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(54) **IMMUNOSORBENT BLOOD TESTS FOR ASSESSING PAROXYSMAL CEREBRAL DISCHARGES**

IMMUNSORBENS-BLUTTESTS ZUR BEURTEILUNG VON PAROXYSMALEN ZEREBRALENTLADUNGEN

ESSAIS SANGUINS IMMUNOADSORBANTS PERMETTANT D'EVALUER LES DECHARGES CEREBRALES PAROXYSMALES

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Description

FIELD OF THE INVENTION

5 **[0001]** The present invention relates to clinical in-vitro diagnostic tests, particularly immuno- and proteomic tests, for diagnosing and assessing the risk associated with nervous and mental diseases. The tests detect specific brain markers of neurotoxicity, evaluate toxicological and neurological brain damage, and assist in the diagnosis, therapy and management of brain disorders.

10 ## BACKGROUND

[0002] Epilepsy is a disease characterized by epileptic seizures - generally defined as those seizures caused by a brief disruption of brain function involving temporary abnormal electrical activity in the nerve cells. The location of this disruption in the brain determines the type of seizure. Epilepsy should be contrasted with syncope which, although both 15 frequently result in unconsciousness, syncope refers to a loss of consciousness due to transient impairment of cerebral blood flow.

[0003] The two main types of epileptic seizures are partial and generalized. Partial seizures involve part of the brain, while generalized seizures involve the whole brain. Partial seizures can become generalized seizures if the epileptic activity spreads to the whole brain.

20 **[0004]** Many people are born with epilepsy. In other cases, epilepsy develops as a result of brain damage from other disorders. For example, brain tumors, head injury, alcoholism, and Alzheimer's disease frequently lead to epilepsy because they alter the normal workings of the brain. Strokes, heart attacks, and other conditions that deprive the brain.of oxygen also can cause epilepsy in some cases. About 32% of all newly developed epilepsy in elderly people appears to be due to cerebrovascular disease. Meningitis, AIDS, viral encephalitis, and other infectious diseases can lead to 25 epilepsy, as can hydrocephalus - a condition in which excess fluid builds up in the brain.

[0005] Epileptiform activity usually begins in vivo with excessive AMPA receptor activation; as the seizure activity intensifies, an increased involvement of NMDA receptors is observed (Dingledine, McBain, Basic Neurochemistry. Philadelphia, PA: Lippincott-Raven; 1998, 315-333). Over activation of NMDA and AMPA receptors allows excessive Ca^{2+} influx into the cell resulting in activation of many enzymes and proteases, which begin to destroy the components of the 30 cell membrane. That includes different Ca++ activated enzymes, including calmodulin-dependent protein kinase, calcineurin, calpain, PKS, phospholipase and number of endonucleases (Whetsell J. Neuropathol. Exp. Neurol. 1996;55: 1-13).

[0006] Seizures from epilepsy can take a number of forms. Generalized seizures include "tonic-clonic," "absence," "atonic," and "mytonic" seizures. A tonic-clonic seizure is the classic and most visible type of seizure associated with 35 epilepsy and refers to a seizure in which the patient loses consciousness, the body stiffens, the patient falls to and experiences jerking movements, "Absence" seizures are generally characterized by momentary unconsciouness; "atonic" seizures are characterized by sudden loss of muscle control that causes person to fall to the ground); and "mytonic" seizures are characterized by brief forceful jerks by the whole body or part of it. Partial seizures are generally classified as "simple partial" (symptoms include twitching; numbness; sweating; dizziness; nausea; disturbances to hearing, vision, 40 smell or taste; strong sense of déjà vu), or "complex partial" (the person appears aware when in fact he/she is not). It is often difficult to distinguish between these types of seizures in the clinic because rarely does the seizure occur in the doctor's office, and the patient usually has no memory of the seizure.

[0007] A single seizure, or even a plurality of seizures, does not mean that the person has epilepsy. Many young children have seizures that are not technically caused by epilepsy, such as convulsions from fevers. Other types of non-45 epileptic seizures are caused by an imbalance of body fluids or chemicals, prenatal brain impairment, or are associated with other disease states such as heart conditions and diabetes. These non-epileptic seizures are often referred to as "pseudo-epilepsy." They are often difficult to distinguish from epileptic seizures because of the multitude of forms that a seizure can take. Seizures can also often be caused by a condition known as non-epileptic attack disorder ("NEAD"). Seizures occurring in this condition are psychogenic in nature, and do not have a physical origin.

50 **[0008]** There are number of different procedures, including electroencephalogram (EEG) and brain scans (i.e. computed tomography) to determine whether a person has epilepsy and, if so, what kind of seizures the person has. Even with these advanced procedures, it is often very difficult to accurately differentiate between epilepsy and non-epilepsy, or to distinguish between the different types of epileptic seizures. Individuals suffering from pseudo-epilepys are often diagnosed as having epilepsy based upon EEG testing, and paroxysmal discharges observed in these patients during 55 the test period. Among seizure patients who display abnormal paroxysmal discharges during testing, a method is needed for distinguishing between epilepsy and non-epilepsy.

[0009] Up-to-now there has been an unmet diagnostic need for an in vitro diagnostic test for distinguishing epilepsy from pseudo-epilepsy. There has also been an unmet diagnostic need for evaluating the risk of a person developing

epilepsy, and for improved targeting, monitoring and adjustment of therapeutic regimens such as anticonvulsant medication and neurosurgery that are directed against epilepsy. Kharitonova, V. et al. (Drug and Alcohol dependence, Vol. 66 (2002), page S93, disclose elevated levels of antibodies against GluR1 as a diagnostic criterion for drug abuse and epilepsy.

OBJECTS OF THE INVENTION

[0010] Therefore, it is an object of the invention to provide clinical utility of blood test and based on immunosorbent and immunochemical methods and kits for diagnosing central nervous system disorders such as paroxysmal cerebral discharges and epilepsy.

[0011] It is another object of the present invention to improve upon the accuracy of currently available methods for diagnosing paroxysmal cerebral discharges and epilepsy, and to improve diagnostic certainty of brain related seizures to the exclusion of non-epileptic seizures.

[0012] Yet another object is to provide assays for detecting free GluR1 peptide fragments in biological samples, not bound in immunoglobulin complexes.

[0013] It is still another object of the present invention to provide methods of diagnosing paroxysmal cerebral discharges and epilepsy using brain markers that distinguish between epilepsy and pseudo-epilepsy.

[0014] Still another object of the invention is to provide immunoassays and immunochemical blood analyses of the risk and progression of paroxysmal cerebral discharges and epilepsy, or the seizures resulting from brain damage.

[0015] It is another object of the present invention to provide rapid immunoassays and kits for diagnosing paroxysmal cerebral discharges and epilepsy, to provide real-time assessments of brain related seizures that permit effective therapeutic intervention.

[0016] It is another object of the present invention to provide rapid and inexpensive immunoassays and kits for diagnosing paroxysmal cerebral discharges and epilepsy, which can be performed at frequent intervals to monitor the progression of brain related seizures, or the effectiveness of anticonvulsant therapy.

SUMMARY OF THE INVENTION

[0017] Methods for evaluating the origin and cause of seizures in patients who display abnormal paroxysmal discharges have been discovered that are based upon the presence and quantity of GluR1 peptides and fragments in the biological fluids of individuals that have experienced seizures. Heretofore EEG measurements of paroxysmal discharges, combined with clinical evaluations of patients, have constituted the "gold standard" for evaluating the origin of seizures in individuals. Because of the established relationship between paroxysmal discharges and epilepsy, seizure patients with abnormal paroxysmal spiking are commonly (and often erroneously) diagnosed as epileptic, and often prescribed anticonvulsant medication or even operated on unnecessarily when in fact the origin of the seizure is not paroxysmal.

[0018] The present inventors have developed methods, compositions and kits, based upon GluR1, for determining whether abnormal paroxysmal activity is truly the origin of a seizure, and for more accurately diagnosing patients who experience seizures that do not have paroxysmal origins despite the existence of abnormal paroxysmal discharges, i.e. "pseudo-epileptic" conditions such as fainting, migraine, loss of consciousness or amnesia and febrile or temperature seizures (in children). Therefore, in a first embodiment the invention provides a method for determining the origin of seizures in patients diagnosed as having paroxysmal discharges comprising directly assaying a biological fluid obtained from said patients for the presence and quantity of GluR1 peptide or an immunogenic fragment thereof.

[0019] The inventors have also discovered improved methods for treating seizure disorders that result from abnormal paroxysmal spiking. In particular, the inventors have determined that medication type, dose and frequency can be adjusted based upon GluR1 changes (or lack thereof) observed in patients treated with a given medication. The inventors have also discovered methods for evaluating the advisability of reducing or ceasing an anticonvulsant medicine based upon GluR1 changes observed when said medication is reduced or ceased. Whereas medication dosage has traditionally been adjusted in a trial and error fashion, based upon whether the individual suffers seizures or exhibit abnormal EEG activity after the change in medication, the present invention provides an in vitro test for measuring response to the medication adjustment, and for evaluating the risk of seizures resulting from the medication adjustment. Described is a method of treating epilepsy in a patient comprising (a) directly or indirectly assaying a biological fluid obtained from said patient for changes in the quantity of GluR1 in a biological fluid, or a fragment or analogue thereof, in response to treatment with an initial dose of an anti-epilepsy medication or a cessation or decrease in dose of an anti-epileptic medication; and (b) altering or maintaining said dose based upon said changes.

[0020] The inventors have still further discovered methods for predicting the risk of future seizures having paroxysmal discharges as their origin. These methods are particularly applicable to patients at high risk for such seizures such as neonates, persons who have experienced traumatic brain injury, and individuals suffering from temporal lobe epilepsy, who can suffer tremendously when they experience seizures, but for whom anticonvulsant therapy is typically adminis-

tered cautiously due to the risks associated with such therapy. Newborns with birth detects such as congenial heart defect are in particular need for monitoring according to the methods of the present invention because these patients often cannot be assessed using EEG, and because heart surgery during infancy is known to cause brain injury manifested as seizures, developmental delay and motor abnormalities (Bellinger et al., Circulation 1999, 100: 526-32). Stroke patients are another particularly suitable at-risk group inasmuch as cerebrovascular diseases are known to be a major cause of common epilepsy late in life (Cleary et al., Lancet 2004, 363:1184-6). Thus in another embodiment the invention provides a method for diagnosing the probability of epilepsy in patients at risk for epilepsy comprising directly assaying a biological fluid in said patient for the presence and quantity of GluR1 or a fragment thereof.

