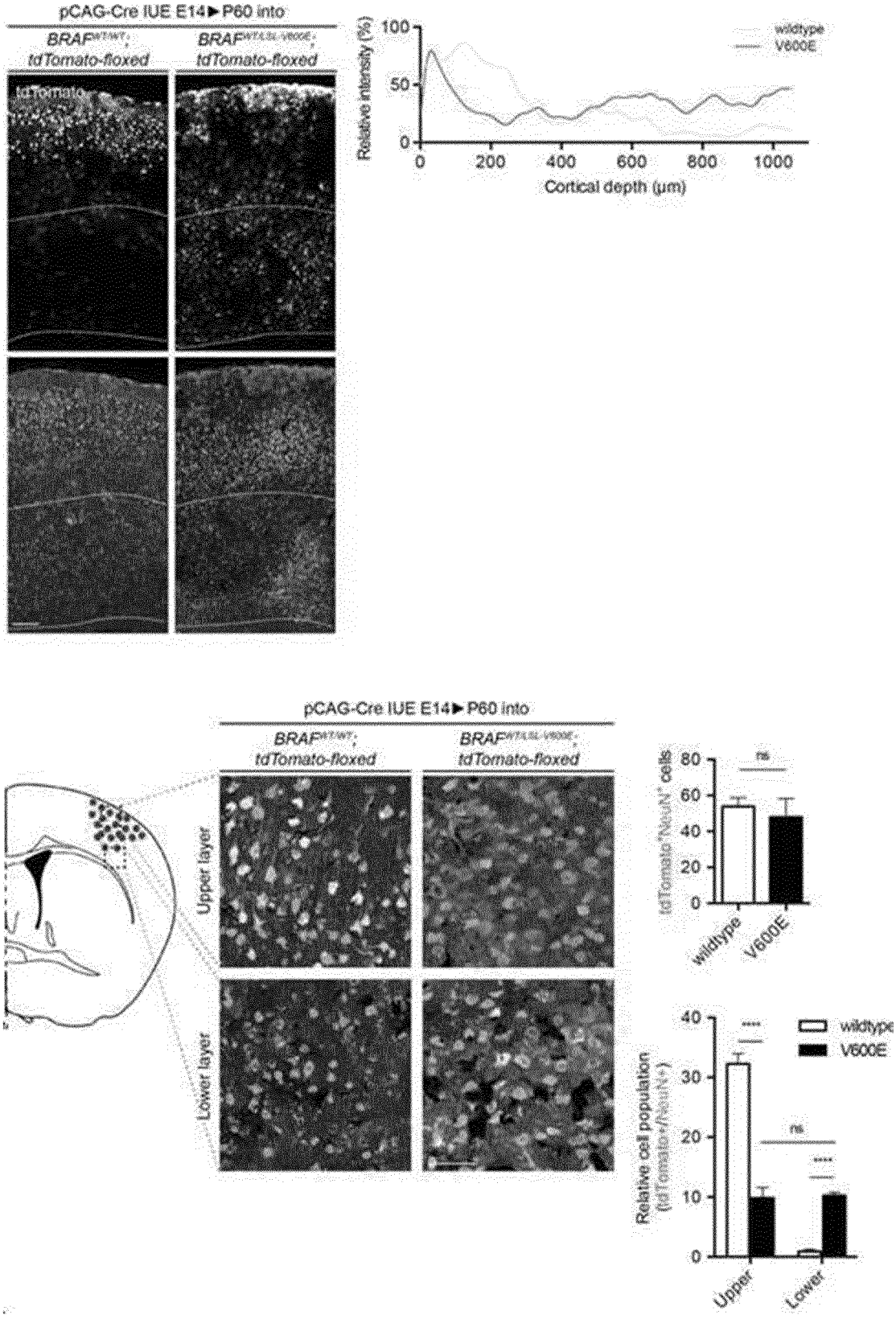
【FIG. 8】


pCAG-Cre IUE E14►P60 into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
tdTomato
CD34
DAPI
SSc
CPu
CD34

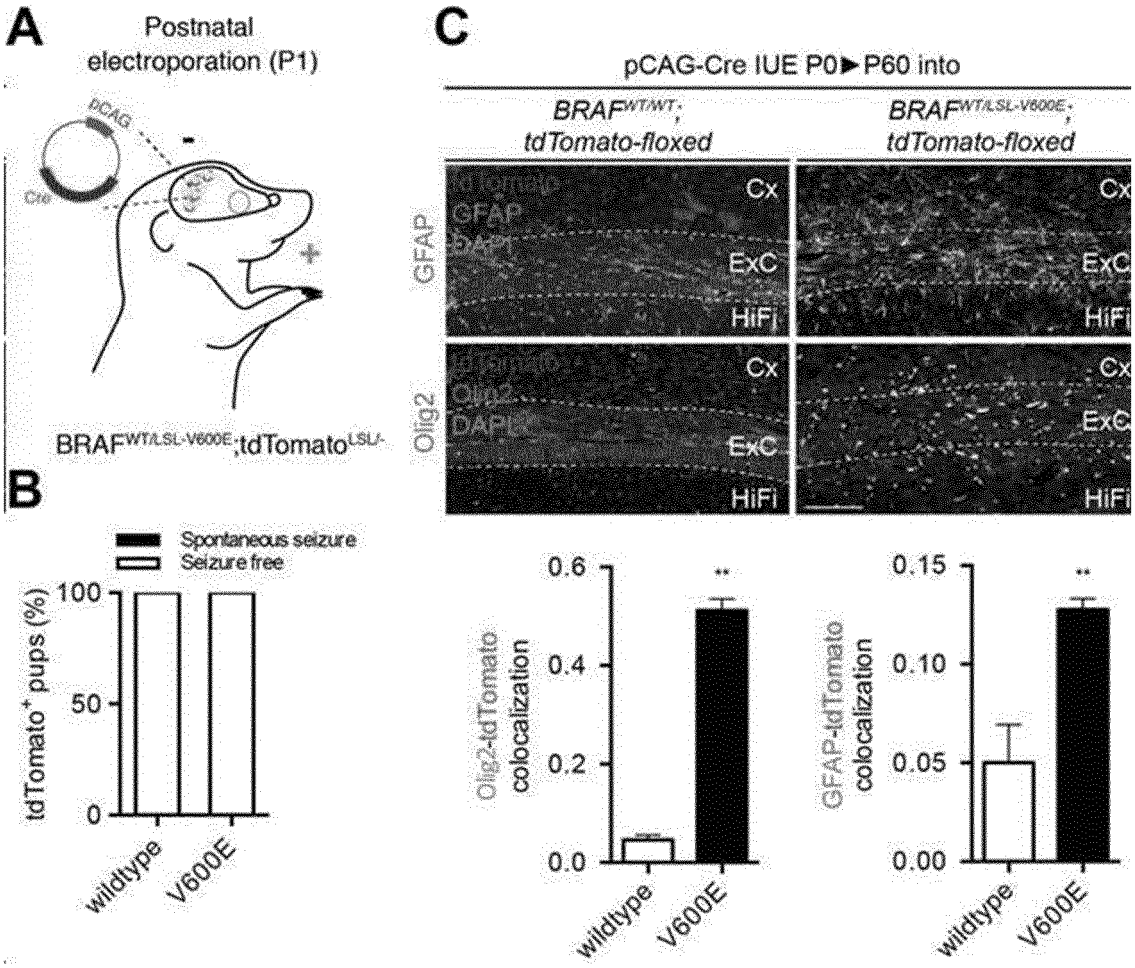
【FIG. 9】


pCAG-Cre IUE E14▶P60 into
BRAF WT/WT, tdTomato-floxed
BRAF WT/LSL-V600E, tdTomato-floxed
Cux1
DAPI
r = -0.1445 (p<0.001)
wildtype
V600E
Relative intensity (%)
100
50
0
0
200
400
600
800
Cortical depth (μm)