[0021] These methods are most particularly carried out in immunoassays using specific GluR1 sequences and antibodies raised against these sequences. Thus, another method for diagnosing epilepsy or paroxysmal discharges in a human patient comprising directly or indirectly assaying a biological fluid from said patient for the presence and quantity of the GluR1 of SEQ ID NO: 5 or 6 (as described below) or an immunogenic fragment or homolog thereof is disclosed. In another embodiment the invention provides compositions, kits, reagents, calibrators and standards based upon said peptide sequences and antibodies against them.

[0022] Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

DISCUSSION

[0023] The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein. Before the present methods and techniques are disclosed and described, it is to be understood that this invention is not limited to specific analytical or synthetic methods as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Definitions and Use of Terms

[0024] The biological sample is blood, blood plasma, blood serum, cerebrospinal fluid, saliva, perspiration or brain tissue. More preferably, the biological sample is a biological fluid. The biological fluid is blood serum and plasma.

[0025] An analogue of a protein, peptide, or polypeptide means a protein, peptide, or polypeptide that contains one or more amino acid substitutions, deletions, additions, or rearrangements. For example, it is well known in the art of protein biochemistry that an amino acid belonging to a grouping of amino acids having a particular size or characteristic (such as charge, hydrophobicity, and hydrophilicity) can often be substituted for another amino acid without altering the activity of the protein, particularly in regions of the protein that are not directly associated with biological activity. Thus, an analogue of a GluR1 receptor or fragment thereof is useful in the present invention if it includes amino acid substitutions, deletions, additions or rearrangements at sites such that antibodies raised against the analogue are still specific against the AMPA receptor or fragment.

[0026] Preferably, a GluR1 recombinant analogue has at least 80%, 85%, 90%, or 95% amino acid identity with naturally occurring AMPA receptor. Amino acid identity is defined by an analogue comparison between the recombinant analogue and naturally occurring ANTA receptor. The two amino acid sequences are aligned in such a way that maximizes the number of amino acids in common along the length of their sequences; gaps in either or both sequences are permitted in making the alignment in order to maximize the number of common amino acids. The percentage amino acid identity is the higher of the following two numbers: (1) the number of amino acids that the two polypeptides have in common with the alignment, divided by the number of amino acids in the GluR1 analogue or fragment thereof, multiplied by 100, or (2) the number of amino acids that the two polypeptides have in common with the alignment, divided by the number of amino acids in naturally occurring AMPA receptor or fragment thereof, multiplied by 100.

[0027] GluR1 derivatives, and derivatives of GluR1 fragments, are also useful in the present invention, and include naturally occurring AMPA and AMPA receptor analogues and fragments thereof that are chemically or enzymatically derivatized at one or more constituent amino acids, including side chain modifications, backbone modifications, and N- and C- terminal modifications, by for example acetylation, hydroxylation, methylation, amidation, phosphorylation or glycosylation. The term also includes GluR1salts such as zinc GluR1 and ammonium GluR 1.

[0028] A protein or peptide is measured "directly" in the sense that the protein or peptide is itself measured in the biological sample, as opposed to some other indirect measure of the protein or peptide such as antigenic fragments, analogs or derivatives of the protein or peptide, or antibodies to the protein or peptide.

[0029] An "antigen" is protein or peptide that evokes an immune response.

[0030] The term "antibody" is synonymous with "immunoglobulin," and includes naturally occurring human antibodies, polyclonal antibodies, and monoclonal antibodies. The term "antibody" is meant to include both the native antibody and biologically active and synthetic derivatives of antibodies, such as, for example, Fab', $F(ab')_2$ or Fv as well as single-domain and single-chain antibodies. A biologically active derivative of an antibody retains the ability to bind antigen.

[0031] The term "immunoassay" is the laboratory approach to detect directly or indirectly protein or peptide in the biological fluid by use of immunological reaction between antigen and antibody.

[0032] The term "calibrator" is used herein, with respect to immunoassays that measure antibodies to GluR1, refers to a solution of GluR1 antibodies containing a known amount of GluR1 antibodies and used for a calibration curve to quantify the concentration of antibodies in an unknown biological fluid.

[0033] The term "standard" is used herein, with respect to immunoassays that measure antibodies to GluR1, refers to a solution of GluR1 antibodies isolated and purified from human biological fluids in a suitable quantitative form to control the quality of reagents containing in an immunoassay kit of the present invention.

[0034] A "negative control" as used herein, with respect to immunoassays that measure GluR1 peptides or fragments directly in biological fluids, refers to GluR1 synthetic peptide or fragment thereof in a suitable quantitative form intended for use as an indicator of GluR1 concentrations in biological fluids from healthy individuals.

[0035] A "positive control" as used herein, with respect to immunoassays that measure GluR1 peptides or fragments directly in biological fluids, refers to GluR1 synthetic peptide or fragments thereof in a suitable quantitative form intended for use as an indicator of GluR1 concentrations in biological fluids from individuals suffering from epileptic paroxysmal discharges.

General Discussion

[0036] The present invention derives from the realization that genetic or accidental increase of expression of AMPA receptors or GluR1 in the brain can be correlated with paroxysmal cerebral discharges to diagnose brain related seizures, non-brain related seizures, and psychogenic related seizure, and to distinguish between brain related seizures that result from paroxysmal discharges and those that do not. Recombinant GluR1 receptors that are abnormally expressed in the brain are quickly metabolized and, following penetration of the blood brain barrier, these metabolic destruction products enter the circulatory system. The immune system recognizes these peptides and protein fragments as foreign antigens and responds by generating antibodies to them. The rapid evaluation of these brain biomarkers in individuals will greatly enhance the confidence of physicians when diagnosing paroxysmal cerebral discharges and epilepsy, and significantly improve diagnostic certainty of brain related seizures and following up anticonvulsant therapy that can be administered. The data can be used independently of other diagnostic strategies, but preferably forms an integral part of a comprehensive diagnostic strategy employing conventional diagnostic techniques.

[0037] The data obtained from the GluR1 biomarkers, especially when combined with EEG or brain scan data, can also be used to monitor the efficacy of a treatment regime. It has surprisingly been found that the GluR1 peptide and antibodies to them provide real time evidence of neurotoxicity, and that reductions in levels of circulating GluR1 peptides or antibodies correspond well with reductions in neurotoxic mechanisms. By obtaining data at appropriate intervals using rapid laboratory techniques such as latex agglutination or lateral flow, one is able to monitor the progression of the episode in response to the anticonvulsant therapeutic regime.

[0038] Immunoassay techniques are generally preferred for measuring the proteins or peptides of the present invention, as discussed in greater detail herein, although other analytical techniques are also available as known to those skilled in the art, such as HPLC. The amino acid sequences of preferred GluR1 subunits, and antigenic fragments thereof, are recited in SEQ ID NO 5 and 6, and any fragment, analogue or derivative of these sequences can be employed in methods for directly detecting the receptors as long as sufficient antigenicity is maintained. However, when using immunoassays it has been found that the antigenic determinants are concentrated in the N-terminal domain of the GluR1, GluR2, GluR3, and GluR4 receptor subunits, and that antibodies raised against the N-terminal domains and fragments thereof should be employed for optimal test results. The inventors have sequenced the amino acid chain of the N-terminal domains for these receptors, and set forth the sequences as SEQ ID NOS. 1, 2, 3 and 4, respectively, at the end of this document.

[0039] Conventional methods can be used to prepare the antibodies. For example, by using a peptide of a GluR1 protein, polyclonal antisera or monoclonal antibodies can be made using standard methods. A mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the peptide (preferably the GluR1 receptor, an antigenic determinant of the GluR1 receptor, or an analogue or derivative thereof) which elicits an antibody response in the mammal. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well known in the art. For example, the peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess the levels of antibodies. Following immunization, antisera can be administered and, if desired, polyclonal antibodies isolated from the sera.

[0040] To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immu-

nized animal and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g., the hybridoma technique originally developed by Kohler and Milstein (Nature 256, 495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol. Today 4, 72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al. Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96), and screening of combinatorial antibody libraries (Huse et al., Science 246, 1275 (1989)]. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the peptide and the monoclonal antibodies can be isolated. Therefore, the invention also contemplates hybridoma cells secreting monoclonal antibodies with specificity for GluR1 proteins or fragments thereof as described herein.

[0041] The biological sample tested for the receptor or fragment can be derived from blood, urine, blood plasma, blood serum, cerebrospinal fluid, saliva, perspiration, or brain tissue. In a preferred embodiment, the biological sample is a blood sample. In an even more preferred embodiment the biological sample is a blood sample diluted to a ratio of from about 1:2 to about 1:32 (v:v).

[0042] Indirect methods for measuring levels of recombinant GluR1 (GluR1) peptide or fragments thereof are disclosed. Thus, analytical techniques can be used to evaluate indirect measures of GluR1 peptide or fragments thereof, such as antibodies specific for the recombinant peptide, or cDNA that encodes for this peptide. In one embodiment, GluR1 peptide and antibodies are simultaneously measured to obtain a reading of the likelihood for seizure onset. Concentrations of higher than 50 or 100 pg/mL (higher than 50 pg/mL in infants) for GluR1, especially when combined with GluR1 antibodies concentration higher than 1.5 ng/ml for men, 1.8 ng/ml for women, 1.0 ng/ml for children, are remarkably predictive of the occurrence of paroxysmal cerebral discharges and epilepsy, and typically justify anticonvulsive therapy, especially when observed in patients who experience paroxysmal spiking as measured by EEG.

[0043] The proposed blood test based on immunosorbent antibodies to GluR1 was tested on the blood serum samples of more than 2300 patients during last seven years, the diagnoses being as follows: epilepsy (1650), brain paroxysmal activity including acute ischemic stroke (187), parkinsonism (148), schizophrenia (manic-depressive psychosis, cyclothymia) (147), Alzheimer's disease (44), drug abuse (morphine, cocaine, hashish) (117), as well as 2150 healthy persons, including cross analyses.

Methods of Treatment

[0044] The diagnostic methods of the present invention are particularly useful when employed in conjunction with treatment regimens that are directed against epileptic seizures. The methods are useful when initiating anticonvulsive therapy, reducing or ceasing the therapy, or considering neurosurgery. The methods are preferably performed in conjunction with additional diagnostic methods such as EEG.

[0045] Thus, for example, intractable epilepsy that warrants neurosurgery can be diagnosed by using EEG and GluR1 monitoring in combination. Neurosurgery may, for example, be warranted based upon the presence of paroxysmal spiking, and abnormally high profiles of concentrations of GluR1 or fragment thereof, that fail to respond to one or more anticonvulsive drug regimens.

[0046] The need to change anticonvulsive medications, or to increase the dose of a prescribed medication, can similarly be assessed based on the ability of a given drug regimen to reduce GluR1 levels, or to reduce those levels below a designated standard based upon population norms. A preferred designated standard is 100, 75 or 50 pg/ml of GluR1 fragment, and/or 2.0, 1.8, 1.5, or 1.0 ng/ml of GluR1 antibody, for adults, and 75, 50, or 35 pg/ml of GluR1 fragment, and/or 1.5, 1.0 or 0.8 ng/ml of GluR1 antibody for children.

[0047] In still another embodiment, the methods disclosed are practiced in conjunction with the cessation or reduction of anticonvulsant therapy. GluR1 levels are monitored in response to the cessation or reduction, and said therapy is reverted to if GluR1 levels either increase or increase above the foregoing designated standards.

Novel Kits of the Present Invention

[0048] Further described are kits for diagnosing central nervous system disorders such as paroxysmal cerebral discharges and epilepsy. Recombinant GluR1 antibodies or antigens may be incorporated into immunoassay diagnostic kits depending upon whether antibodies or GluR1 are being measured. A kit may include a composition comprising an antigen or antibody preparation. Both antibody and antigen preparations should preferably be provided in a suitable quantitative form, with antigen and/or antibody concentrations given for easy reference in quantitative applications.

[0049] The kits may also include an immunodetection reagent or label for the detection of specific immunoreaction between the provided antigen and/or antibody, as the case may be, and the diagnostic sample. Suitable detection reagents are well known in the art as exemplified by radioactive, enzymatic or otherwise chromogenic agents, which are typically employed in association with the antigen and/or antibody, or in association with a second antibody having specificity for first antibody. Thus, the reaction is detected or quantified by means of detecting or quantifying the label.

Immunodetection reagents and processes suitable for application in connection with the novel methods disclosed herein are generally well known in the art.

[0050] The reagents may also include ancillary agents such as buffering agents and protein stabilizing agents, e.g., polysaccharides and the like. The diagnostic kit may further include where necessary agents for reducing background interference in a test, agents for increasing signal, apparatus for conducting a test, calibration curves and charts, standardization curves and charts, and the like.

[0051] In a more particular aspect the disclosure relates to an immunosorbent containing antibodies to GluR1 or synthetic peptide GluR1, present in an ELISA or latex agglutination format. Thus, kits are described that contain a microtiter plate comprising GluR1 or fragments thereof or antibodies to GluR1, and a human or synthetic calibrator. The reagents may also include ancillary agents such as buffering agents and protein stabilizing agents, e.g., polysaccharides and the like. The diagnostic kit may further include, where necessary, other members of the signal-producing system of which system the detectable group is a member (e.g., enzyme and non-enzyme substrates), agents for reducing background interference in a test, agents to increase the signal, apparatus for conducting a test, calibration and standardization information or instructions, and the like.

[0052] Calibration is typically accomplished by generating a standard curve from the measurement of samples of known value. Specimens with unknown levels of analyte are then measured and compared to the standard curve using mathematically derived relationships. The standard curve may be determined prior to or concurrently with analysis of the sample specimens, depending on the stability and reproducibility of the assay. Further described is a kit comprising, as a calibrator or control, antibodies raised against the GluR1 fragment of SEQ ID NO: 5 or 6, or an immunogenic fragment, homolog or derivative thereof. In yet another embodiment the kit is manufactured against an antibody standard comprising a specific fraction of immunoglobulins G purified from human blood, optionally immunoglobulins of 95% purity that specifically bind the GluR1 peptide without significant cross-reaction with other glutamate receptor fragments or other neuroreceptors (e.g. D1, D2, D3, NMDAR, opiate, etc).

Latex Agglutination

[0053] A latex agglutination technique or lateral flow format has also been developed which dramatically increases the speed of diagnosis obtained by the methods described herein, and thereby improves the diagnostic certainty. Latex agglutination assays have been described in Beltz, G. A. et al., in Molecular Probes: Techniques and Medical Applications, A. Albertini et al., eds., Raven Press, New York, 1989. In the latex agglutination assay, antibody raised against a particular biomarker is immobilized on latex particles. A drop of the latex particles is added to an appropriate dilution of the serum to be tested and mixed by gentle rocking of the card. With samples lacking sufficient levels of the biomarkers, the latex particles remain in suspension and retain a smooth, milky appearance. However, if biomarkers reactive with the antibody are present, the latex particles clump into visibly detectable aggregates.

[0054] An agglutination assay can also be used to detect biomarkers wherein the corresponding antibody is immobilized on a suitable particle other than latex beads, for example, on gelatin, red blood cells, nylon, liposomes, gold particles, etc. The presence of antibodies in the assay causes agglutination, similar to that of a precipitation reaction, which can then be detected by such techniques as nephelometry, turbidity, infrared spectrometry, visual inspection, colorimetry, and the like.

[0055] The term latex agglutination is employed generically herein to refer to any method based upon the formation of detectable agglutination, and is not limited to the use of latex as the immunosorbent substrate. While preferred substrates for the agglutination are latex based, such as polystyrene and polypropylene, particularly polystyrene, other well-known substrates include beads formed from glass, paper, dextran, and nylon. The immobilized antibodies may be covalently, ionically, or physically bound to the solid-phase immunoadsorbent, by techniques such as covalent bonding via an amide or ester linkage, ionic attraction, or by adsorption. Those skilled in the art will know many other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

[0056] The technique can be adapted for use in the detection of GluR1 receptors, antibodies, or any other suitable biomarker against central nervous system disorders. Using the latex agglutination technique, one is able to provide real-time biochemical diagnosis and monitoring of patients with epilepsy (within about 10 minutes), and thereby dramatically improve follow-up anticonvulsant treatment. This is surprising because these biomarkers are naturally occurring and, in contrast to viruses for which latex agglutination methods were originally developed, show much lower strengths of association with their corresponding antibodies.

[0057] Thus, further described is a method of measuring the GluR1 receptor, fragment therof, or other biomarker is by latex agglutination comprising: (i) contacting the biological sample with an agglutinating carrier that comprises an antibody to GluR1 or an antigenic determinant of GluR1, for a sufficient time period and under conditions to promote agglutination; and (ii) reading a signal generated from the agglutination; wherein the amount of signal detected correlates to the titer of biomarkers present in the sample.

[0058] The reaction is preferably read macroscopically against a dark background for a sufficient time period. The

method preferably yields a clinically useful reading within about 30 minutes or less. It has been experimentally found that latex beads having a mean diameter of from about 0.25 to about 0.4 micrometers are particularly preferred in the practice of this invention. Latex beads can be prepared generally by adding antibodies to the target biomarker to a carrier solution that contains a 1% concentration (by weight) of latex beads, until the concentration of the antibodies in the carrier solution reaches about 2 mg/ml, and allowing the ingredients a sufficient time to covalently link, typically about 1 hour, in the presence of a linking agent such as glutaraldehyde.

[0059] Further described is a latex agglutination kit that comprises: (1) latex beads that comprise GluR1 or fragments thereof or antibodies to GluR1, and (2) positive and negative controls.

Novel Compositions of the Invention

[0060] The methods of the present invention rely upon a series of compositions.

[0061] Thus, in one series it is provided. a recombinant polypeptide fragment of the GluR1, GluR2, GluR3 and GluR4 subunits of AMPA receptor, comprising: .

1. The N-terminal domain of the GluR1, SEQ ID NO. 1;
2. The N-terminal domain of the GluR2, SEQ ID NO. 2;
3. The N-terminal domain of the GluR3, SEQ ID NO. 3;
4. The N-terminal domain of the GluR4, SEQ ID NO. 4;
5. Recombinant GluR1, SEQ ID NOs. 5 and 6,

or an antigenic fragment, analog, or derivative thereof. Another series provides any of the foregoing polypeptides linked covalently to a distinct antigenic determinant, such as human serum albumin. In still another series it is provided any of the foregoing polypeptides linked to any of the immunosorbent materials discussed above. The immunosorbent can be in the form of a bead for latex agglutination, in the size ranges discussed above, or in the form of a synthetic plate for conventional immunoassay analysis. The polypeptide can be linked to the immunosorbent using any conventional means of linkage, including covalent linkage, ionic linkage, and adsorption.

[0062] Another series relates to the monoclonal and polyclonal antibodies specific for and/or raised against the foregoing polypeptides, including the foregoing polypeptides linked to distinct antigenic determinants. Thus, provided are non-human antibodies against any of the foregoing peptides or polypeptides or antigenic fragment, analog, or derivative thereof. Further provided are immunosorbents to which such antibodies are linked.

Brief Description of the Sequence Listings

[0063] The features, aspects, and advantages of the present invention will become better understood with regard to the following sequence listings where, in the sequence the recited amino acid position numbering reflects that used throughout this document.