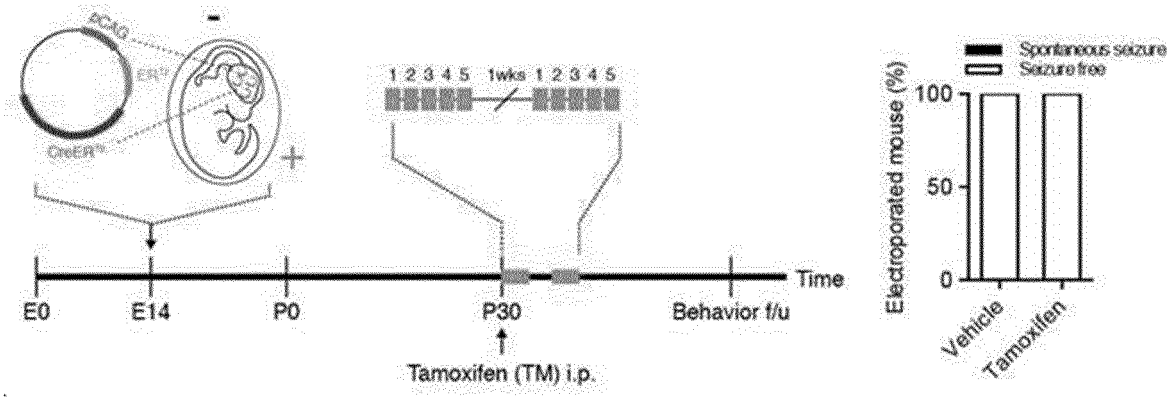
【FIG. 10】


pCAG-Cre IUE E14►P60 into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
tdTomato
Relative intensity (%)
100
50
0
0
200
400
600
800
1000
Cortical depth (μm)
wildtype
V600E
pCAG-Cre IUE E14►P60 into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
Upper layer
Lower layer
tdTomato+NeuN+ cells
80
60
40
20
0
ns
wildtype
V600E
Relative cell population (tdTomato+/NeuN+)
40
30
20
10
0
****
ns
****
wildtype
V600E
Upper
Lower

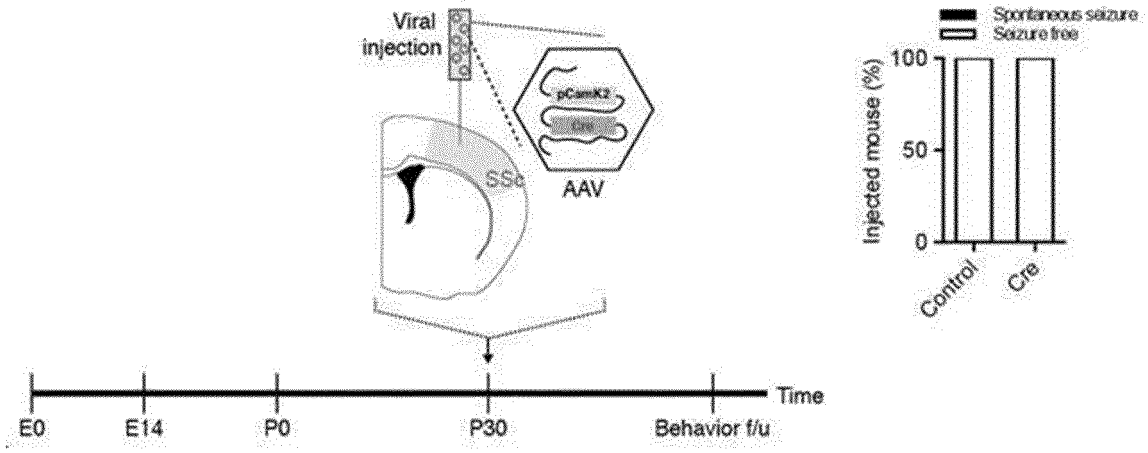
【FIG. 11】


A
Postnatal electroporation (P1)
pCAG
Cre
BRAF^WT/LSL-V600E;tdTomato^LSL/-
B
Spontaneous seizure
Seizure free
tdTomato+ pups (%)
100
50
0
wildtype
V600E
C
pCAG-Cre IUE P0►P60 into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
GFAP
Olig2
Cx
ExC
HiFi
Olig2-tdTomato colocalization
GFAP-tdTomato colocalization
0.6
0.4
0.2
0.0
0.15
0.10
0.05
0.00
**
wildtype
V600E