SEQ ID NO:1. shows the amino acid sequence of the mature N-terminal domain of GluR1 receptor subunit, as follows:
SEQUENCE LISTING
PEPTIDE Homo sapiens glutamate receptor ionotropic, GluR1
Proc. Natl. Acad. Sci. U.S.A. 88:7557-7561(1991)

19 AN FPNNIQIGGL FPNQQSQEHA AFRFALSQLT EPPKLLPQID 60

61 IVNISDSFEM TYRFCSQFSK GVYAIFGFYE RRTVNMLTSF CGALHVCFIT PSFPVDTSNQ 120

121 FVLQLRPELQ DALISIIDHY KWQKFVYIYD ADRGLSVLQK VLDTAAEKNW QVTAVNILTT 180

181 TEEGYRMLFQ DLEKKKERLV VVDCESERLN AILGQIIKLE KNGIGYHYIL ANLGFMDIDL 240

241 NKFKESGANV TGFQLVNYTD TIPAKIMQQW KNSDARDHTR VDWKRPKYTS ALTYDGVKVM 300

301 AEAFQSLRRQ RIDISRRGNA GDCLANPAVP WGQGIDIQRA LQQVRFEGLT GNVQFNEKGR 360

361 RTNYTLHVIE MKHDSIRKIG YWNEDDKFVP AATDAQAGGD NSSVQNRTYI VTTILEDPYV 420

421 MLKKNANQFE GNDRYEGYCV ELAAEIAKHV GYSYRLEIVS DGKYGARDPD TKAWNGMVGE 480

481 LVYGRADVAV APLTITLVRE EVIDFSKPFM SLGISIMIKK PQKSKPGVFS FLDPLA

SEQ ID NO:2. shows the amino acid sequence of the N-terminal domain of GluR2 subunit, as follows:
SEQ ID NO:2
PEPTIDE Homo sapiens glutamate receptor. ionotropic, GluR2
NeuroReport 5:441-444(1994)

22 VSSNSIQIG GLFPRGADQE YSAFRVGMVQ FSTSEFRLTP 60

61 HIDNLEVANS FAVTNAFCSQ FSRGVYAIFG FYDKKSVNTI TSFCGTLHVS FITPSFPTDG 120

121 THPFVIQMRP DLKGALLSLI EYYQWDKFAY LYDSDRGLST LQAVLDSAAE KKWQVTAINV 180

181 GNINNDKKDE MYRSLFQDLE LKKERRVILD CERDKVNDIV DQVITIGKHV KGYHYIIANL 240

241 GFTDGDLLKI QFGGANVSGF QIVDYDDSLV SKFIERWSTL EEKEYPGAHT TTIKYTSALT 300

5

301 YDAVQVMTEA FRNLRKQRIE ISRRGNAGDC LANPAVPWGQ GVEIERALKQ VQVEGLSGNI 360

10

361 KFDQNGKRIN YTINIMELKT NGPRKIGYWS EVDKMVVTLT ELPSGNDTSG LENKTVVVTT 420

15

421 ILESPYVMMK KNHEMLEGNE RYEGYCVDLA AEIAKHCGFK YKLTIVGDGK YGARDADTKI 480

20

481 WNGM

SEQ ID NO:3; shows the amino acid sequence of the N-terminal domain of GluR3 subunit, as follows:

SEQ ID NO:3

25

PEPTIDE Homo sapiens glutamate receptor. ionotropic, GluR3

Biochim. Biophys. Acta 1219:563-566(1994)

29 GF PNTISIGGLF MRNTVQEHSA

30

FRFAVQLYNT 60

61 NQNTTEKPFH LNYHVDHLDS SNSFSVTNAF CSQFSRGVYA IFGFYDQMSM

35

NTLTSFCGAL 120

121 HTSFVTPSFP TDADVQFVIQ MRPALKGAIL SLLGHYKWEK FVYLYDTERG

40

FSILQAIMEA 180

181 AVQNNWQVTA RSVGNIKDVQ EFRRIIEEMD RRQEKRYLID CEVERINTIL

45

EQVVILGKHS 240

241 RGYHYMLANL GFTDILLERV MHGGANITGF QIVNNENPMV

50

QQFIQRWVRL DEREFPEAKN 300

301 APLKYTSALT HDAILVIAEA FRYLRRQRVD VSRRGSAGDC

55

LANPAVPWSQ GIDIERALKM 360

361 VQVQGMTGNI QFDTYGRRTN YTIDVYEMKV SGSRKAGYWN EYERFVPFSD QQISNDSASS 420

421 ENRTIVVTTI LESPYVMYKK NHEQLEGNER YEGYCVDLAY EIAKHVRIKY KLSIVGDGKY 480

481 GARDPETKIW NGMVGELVYG RADIAVAPLT ITLVREEVID FSKPLMSLGI SIMIKKPQKS 540

541 KPGVFSFLDP LA

SEQ ID NO:4; shows the amino acid sequence of the N-terminal domain of GluR4 subunit, as follows:

SEQ ID NO:4

PEPTIDE Homo sapiens glutamate receptor. ionotropic, GluR4

Recept. Channels 3:21-31(1995)

21 GAFPSSVQIG GLFIRNTDQE YTAFRLAIFL HNTAPNASEA 60

61 PFNLVPHVDN IETANSFAVT NAFCSQYSRG VFAIFGLYDK RSVHTLTSFC SALHISLITP 120

121 SFPTEGESQF VLQLRPSLRG ALLSLLDHYE WNCFVFLYDT DRGYSILQAI MEKAGQNGWH 180

181 VSAICVENFN DVSYRQLLEE LDRRQEKKFV IDCEIERLQN ILEQIVSVGK HVKGYHYIIA 240

241 NLGFKDISLE RFIHGGANVT GFQLVDFNTP MVTKLMDRWK KLDQREYPGS ETPPKYTSAL 300

301 TYDGVLVMAE TFRSLRRQKI DISRRGKSGD CLANPAAPWG QGIDMERTLK QVRIQGLTGN 360

361 VQFDHYGRRV NYTMDVFELK STGPRKVGYW NDMDKLVLIQ DVPTLGNDTA AIENRTVVVT 420

421 TIMESPYVMY KKNHEMFEGN DKYEGYCVDL ASEIAKHIGI KYKIAIVPDG KYGARDADTK 480

481 IWNGMVGELV YGKAEIAIAP LTITLVREEV IDFSKPFMSL GISIMIKKPQ KSKPGVFSFL 540

541 DPLAYE

SEQ ID NO:5; shows the amino acid sequence of recombinant GluR1, as follows:

SEQ ID NO:5

PEPTIDE Recombinant GluR1

LANLGFMDIDLNSGAVYGRAEIAGYCV

SEQ ID NO:6; shows the amino acid sequence of the recombinant GluR1, another such peptide (27 amino acids derived from the GluR1 sequence and an N-terminal Cys for attachment to a carrier protein) as follows:

SEQ ID NO:6

PEPTIDE **Artificial Sequence**

CN LANLGFMDIDLNSGAVYGRAEIAGYCV

EXAMPLES

Example 1 - Clinical Assessment of Epilepsy in Children (comparative)

[0064] This randomized and double-blinded trial was conducted at 4 Children Epilepsy Centers in Moscow and St. Petersburg (Russia) from, January 1995 through December 1999. Although our study is not strictly population-based, we recruited individuals to achieve a group representative of children with epilepsy in the western part of the country.

[0065] Eligible for the trials patients were at 4 mo.-14 years of age and had been diagnosed with epilepsy syndromes and seizures that were classified based on all information available at diagnosis and according to International League Against Epilepsy's (ILAE) guidelines by four pediatric epilepsy specialists. These include the localization-related and generalized idiopathic syndromes (e.g., benign rolandic and childhood absence epilepsy), and the cryptogenic and symptomatic generalized syndromes. The symptomatic and cryptogenic localization-related epilepsies represent a broad spectrum of syndromes defined by cause and localization, to the extent that these are known. The outcome in this group was mixed.

[0066] Etiology was classified separately from syndrome although it partially depends on the syndrome. Remote symptomatic refers to the presence of an underlying neurologic condition or insult associated with an increased risk of epilepsy (e.g., history of bacterial meningitis, stroke, cerebral palsy). Idiopathic syndromes are almost always assigned an idiopathic etiology. Occasionally, a neurologic abnormality coexists with an idiopathic syndrome (e.g., childhood absence epilepsy with mental retardation), in which case the etiology as remote symptomatic despite the idiopathic syndrome was classified. Results of neuroimaging studies were used in classifying etiology. Cryptogenic etiology refers to epilepsy that does not meet the criteria for an idiopathic syndrome and for which there is no identified significant underlying neurologic abnormality or condition. Such individuals appear to be otherwise neurologically normal.

[0067] Initial seizure frequency was defined as the ratio of the total number of unprovoked seizures since epilepsy diagnosis divided by the time between the dates of first unprovoked seizure and formal diagnosis. The seizure frequency was presented as 1 seizure per day to 1-2 fits per year. The follow up calls and observations for GluR1 aAb levels vs. seizures occurrence where performed through 7 days and 6 months (optional).

[0068] A definite diagnosis of epilepsy was based on two witnessed and well-described seizures onset or one witnessed and well-described seizure plus either EEG tracing or MRI/CT scan with evidence of a focal abnormality consistent with localization-related epilepsy.

[0069] Patients were excluded from the trial for nonepileptic seizures (e.g., pseudoseizures) or a treatable cause of seizures; progressive or degenerative disorder; psychiatric or mood disorder requiring medication, suicide attempt.

[0070] Children with non-epileptic neurological disorders and healthy individuals represented in age- and gender-matched groups where used as controls.

[0071] The trial was conducted in accordance with the international rules of good clinical practice. Written informed consent was obtained from each patient's parent or legal guardian before trial-related procedures were initiated.

[0072] Chi-square and *t*-tests were used for bivariate comparisons. Log transformations were employed when necessary to normalize a highly skewed distribution. For some continuous variables, categories were constructed to facilitate

presentation of data and testing of the assumption of linearity.

[0073] A total of 605 children (age of 4 mo.-14 years, 302 girls and 303 boys) were recruited into the study. The initial age at onset was 1-2 years. The median follow-up was 1.0 year.

[0074] In this clinical study GluR1 aAb concentration in blood samples of healthy children and those with non-epileptic neurological disorders depended on age and steady increased as children aged from neonates to adolescents (Fig. 1). It is possibly due to developing immune system and to increase of naturally circulating autoantibodies during maturation. The amounts of GluR1 aAb differ insignificantly for healthy controls and children with non-epileptic disorders (Fig.8). The comparison of mean values of GluR1 aAb in independent, age- and gender- matched groups demonstrated that aAb values for the healthy children, and patients with other neurological disorders belong to the same distribution with mean value of 0.9-1.1 ng/mL.

[0075] The detection of GluR1 aAb concentrations in blood specimens from patients with epilepsy and epilepsy syndromes showed that independently from age group all children had significantly elevated amounts of GluR1 aAb compared with that for controls (Fig.9). Autoantibody levels were higher for children with age of 4 mo.- 3 years when the most patients had the first unprovoked seizures and were diagnosed as having epilepsy or epi-syndromes. Pediatric patients at age of 3-14 years demonstrated decreased levels of GluR1 aAb compared with those at smaller age (Fig. 2).

[0076] The levels of GluR1 aAb were significantly higher for patients with generalized type of seizures compared with that for partial ones (Fig. 3). Significant correlation of spiking activity on EEG and GluR1 aAb concentrations (Spearman's coefficient 0.89, $p<0.01$) was demonstrated in all study centers. It was established that measuring GluR1 aAb in children have two potential uses: 1) epilepsy risk assessment; and 2) to assist to better clinical diagnosis of a patient with 'epilepsy like' symptoms. This premise is supported by the high predictive value of the test for recognizing individuals with epilepsy and epi-syndromes (84 % at 1.0 ng/mL cutoff).