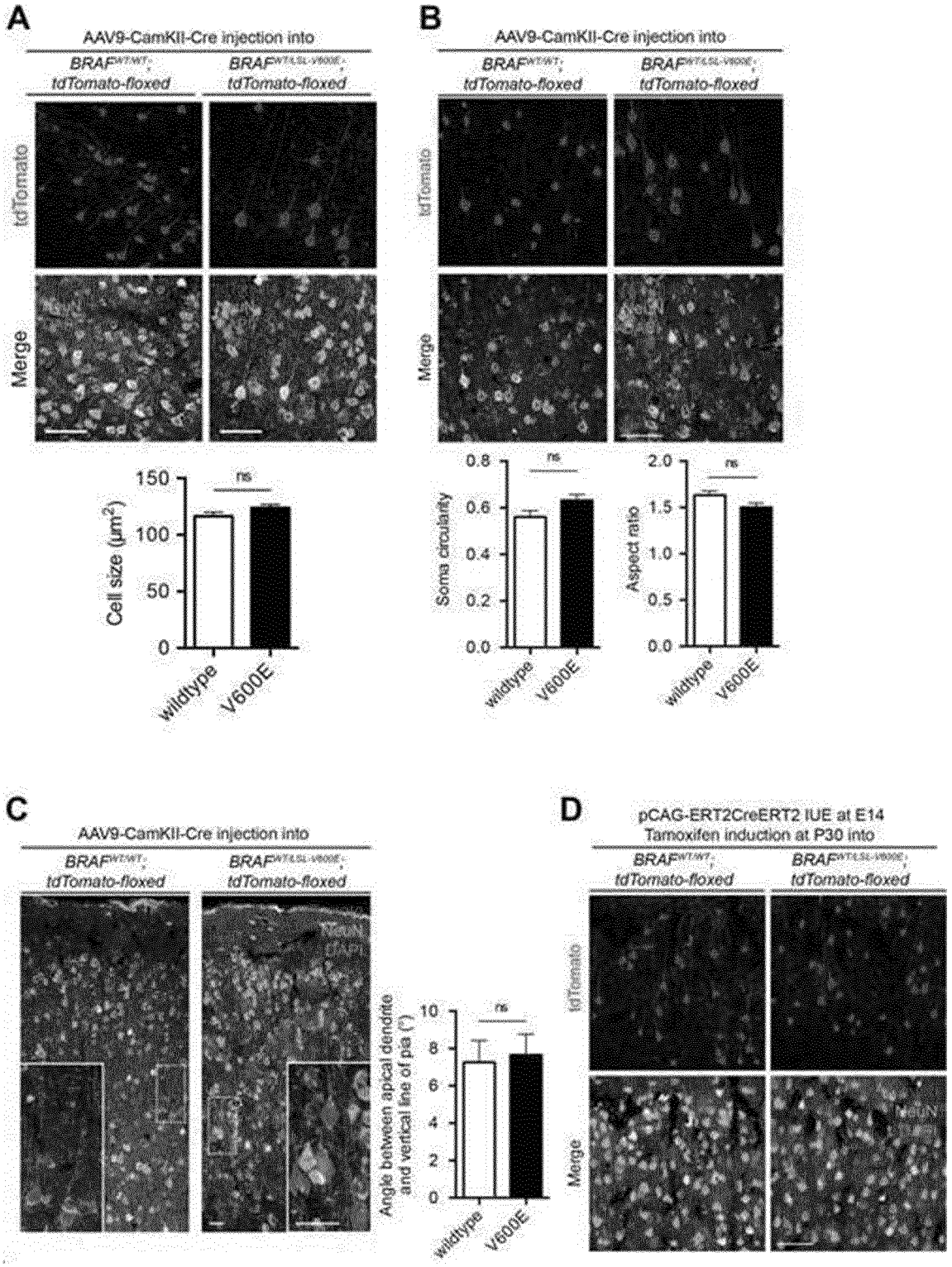
【FIG. 12】


pCAG
ER^T2
CreER^T2
1 2 3 4 5 1wks 1 2 3 4 5
E0
E14
P0
P30
Time
Behavior f/u
Tamoxifen (TM) i.p.
Spontaneous seizure
Seizure free
Electroporated mouse (%)
100
50
0
Vehicle
Tamoxifen

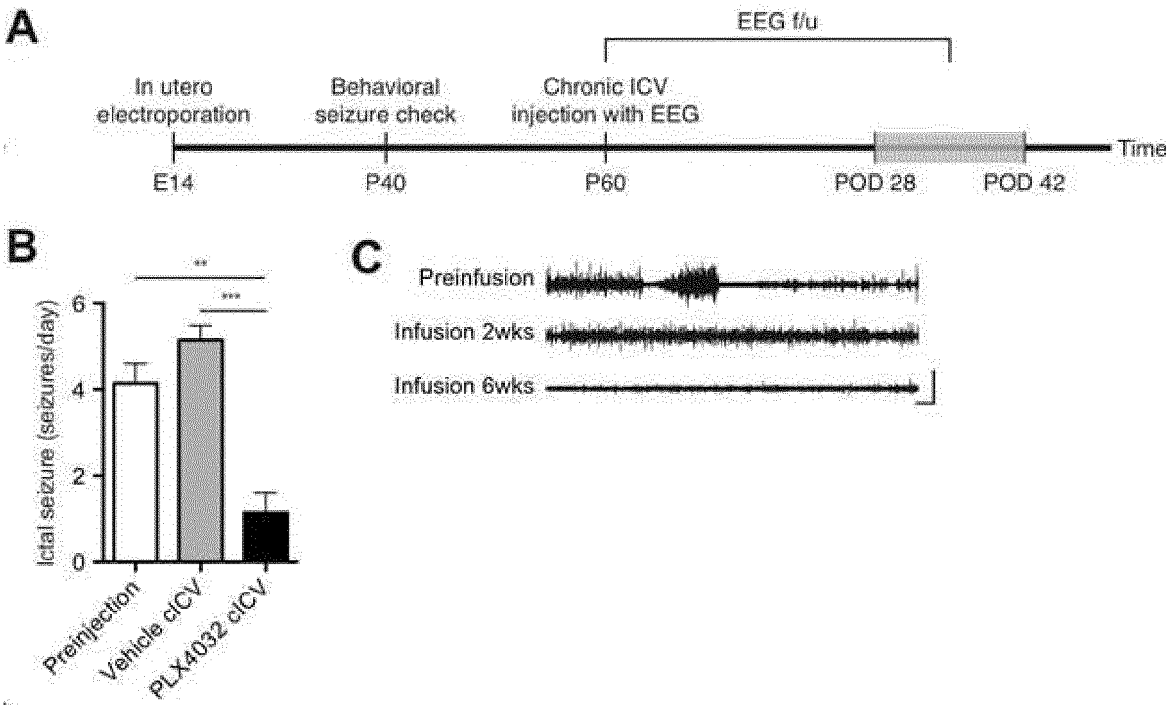
【FIG. 13】


Viral injection
pCamK2
Cre
AAV
SSc
Time
E0
E14
P0
P30
Behavior f/u
Spontaneous seizure
Seizure free
Injected mouse (%)
100
50
0
Control
Cre