[0077] Clinically predetermined cutoff for GluR1 aAb allowed us to differentiate patients according to seizures frequency. Monitoring of GluR1 aAb in 41 patients with epilepsy within 1 month of hospital admission showed that GluR1 aAb levels (2.6-2.7 ng/mL) at frequency of seizures 1 per day or week were higher than that (2.2 ng/mL) at seizures frequency 1 per month. The tendency maintained the same for patients with epilepsy and epi-syndromes independently from age and type of seizures.

[0078] It was established the correlation of GluR1 aAb with data obtained from CT and MRI scans that were interpreted by neuroradiologists blinded to test results for children with intractable seizures assigned for neurosurgery. Maximal concentrations of GluR1 aAb in patient with right hemispheric localization of epileptic focus were detected (Iatsuk et al., Zh. Nevropat. Psikhiat. Im. SS Korsakova. 1999, 99:34-6). The etiology of disease affects on appearance of increased GluR1 aAb levels: prenatal trauma (100 % cases), history of bacterial meningitis (85.8 % cases) and tumor (55.6 % cases).

[0079] The follow up investigation (6 mo.) of anti-epileptic therapy, seizures frequency, changes of EEG and GluR1 aAb in 19 children with mix type of seizures resulted in good correlation of detected parameters. The improvement of patient state (declining or absence of seizures) accompanied by down-regulation of GluR1 up to the control level in 84 % of cases. The correlation of EEG data with GluR1 aAb values was about 95 % in this study.

Example 2 - Clinical Assessment of Adult Epilepsy (comparative)

[0080] Double-blinded trial was conducted at Dept. of Epilepsy, V.M Bechterev's Institute on Psychiatry and Neurology and Dept. of Neurology, Russian Military Medical Academy in St. Petersburg (Russia) from February 1994 to December 1997. Two hundred thirty seven consecutive patients with epilepsy aged 18 to 40 years (130 women, 107 men) admitted to hospitals of aforementioned institutions due to increased frequency of seizures were considered for participation. Epilepsy was classified according to ILAE guidelines and the following inclusion criteria were considered in the protocol: the duration of disorder from 1 year to 20 years; the frequency of seizures from 1 per day to one per a year; type of seizures: partial simple, partial multiple, generalized with absences, generalized tonic-clonic of different etiology (e.g., history of bacterial meningitis, stroke, cerebral palsy, prenatal trauma etc.). A definite diagnosis of epilepsy was based on well-described history of seizures plus either EEG or MRI/CT scan with evidence of a focal abnormality consistent with localization-related epilepsy. Patients were excluded from the trial for progressive or degenerative disorder; psychiatric or mood disorder requiring medication, suicide attempt.

[0081] Patients (n=193, 79 women, 114 men) with non-epileptic neurological disorders (brain trauma without seizures, low back pain, arachnoidid), nonepileptic seizures (e.g., pseudoseizures) and healthy individuals (n=93) represented in age- and gender-matched groups to epileptic patients where used as controls.

[0082] The level of GluR1 aAb in healthy patients measured by use of PA-ELISA test was 1.5 ± 0.3 ng/mL and for patients with non-epileptic neurological disorders (NED) was 1.7 ± 0.2 ng/mL (Fig. 4). The comparison of mean values of GluR1 aAb in independent, age- and gender-matched groups demonstrated that aAb values for the control and patients with non-epileptic neurological disorders belong to the same distribution. GluR1 aAb-positive patients with epilepsy had a mean concentration of 3.02 ± 0.4 ng/L (range 2.1-4.1). Different control values for GluR1 aAb were revealed for women (1.8 ± 0.1 ng/mL) and men (1.5 ± 0.1 ng/mL) in total control group and patients with epilepsy as well (Fig. 5).

[0083] Our studies demonstrated the increased amount of autoantibodies to glutamate binding proteins of healthy volunteers who have had instable spiking activity on EEG (25% of tested cases). These results indicate that the raised level of autoantibodies to both fragments of glutamate receptors might be blood marker of cerebrovascular abnormalities registered by EEG without any neurological signs. In addition, cross-reaction of both autoantibodies to NMDA and AMPA receptors was revealed in some cases of patients (32%) with epilepsy and stroke (Dambinova et al. J.Neurol. Sci. 1997, 152: 93-7).

[0084] It was demonstrated negative correlation between the duration of disease and value of GluR1 aAb (Odinak et al., Zh Nevropatol Psikhiatr Im S S Korsakova. 1996, 96: 45-48). Patients with duration of epilepsy less than 5 years had higher levels of GluR1 aAb then those with longer term of disease.

[0085] The investigation of GluR1 aAb values in patients with different seizures types and frequency is depicted in Figure 6. The highest GluR1 concentrations were registered in patients with daily generalized tonic-clonic and partial multiple types of seizures in more than 86 % cases. In patients with rare fits, less than 1 per a half year, increased above cut-off aAb value (1.5 ± 0.3 ng/mL for all adults) was detected in more than 80 % cases (Odinak et al. Zh Nevropatol Psikhiatr Im S S Korsakova. 1996, 96: 45-48; Gromov et al., Zh Nevropatol Psikhiatr Im S S Korsakova 1997, 97:46-9). The correlation of seizures frequency only with concentration of GluR1 aAb was not high as expected: Spearman's coefficient 0.34 ($p<0.01$).

[0086] The comparison of results from GRACE-NeuroTest-Epilepsy ELISA test with appearance of spiking activity on EEG allowed diagnose epilepsy and support epileptic nature of spiking activity in range 84 % - 95 % cases (Odinak et al., Zh Nevropatol Psikhiatr Im S S Korsakova. 1996,96: 45-48).

[0087] The investigation of time course prior and following seizures occurrence was performed in collaboration with Dr. J. Majkowsky (Clinic of Epilepsy, Warsaw, Poland, 1994-1995) and Dr. P.Wolf (Epilepsy Center, Bielefield, Germany, 1995-1996). It was demonstrated the sudden increase of GluR1 aAb prior seizures manifestations and aAb values maintained the on high level during the followed day. These results were supported by increased spiking activity defined by daily EEG

Example 3 -- GRACE-NeuroTest-Epilensy ELISA tests performance

[0088] The GRACE-NeuroTest-Epilepsy ELISA kits for detecting GluR1 antibodies comprises (i) an immunosorbent for GluR1 peptide or antibodies to GluR1; and (ii) an indicator reagent comprising secondary antibodies attached to a signal-generating compound. The test intended to be used to assess persons undergoing paroxysmal cerebral discharges and epilepsy.

[0089] The quality of microplates covered by GluR1 peptide or antibodies to GluR1 was controlled by use of calibrators and sera specimens from healthy persons in presence and absence of calibrators (Gromova et al., Neirokhimiia. 1997, 1:23-7). The assessments of intra-assay variability, batch-to-batch variation and stability of the ELISA reaction for antibody or GluR1 calibrators were performed at various storage conditions (temperature, type of packing, storage duration). Kinetic of reaction was studies to reach optimal characteristics of variables. The concentrations of GluR1 peptide or GluR1 antibodies were then assessed in blood serum or plasma samples from patients with neurological disorders (stroke, Parkinson's and Alzheimer's diseases, palsies, and multiple sclerosis), infection diseases (TB, encephalitis, meningitis), non-infection disorders (phenylketonuria, lupus erythromatosis, diabetes, drug abuse) and healthy volunteers collected according to approved human investigative protocol.

Linearity

[0090] Blood specimens from four apparently healthy individuals were spiked with GluR1 antibodies to final concentrations of 200 ng/mL (serum) or GluR peptide to final concentration of 2.0 ng/mL (plasma). Each spiked specimen was diluted gravimetrically with unspiked one to obtain GluR1 antibodies or GluR1 peptide values throughout the range of GRACE-NeuroTest-Epilepsy assay. A correction was made for the small amount (< 0.1 ng/mL for antibodies and < 10 pg/mL for peptide) of endogenous GluR1 antibodies or GluR1 peptide in the unspiked sample. Linear regression analysis of the data indicates that the assays have linear range of 0-2.5 ng/mL for the GluR1 antibodies test and of 0-200 pg/mL.

Analytical Sensitivity

[0091] The analytical sensitivity or lowest detectable concentration that is distinguishable from zero for the GRACE-NeuroTest-Epilepsy ELISA was determined by testing a zero calibrator 20 times each using 4 lots of reagents on 5 days. The average 95% confidence limit of the analytical sensitivity of the GluR1 antibodies test was less than 0.05 ng/mL (95% confidence interval 0.01 - 0.06 ng/mL) and GluR1 peptide test was less than 5 pg/mL (95% confidence interval 0.2 - 4.9 pg/mL).

Interfering Substances

[0092] Hemoglobin (up to 10,000 mg/dL) and lipids (cholesterol up to 1000 mg/dL and triglycerides up to 1000 mg/dL) or bilirubin (up to 20 mg/dL) added to serum specimens containing GluR1 antibodies or GluR1 peptide did not interfere with the recovery of GluR1 antibodies or GluR1 peptide. However, severely hemolyzed specimens should be avoided whenever possible. When a sample appears to be severely hemolyzed, another specimen (serum or plasma) should be obtained and tested.

Analytical Specificity

Antibodies

[0093] The immunoactive peptides from N-terminal fragments of μ- (MOR) or δ-opioid receptors (DOR), glutamate receptors (NR1, GluR4), and dopamine receptors (D2, D3, D4) or their specific antibodies (IgG) were evaluated for potential cross-reactivity and interference in the GRACE-NeuroTest-Epilepsy ELISA assay at the concentrations indicated below. There was no significant interference with the GluR1 antibodies measurement, nor was there any significant assay cross-reactivity.

Neuroreceptor Type	Concentration of Substance		% Recovery	Reference
	Antibodies, μg/mL	Peptide, ng/mL		
MOR	1.0	100	105%	Dambinova, Izykenova, 2002
DOR	1.0	100	107%	Dambinova, Izykenova, 2002
GluR4	0.5	100	99%	Dambinova et al., 1997
NR1	0.5	100	101%	Izykenova et al., 2000
D2	1.0	100	104%	fragment 8-31
D3	1.0	100	109%	fragment 6-27
D4	1.0	100	103%	fragment 1-18

Example 4 -- GRACE-NeuroTest-Epilepsy tests results deviation

[0094] Within-day and total imprecision were determined using the ANOVA model by testing controls and human specimen pools that had the respective analytes added at concentrations near the decision points of the assay and throughout the range of the standard curve. The study was conducted over 20 days, testing each control 5 times per day.