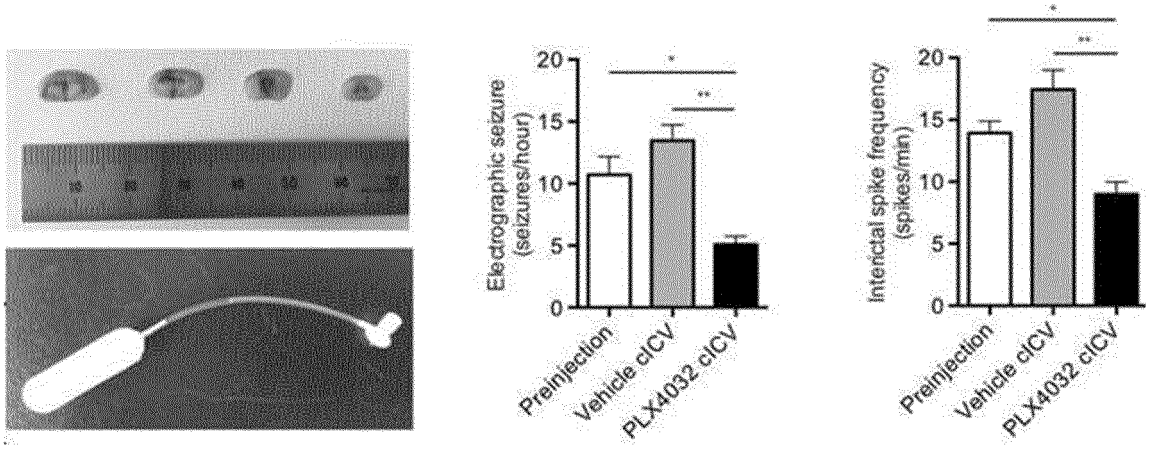
【FIG. 14】


A
AAV9-CamKII-Cre injection into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
tdTomato
Merge
Cell size (μm²)
150
100
50
0
ns
wildtype
V600E
B
AAV9-CamKII-Cre injection into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
tdTomato
Merge
Soma circularity
0.8
0.6
0.4
0.2
0.0
ns
wildtype
V600E
Aspect ratio
2.0
1.5
1.0
0.5
0.0
ns
wildtype
V600E
C
AAV9-CamKII-Cre injection into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
Angle between apical dendrite and vertical line of pia (°)
10
8
6
4
2
0
ns
wildtype
V600E
D
pCAG-ERT2CreERT2 IUE at E14
Tamoxifen induction at P30 into
BRAF^WT/WT; tdTomato-floxed
BRAF^WT/LSL-V600E; tdTomato-floxed
tdTomato
Merge

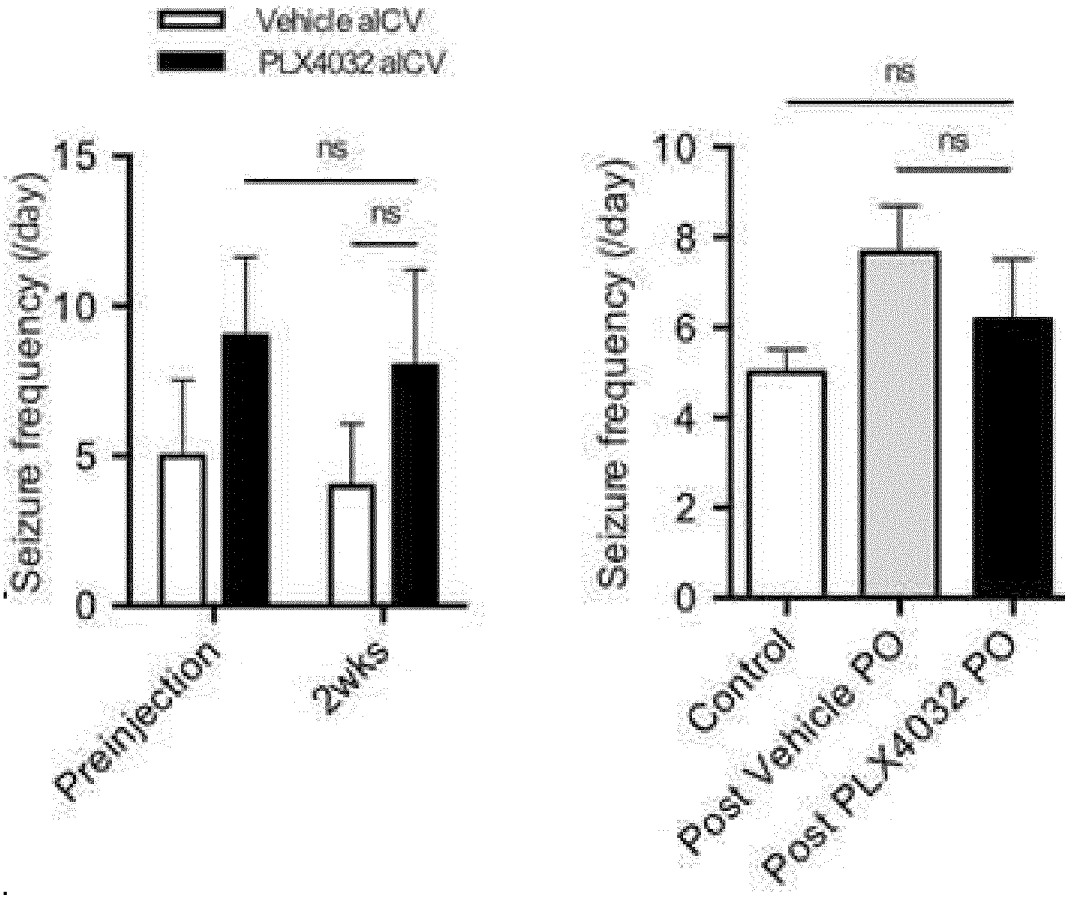
【FIG. 15】


A
EEG f/u
In utero electroporation
Behavioral seizure check
Chronic ICV injection with EEG
Time
E14
P40
P60
POD 28
POD 42
B
Ictal seizure (seizures/day)
Preinjection
Vehicle cICV
PLX4032 cICV
C
Preinfusion
Infusion 2wks
Infusion 6wks