[0095] Average intra-assay imprecision

Mean		Standard Deviation		Coefficient of variation	
GluR1 Antibodies, ng/mL	GluR1 Peptide, pg/mL	GluR1 Antibodies, ng/mL	GluR1 Peptide, pg/mL	GluR1 Antibodies, %	GluR1 Peptide, %
1.5	50	0.1	3.0	7.0	6.0
3.2	100	0.2	5.0	6.5	5.0
12.5	500	0.5	23.0	6.0	4.6

[0096] Average inter-assay imprecision

Mean		Standard Deviation		Coefficient of variation	
GluR1 Antibodies, ng/mL	GluR1 Peptide, pg/mL	GluR1 Antibodies, ng/mL	GluR1 Peptide, pg/mL	GluR1 Antibodies, %	GluR1 Peptide, %
1.5	50	0.2	6.9	13.0	14.0
3.2	100	0.3	11.2	9.5	11.2
12.5	500	0.8	49.4	6.5	9.9

Example 5. GRACE-NeuroTest-Epilepsy ELISA Expected Values in Individuals Without Epilepsy

[0097] The circulating GluR1 peptide and GluR1 antibodies concentration were determined in blood specimens from 214 children (age of 4mo.-14 years, 111 girls and 103 boys) and 286 individuals (126 women and 160 men) without epilepsy. This population included individuals with neurological disorders (brain trauma without seizures, low back pain, arachnoidid, Parkinson's and Alzheimer' diseases, pseudoepilepsy), infection diseases (TB, encephalitis, meningitis), non-infection disorders (phenylketonuria, lupus erythromatosis, diabetes, drug abuse) and healthy volunteers. There are no statistically significant changes in GluR antibodies concentration associated with brain trauma without seizures, low back pain, arachnoidid, phenylketonuria, lupus erythromatosis, drug abuse, TB, encephalitis, and meningitis. The descriptive statistics for GluR1 antibodies concentrations in individuals without epilepsy are shown in the following table. The values are representative of the values obtained from clinical studies. The decision threshold was determined by the 95% confidence limit of GluR1 antibodies concentration in the non-epilepsy population different ages. These values translate into a general specificity of the test of greater than 89 % for GluR1 antibodies and greater than 92 % for GluR1, i.e. less than 10 % expected false positives in individuals without epilepsy.

[0098] GluR1 antibodies concentration (ng/mL) in Non-Epilepsy Population

Index	All				
	Children			Adults, Age 18-40	
	Age <1	Age 1-3	Age 3-14	Women	Men
Mean	0.70	1.02	1.25	1.8	1.5
SD	0.15	0.34	0.29	0.1	0.2
Median	0.60	0.93	1.1	1.7	1.4
Percent<1.0 ng/mL <1.5 ng/mL <1.8 ng/mL	99.0% - -	98.0% - -	96 % 3% -	- 5 % 90 %	5% 89% 2%
Minimum	0.5	0.5	0.5	1.0	0.9
Maximum	0.9	1.2	1.4	1.9	1.5
N	70	81	63	126	160

[0099] GluR1 peptide concentration (pg/mL) in Non-Epilepsy Population

Index	All		
	Children		Adults, Age 18-40
	Age <3	Age 3-14	Women/Men
Mean, pg/mL	45.2	84	97
SD	5.1	7.7	8.2
Median	48.3	89.5	99
Percent			

(continued)

Index	All		
	Children		Adults, Age 18-40
	Age <3	Age 3-14	Women/Men
<50 pg/mL	94.0 %	1.0 %	0.5 %
<100 pg/mL	3.0 %	96.5 %	92.1 %
Minimum	38.2	75.3	88.4
Maximum	51.0	92.0	106.1
N	151	63	286

[0100] Based on routinely EEG and clinical evaluation the diagnosis and progression of epilepsy can only partially be predicted. Up-to-now there was an unmet diagnostic need for a laboratory test with blood samples. The GRACE-NeuroTest-Epilepsy gives an answer to this need. It is recommended to use this blood test support for all neurological considerations with respect to the differential diagnosis of epilepsy. The GRACE-NeuroTest-Epilepsy assay has been evaluated and is proposed for the following clinical indications in children:

- Rule in brain related seizures and epilepsy to increase the degrees of diagnosis certainty
- Rule out pseudo-epilepsy and epilepsy-like disorders.

[0101] ***Reference ranges in blood:*** (Iatsuk et al. Zh Nevropat Psikhiat. SS Korsakova 1999, 99:34-6)

Children, age dependant	*Normal reference ranges*		
	GluR1 antibody ng/mL	*GluR1 peptide pg/mL*	*Antibody/peptide ratio*
<3 years old	<0.7	<50	<14
Between 3 and 14 years	<1.0	<90	<11

[0102] The GRACE-NeuroTest-Epilepsy Assay has been evaluated and is proposed for the following clinical indications in adults:

- Rule in brain related seizures and epilepsy to increase the degrees of diagnosis certainty
- Risk factor for paroxysmal cerebral discharges and epilepsy after other disorders
- Prognosis of brain related seizures
- Follow-up after treatment and for the adjustment of the adequate therapy and doses

[0103] ***Reference ranges in serum:*** (Gromov et al. Zh Nevropatol Psikhiatr Im S S Korsakova 1997, 97:46-49)

Adult	*Normal reference ranges*		
	GluR1 antibody ng/mL	*GluRl peptide pg/mL*	*Antibody/peptide ratio*
Men	<1.5	<100	<15
Women	<1.8	<100	<18

Example 6. GRACE-NeuroTest-Epilepsy ELISA Expected Values in Individuals With Epilepsy

[0104] Blood samples were obtained from 391 children (age of 4 mo.-14 years, 191 girls and 200 boys) and 237 individuals (130 women and 107 men) with epilepsy and epilepsy syndromes. The descriptive statistics for GluR1 aAb concentrations in patients with epilepsy and epilepsy syndromes are presented in the table below. These values are representative of the values obtained from clinical studies.

[0105] GluR1 Antibodies Concentration (ng/mL) in Patients with Epilepsy and Epi-syndromes

	All				
	Children			Adults, Age 18-40	
	Age <1	Age 1-3	Age 3-14	Women	Men
Mean	2.0	2.5	2.8	3.2	2.7
SD	0.2	0.3	0.3	0.2	0.2
Median	1.9	2.2	2.6	2.7	2.4
Percent>1.0 ng/mL >1.5 ng/mL >1.8 ng/mL	16 % 57% 21 %	2% 59% 34%	3% 55% 37%	2% 3% 90 % 90 %	2% 4% 88 %
Minimum	1.0	1.0	1.0	1.0	1.0
Maximum	2.4	3.0	3.3	4.1	3.1
N	119	132	140	130	107

[0106] GluR1 peptide concentration (pg/mL) in Patients with Epilepsy and Epilepsy Syndromes

Index	All		
	Children		Adults, Age 18-40
	Age <3	Age 3-14	Women/Men
Mean, pg/mL	298.2	307.1	452.9
SD	91.1	146.4	152.2
Median	304.3	332.0	490.4
Percent >50 pg/mL >100 pg/mL	91.0 % 8.0 %	1.5 % 96.5 %	3% 93.2 %
Minimum	48.2	47.5	99.0
Maximum	495.4	481.0	905.2
N	251	140	237

Example 7. The Sensitivity and Specificity of GRACE-NeuroTest-Epilepsy ELISA

[0107] The 2 by 2 table for entire children groups assessed
by GRACE-NeuroTest-Epilepsy assay detecting GluR antibodies

	Epi	No Epi	
Positive	330 (TP)	10 (FP)	340
Negative	61 (FN)	204 (TN)	265
	391	214	605

Total
Sensitivity: 330/391 = 84 %
Specificity: 204/214 = 95 %

[0108] The 2 by 2 table for entire children groups assessed
by GRACE-NeuroTest-LA assay detecting GluR peptide

	Epi	No Epi	
Positive	379 (TP)	11 (FP)	390
Negative	12 (FN)	203 (TN)	215
	391	214	605

Total
Sensitivity: 379/391= 97 %
Specificity: 203/214 = 95 %

[0109] The 2 by 2 table for entire adult groups assessed by GRACE-NeuroTest-Epilepsy assay detecting GluR antibodies

	Epi	No Epi	
Positive	204 (TP)	26 (FP)	230
Negative	33 (FN)	260 (TN)	293
	237	286	523

Total
Sensitivity: 204/237 = 86 %
Specificity: 260/286 = 91 %

[0110] The 2 by 2 table for entire adult groups assessed by GRACE-NeuroTest-LA assay detecting GluR peptide

	Epi	No Epi	
Positive	221 (TP)	21 (FP)	242
Negative	16 (FN)	265 (TN)	281
	237	286	523

Total
Sensitivity: 221/237 = 93 %
Specificity: 265/286 = 93 %

Example 8. Interpretation of Results GRACE-NeuroTest-Epilepsy ELISA

[0111] The Receiver Operating Characteristic Curve (ROC) of GluR1 antibodies cut-offs versus clinical sensitivity and specificity provided the area under the curve > 0.95 $\pm$ 0.01 and cut-offs of 1 ng/mL for children (age <14 years), of 1.5 ng/mL for men and of 1.8 ng/mL for women. ROC of GluR1peptide cut-offs versus clinical sensitivity and specificity provided the area under the curve > 0.97 $\pm$ 0.01 and cut-offs of 50 pg/mL for children (age <3 years) and of 100 pg/mL for adolescent and adults. The clinical sensitivity and specificity of the GRACE-NeuroTest-Epilepsy Test using set cutoffs for various age and gender groups is described in the table below.

[0112] Sensitivity and Specificity vs. Age & Gender

Index	All			
	Children		Adult, age 18-40	
	GluR antibodies	GluR peptide	GluR antibodies	GluR peptide
Sensitivity, % at 95 % CI	84.0 78.0 - 88.5	97.0 95.4 - 99.1	85.0 83.3 - 95.5	93.0 90.4 - 96.2
Specificity, % at 95 % CI	95.0 93.5 - 98.0	95.0 92.1-97.7	91.0 85.2 - 98.7	93.0 89.9 - 95.4

Clinical Specificity

[0113] Serum samples from persons without paroxysmal cerebral discharges and epilepsy were assayed for the presence of GluR1 antibodies. Total specificity was shown to be 91% for adult and 95 % for children.