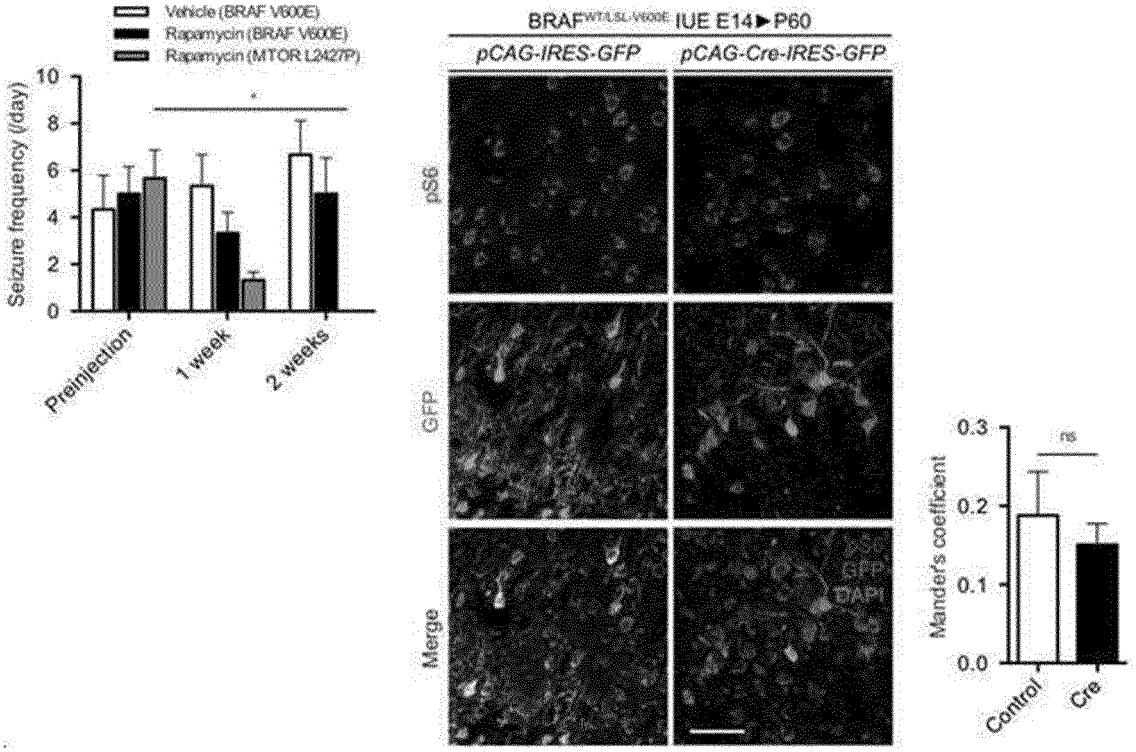
【FIG. 16】


Electrographic seizure (seizures/hour)
Interictal spike frequency (spikes/min)
Preinjection
Vehicle cICV
PLX4032 cICV

【FIG. 17】


Vehicle alCV
PLX4032 alCV
Seizure frequency (/day)
15
10
5
0
ns
ns
Preinjection
2wks
Seizure frequency (/day)
10
8
6
4
2
0
ns
ns
Control
Post Vehicle PO
Post PLX4032 PO

【FIG. 18】


Vehicle (BRAF V600E)
Rapamycin (BRAF V600E)
Rapamycin (MTOR L2427P)
Seizure frequency (/day)
10
8
6
4
2
0
*
Preinjection
1 week
2 weeks
BRAFWT/LSL-V600E IUE E14►P60
pCAG-IRES-GFP
pCAG-Cre-IRES-GFP
pS6
GFP
Merge
GFP
DAPI
Mander's coefficient
0.3
0.2
0.1
0.0
ns
Control
Cre

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2018/007234

A. CLASSIFICATION OF SUBJECT MATTER
A61K 31/437(2006.01)i, A61K 31/506(2006.01)i, C12N 15/85(2006.01)i, C07K 14/435(2006.01)i, A01K 67/033(2006.01)i, G01N 33/68(2006.01)i, C12Q 1/6883(2018.01)i
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/437; A01K 67/027; C07K 14/47; C07K 16/32; C12N 15/11; C12Q 1/68; G01N 33/574; A61K 31/506; C12N 15/85; C07K 14/435; A01K 67/033; G01N 33/68; C12Q 1/6883

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Utility models and applications for Utility models: IPC as above
Japanese Utility models and applications for Utility models: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS (KIPO internal) & Keywords: BRAF, V600E, ganglioglioma, epilepsy, Vemurafenib

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	BUFALO, F. et al., "Response of Recurrent BRAFV600E Mutated Ganglioglioma to Vemurafenib as Single Agent", Journal of Translational Medicine, 2014, 12:356, pages 1-7 See abstract; and inner page 1.	14-20
A	KR 10-2012-0099630 A (RESPONSE GENETICS, INC.) 11 September 2012 See the entire document.	1-34
A	JP 2013-543117 A (DEUTSCHES KREBSFORSCHUNGSZENTRUM) 28 November 2013 See the entire document.	1-34

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 NOVEMBER 2018 (26.11.2018)	**27 NOVEMBER 2018 (27.11.2018)**
Name and mailing address of the ISA/KR Korean Intellectual Property Office Government Complex Daejeon Building 4, 189, Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea Facsimile No. +82-42-481-8578	Authorized officer Telephone No.

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Information on patent family members

International application No.
PCT/KR2018/007234

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/KR2018/007234

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		US 9297812 B2	29/03/2016
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REFERENCES CITED IN THE DESCRIPTION

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