Clinical Sensitivity

[0114] The clinical sensitivity of the GluR1 antibodies assay to assess patients with epilepsy in a random population was determined to be 84-85%.

[0115] The clinical sensitivity of the assay for epilepsy was determined by comparing GluR1 test results from seven groups of selected patients: with epilepsy, both definite and uncertain; with loss of consciousness; fainting/ syncope; migraine; brain trauma; and cerebrovascular disease.

TABLE GluR1 Peptide Assay Clinical Performance

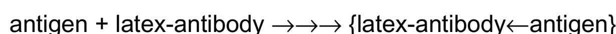
Disease	Number of patients	Number of correct "+" results	Number of false "-" results	Clinical Sensitivity* * %	Clinical Specificity %¶
Epilepsy: Definite Uncertain	976* 136*	839 106	137 30	75 10	- -
Loss of consciousness	32	31	1	-	100
Fainting/ syncope	19	16	3	-	100
Migraine	17	13	4	-	99
Traumatic Brain Injury	71*	59	12	-	99
Cerebrovascular disease: Stroke TIA	31 14	27 12	4 2	- -	99 100
Brain Tumor	19*	14	5	-	100
Parkinsonism	30	28	2	-	100
Alzheimer's Disease	15	12	3	-	100
Multiple sclerosis	15	15	0	-	100
Other Diseases: TB Phenylketonuria Lupus erythromatosis Diabetes mellitus Drug Abuse	18 19 31* 21* 33*	18 19 31 19 32	0 0 0 2 1	- - - - -	100 100 100 100 100
Healthy Persons	505*	461	44	-	95
Total	2002	1754	248	85	91

Example 9. GRACE-NeuroTest-Epilepsy LA

[0116] A rapid assay of GluR1 peptide for epilepsy assessment based on a latex agglutination technique directed on improvement of power of diagnostic certainty.

[0117] The GRACE-NeuroTest-Epilepsy LA assay employs triple concave slides with a built-in magnification device to detect the reaction visually, providing an immediate "yes" or "no" response. In this assay, plasma samples are mixed

with antibody coupled with colored latex particles and agglutination is indicated in between 2 and 5 minutes. The reaction occurs in a homogeneous phase and can be detected visually:

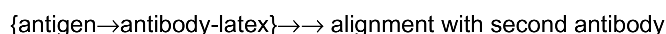

antigen + latex-antibody →→→ {latex-antibody←antigen}

[0118] We are developing the GRACE-NeuroTest-Epilepsy Flow microassay based on lateral-flow technique using colored latex particles containing antibodies to GluR1. The blood or plasma "reconstitutes" the latex-reagent and transports it to the detection line. In most cases, sandwich assays are performed. The test is a heterogeneous assay; ie, reactions in both solution and solid phase occur. This test procedure is as follows:

Step 1. Blood is dropped on a specific site on the lateral-flow device.
Step 2. Blood reconstitutes the colored latex reagent.
Step 3. If the analyte in question is in blood, then the first reaction takes place:

Antigen is bound to the antibody on the latex particle→→ {antigen→antibody-latex}-complex.

Step 4. In parallel with the reaction in Step 3, transport to the detection line of the complex: {antigen→antibody-latex} with another antibody occurs. The following reaction then takes place:


{antigen→antibody-latex}→→ alignment with second antibody

[0119] The concentration of this complex can be quite high at the detection line and may be visually detected (ie, by color) or measured by device (fluorometric. method). The analytical sensitivity is high because of the concentration-process of the colored particle (the "catching principle"). Healthy people generally have GluR1 peptide concentration of 50 pg/mL.
[0120] Clinical trials of GRACE-NeuroTest-Epilepsy in ELISA and LA formats combined with clinical observations and EEG data demonstrated its value. This could be shown very impressively by the comparison of the pre- and post-test probabilities of epilepsy in observed patient groups:

Diagnostic Indication	Pre-test probability of seizures and spiking activity on EEG, %	GluR1 antibodies LR*	GluR1 peptide LR*	Post-test Probability**, %
Epilepsy: Definite	63.5	9.4	- 28.1	83.5 (80, 95 CI) 95.0 (90, 98 CI)
Uncertain	35.0	1.1	- 5.0	35.0 (21, 44 CI) 42.0 (32, 51 CI)
Loss of consciousness	15.0	<0.04	- <0.01	0.15 <0.1
Fainting/syncope	14.2	<0.04	- <0.01	0.11 <0.1
Migraine	<10	0.02	- <0.01	<0.1 <0.05
Brain trauma	32.3	0.9	- <0.01	3.5 <0.02
Stroke	25.1	0.5	- <0.01	1.5 <0.01

*positive likelihood ratio, LR = Sensitivity/1-Specificity; ** Post-test probabilities arrived from pre-test probabilities defined from clinical observations of seizure manifestations combined with EEG data and likelihood ratios (LR) for GluR1 autoantibodies positive test according to http://www.med.nagoya-cu.ac.jp/psych.dir/graphical.htm ; CI - confidence interval

[0121] The latex agglutination method is especially well suited for POC use because GluR1 peptide levels are elevated at a very early stage of paroxysmal cerebral discharges and, thus provide real-time indication of neurotoxic events. In addition, results can be processed in less than 10 minutes, allowing timely and appropriate intervention.
[0122] This method provides reliable data in a format that is simple to interpret. The application of the latex agglutination technique to analysis of brain biomarkers for epilepsy will decrease the cost of analysis, provide the opportunity to monitor real-time progress of a treatment procedure, and allow physicians to determine the efficacy of medication

administered in the treatment of epilepsy.

Claims

1. A method for determining the origin of seizures in a patient diagnosed as having paroxysmal discharges comprising directly assaying a biological fluid obtained from said patient for the presence and quantity of GluR1 peptide and comparing said quantity of GluR1 to a baseline level selected from population norms and prior levels measured in said patient

2. The method of claim 1 wherein said origin is paroxysmal or non-paroxysmal.

3. The method of claim 1 wherein a concentration of free GluR1 or fragment thereof in blood of greater than 50 pg/ml indicates a paroxysmal origin of said seizures, and a GluR1 concentration in blood of less than 50 pg/ml indicates a non-paroxysmal origin of said seizures.

4. The method of claim 1, wherein said origin is paroxysmal, further comprising:

   a) directly assaying said biological fluid for the presence and quantity of GluR1 after having treated said patient with an initial dose of anticonvulsive drug therapy; and
   b) directly assaying said biological fluid for the presence and quantity of GluR1 after having increased the dose, changed the drug, or treated with multiple drugs, if the presence or quantity of GluR1 fails to fall below a designated standard.

5. The method of claim 4 further comprising correlating changes in GluR1 to alterations in paroxysmal spiking activity as measured by EEG.

6. The method of claim 1, comprising:

   a) contacting said biological sample with a solid phase comprising antibodies to GluR1, for a time sufficient to form a complex between GluR1 or fragment thereof and GluR1 antibodies;
   b) contacting said complex with an indicator reagent attached to a signal-generating compound; and
   c) measuring the signal generated; wherein the amount of signal detected correlates to the amount of said GluR1 present in said sample.

7. The method of claim 1 comprising:

   a) contacting said biological sample with an agglutinating carrier comprising antibodies to GluR1, for a time sufficient to form a complex between GluR1 or fragment thereof and GluR1 antibodies; and
   b) reading a signal generated from the agglutination; wherein the signal correlates to an abnormally high amount of GluR1,.

8. A method for diagnosing the probability of epilepsy in patients at risk for epilepsy comprising directly assaying a biological fluid from said patient for the presence and quantity of GluR1 or a fragment thereof.

9. Use of the method of claim 8 for preparing a medication for adjusted anticonvulsant therapy in response to changes in quantities of GluR1 or GluR1 quantities above said designated standard.

Patentansprüche

1. Verfahren zur Bestimmung der Ursache von Anfällen bei einem Patienten, bei welchem paroxysmale Entladungen diagnostiziert wurden, das das direkte Untersuchen der Präsenz und Menge von GluR1-Peptid in einem biologischen Fluid umfasst, welches vom Patienten erhalten wurde, und das Vergleichen von dieser Menge an GluR1 mit einem Grundpegel, welcher von Bevölkerungsnormen und den bereits zu früheren Zeitpunkten in dem Patient gemessenen Pegeln aus ausgewählt wurde, umfasst.

2. Verfahren nach Anspruch 1, wobei der Ursprung der Anfälle paroxysmal oder nicht-paroxysmal ist.

3. Verfahren nach Anspruch 1, wobei eine Konzentration von freiem GluR1 oder GluR1-Fragmenten im Blut von größer als 50 pg/ml eine paroxysmale Ursache der Anfälle und eine GluR1-Konzentration im Blut von weniger als 50 pg/ml eine nicht-paroxysmale Ursache der Anfälle anzeigt.

4. Verfahren nach Anspruch 1, wobei die Ursache paroxysmal ist, das weiterhin umfasst:

a) das direkte Untersuchen der Präsenz und Menge von GluR1-Peptid in einem biologischen Fluid, nachdem der Patient mit einer Initialdosis eines Antikonvulsivums behandelt wurde; und
b) das direkte Untersuchen der Präsenz und Menge von GluR1-Peptid in einem biologischen Fluid nachdem die Dosis erhöht worden ist, das Medikament gewechselt worden ist, oder mit mehreren Medikamenten behandelt worden ist, wenn es nicht gelungen ist die Präsenz oder Menge von GluR1 unter einen gewünschten Standard zu senken.

5. Verfahren nach Anspruch 4, welches weiterhin die Korrelierung der Änderungen von GluR1 mit der Änderung der Aktivität des Auftretens von paroxysmalen Anfällen, welche in einem EEG gemessen wird, umfasst.

6. Verfahren nach Anspruch 1, welches umfasst:

a) das Kontaktieren der biologischen Probe mit einer Festphase, welche Antikörper gegen GluR1 enthält, für eine ausreichend lange Zeit, um die Bildung von Komplexen zwischen GluR1 oder GluR1-Fragmenten und den GluR1-Antikörpern zu ermöglichen;
b) das Kontaktieren des Komplexes mit einem Nachweisreagens, welches an eine signalgebende Substanz gebunden ist; und
c) das Messen des entstandenen Signals, wobei die Stärke des erkannten Signals mit der Menge des in der Probe enthaltenen GluR1 korreliert.

7. Verfahren nach Anspruch 1, welches umfasst:

a) das Kontaktieren der biologischen Probe mit einem agglutinierendem Trägermaterial, das Antikörper gegen GluR1 enthält, für eine ausreichend lange Zeit, um die Bildung von Komplexen zwischen GluR1 oder GluR1-Fragmenten und den GluR1-Antikörpern zu ermöglichen; und
b) das Auslesen eines Signals, welches durch das Agglutinieren entstanden ist, wobei das Signal mit einer abnormal erhöhten Menge von GluR1 korreliert.

8. Verfahren zur Diagnose der Wahrscheinlichkeit des Auftretens von Epilepsie in Patienten einer Epilepsierisikogruppe, welches das direkte Untersuchen der Präsenz und Menge von GluR1 oder GluR1-Fragmenten in einem biologischen Fluid vom Patienten umfasst.

9. Anwendung des Verfahrens nach Anspruch 8 zur Herstellung eines Medikaments zur angepassten antikonvulsiven Therapie, um auf Änderungen der Menge von GluR1 oder auf Mengen von GluR1, die einen gewünschten Standard überschreiten, zu antworten.

Revendications

1. Procédé destiné à déterminer l'origine de crises chez un patient ayant fait l'objet d'un diagnostic selon lequel il présente des décharges paroxystiques, comprenant le dosage direct d'un fluide biologique obtenu auprès dudit patient pour déterminer la présence et la quantité du peptide GluR1 et la comparaison de ladite quantité de GluR1 à un niveau de base sélectionné à partir de normes de la population et de niveaux antérieurs mesurés chez ledit patient.

2. Procédé selon la revendication 1, dans lequel ladite origine est paroxystique ou non paroxystique.

3. Procédé selon la revendication 1, dans lequel une concentration de GluR1 libre ou d'un fragment de celui-ci dans le sang supérieure à 50 pg/ml indique une origine paroxystique desdites crises, et une concentration de GluR1 dans le sang inférieure à 50 pg/ml indique une origine non paroxystique desdites crises.

4. Procédé selon la revendication 1, dans lequel ladite origine est paroxystique, comprenant en outre les étapes

consistant à :

a) doser directement ledit fluide biologique pour déterminer la présence et la quantité de GluR1 après avoir traité ledit patient avec une dose initiale de traitement par médicaments anticonvulsifs, et
b) doser directement ledit fluide biologique pour déterminer la présence et la quantité de GluR1 après avoir augmenté la dose, modifié le médicament, ou traité avec plusieurs médicaments, si la présence ou la quantité de GluR1 ne réussit pas à tomber au-dessous d'une norme désignée.

5. Procédé selon la revendication 4, comprenant en outre la corrélation des variations de GluR1 avec des modifications de l'activité paroxystique de pointe telle que mesurée par électroencéphalogramme.

6. Procédé selon la revendication 1, comprenant les étapes consistant à :

a) mettre en contact ledit échantillon biologique avec une phase solide comprenant des anticorps vis-à-vis du GluR1, pendant un temps suffisant pour former un complexe entre le GluR1 ou un fragment de celui-ci et les anticorps vis-à-vis du GluR1,
b) mettre en contact ledit complexe avec un réactif indicateur fixé à un composé générant un signal, et
c) mesurer le signal généré, où l'intensité du signal détecté présente une corrélation avec la quantité dudit GluR1 présent dans ledit échantillon.

7. Procédé selon la revendication 1, comprenant les étapes consistant à :

a) mettre en contact ledit échantillon biologique avec un vecteur agglutinant comprenant des anticorps vis-à-vis du GluR1, pendant un temps suffisant pour former un complexe entre le GluR1 ou un fragment de celui-ci et les anticorps vis-à-vis du GluR1, et
b) lire un signal généré à partir de l'agglutination, où le signal présente une corrélation avec une quantité anormalement élevée de GluR1.

8. Procédé destiné à diagnostiquer la probabilité d'épilepsie chez des patients présentant un risque d'épilepsie, comprenant le dosage direct d'un fluide biologique provenant dudit patient pour déterminer la présence et la quantité de GluR1 ou d'un fragment de celui-ci.

9. Utilisation du procédé selon la revendication 8 pour préparer un médicament dans le but d'un traitement anticonvulsif ajusté en réponse à des variations des quantités de GluR1 ou à des quantités de GluR1 supérieures à ladite norme désignée.

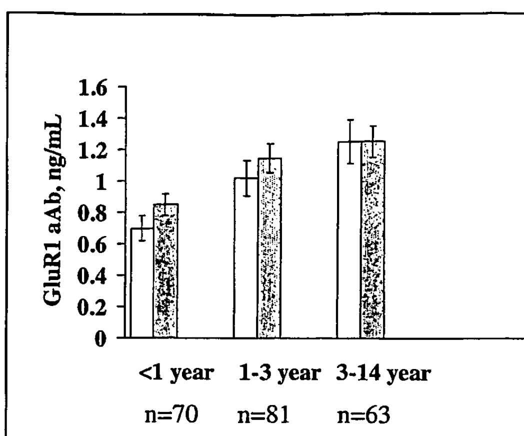


Fig. 1. GluR1autoantibodies amounts in blood serum of healthy children (white bars) and those with non-epileptic disorders (shaded bars) depending on age.

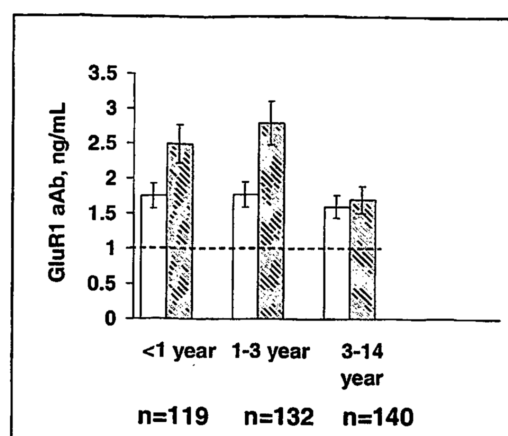


Fig. 2. GluR1autoantibodies in blood serum of children with epilepsy syndrome (white bars) and epilepsy (stroked bars). Dotted line shows the cut off for GluR1 aAb.

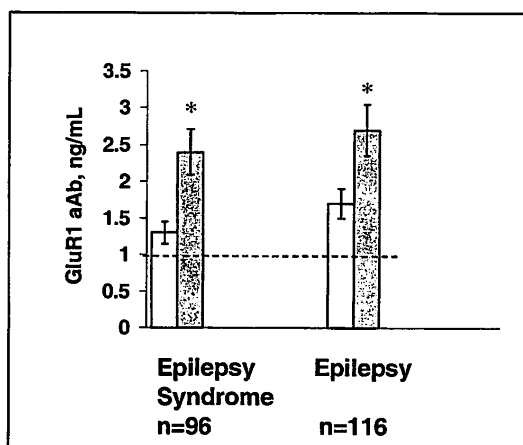


Fig.3. GluR1autoantibodies in blood serum of children with partial (white bars) and generalized seizures (shaded bars). Dotted line shows the cut off for GluR1 aAb.

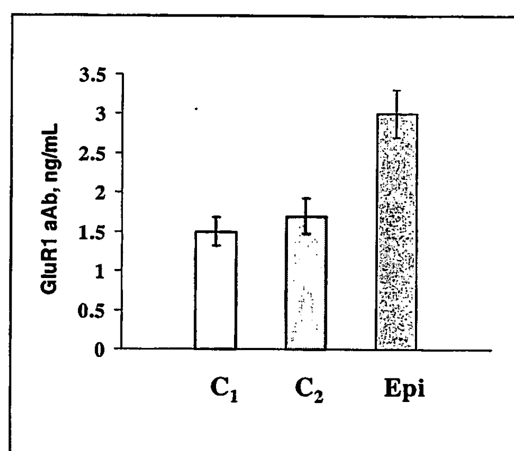


Fig. 4. Detection of GluR1 aAb in blood serum of healthy persons (C_1), patients with non-epileptic neurological disorders (C_2) and patients with epilepsy (Epi).

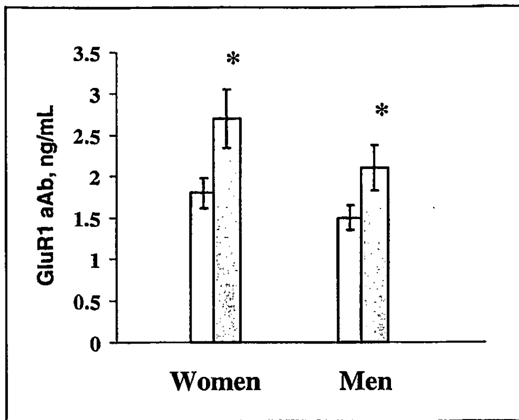


Fig. 5. GluR1 aAb dependence from gender: in total controls (white bars) and patients with epilepsy (shaded bars).

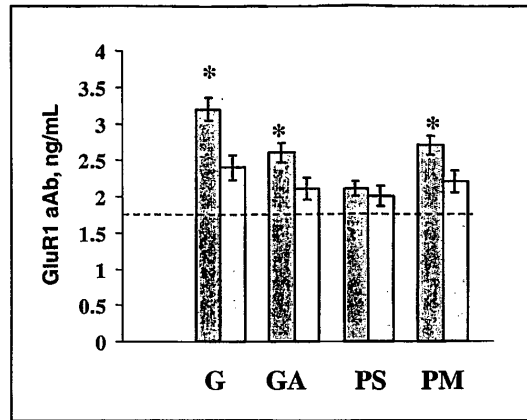


Fig. 6. GluR1 aAb dependence from seizures type and frequency: dark bars – daily seizures; light bars – 1 per half year. G– generalized tonic-clonic (n=98); GA – generalized with absences (n=50); PS – partial simple (n=33) and PM – partial multiple (n=56).

REFERENCES CITED IN THE DESCRIPTION

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patsnap

专利名称(译)	用于评估阵发性脑放电的免疫吸附血液测试		
公开(公告)号	EP1689288B1	公开(公告)日	2010-04-21
申请号	EP2004818645	申请日	2004-11-08
申请(专利权)人(译)	GRACE实验室，INC.		
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摘要(译)

用于诊断中枢神经系统疾病，尤其是阵发性脑放电和癫痫的免疫吸附剂，试剂盒和组合物，包括测量来自人类受试者的生物样品中GluR1或其片段和/或GluR1抗体的浓度。 该方法对于识别有脑相关癫痫和癫痫风险的个体，将癫痫与假性癫痫和癫痫样疾病区分开来，在抗惊厥治疗后进行随访以及调整适当的治疗方法和剂量特别有用。

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