US 20080318220A1

(19) **United States**

(12) **Patent Application Publication**

(10) **Pub. No.: US 2008/0318220 A1**

(43) **Pub. Date: Dec. 25, 2008**

Rosell Vives et al.

(54) **METHOD FOR THE DIAGNOSIS AND/OR PROGNOSIS OF ALZHEIMER'S DISEASE**

(75) Inventors: **Elisabet Rosell Vives**, Barcelona (ES); **Marta Barrachina Castillo**, Barcelona (ES); **Isidro Ferrer Abizanda**, Barcelona (ES); **Tamara Maes**, Castelldefels (ES)

Correspondence Address:
SUGHRUE MION, PLLC
2100 PENNSYLVANIA AVENUE, N.W., SUITE 800
WASHINGTON, DC 20037 (US)

(73) Assignees: **FINA BIOTECH, S.L.**, POZUELO DE ALARCON (Madrid) (ES); **ORYZON GENOMICS, S.A.**, BARCELONA (ES)

(21) Appl. No.: **11/765,669**

(22) Filed: **Jun. 20, 2007**

Publication Classification

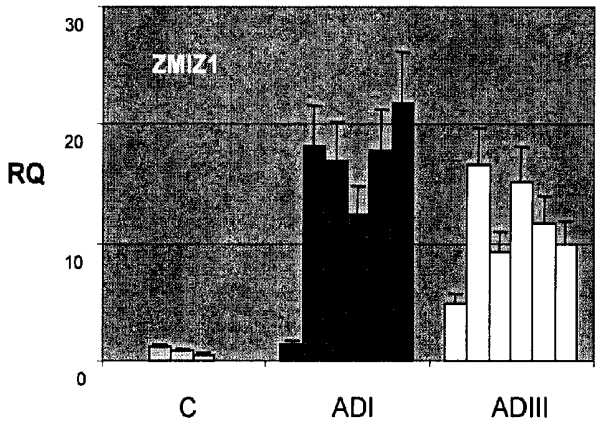
(51) **Int. Cl.**
C12Q 1/68 (2006.01)
G01N 33/53 (2006.01)

(52) **U.S. Cl.** **435/6**; 435/7.1

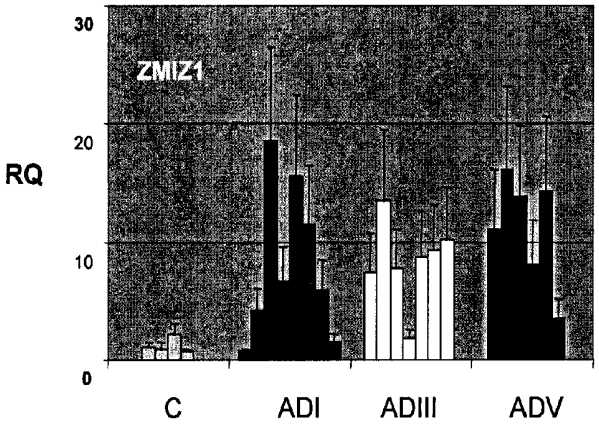
(57) **ABSTRACT**

The present invention relates to a method for the diagnosis and/or prognosis of Alzheimer's disease by means of determining the ZMIZ1 gene expression level in a biological sample and comparing said level with a reference value, in which the alteration of said level is indicative of Alzheimer's disease.

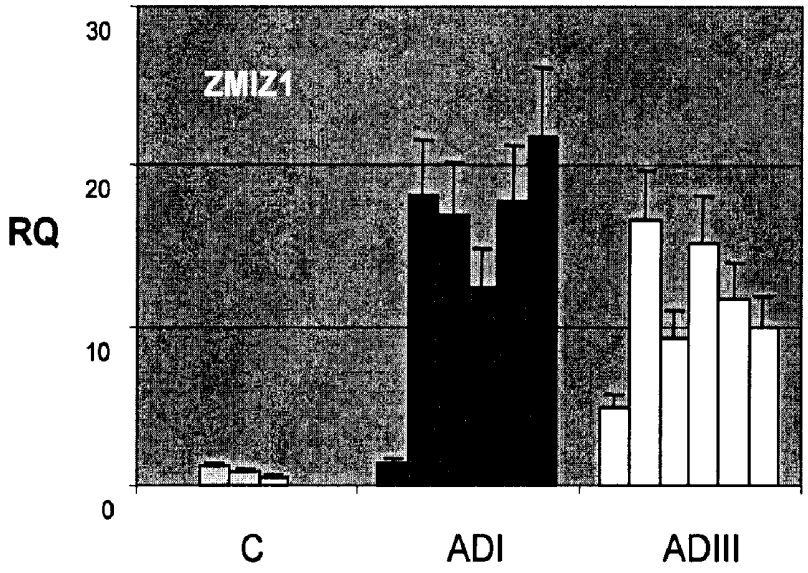
A)



B)



A)



B)

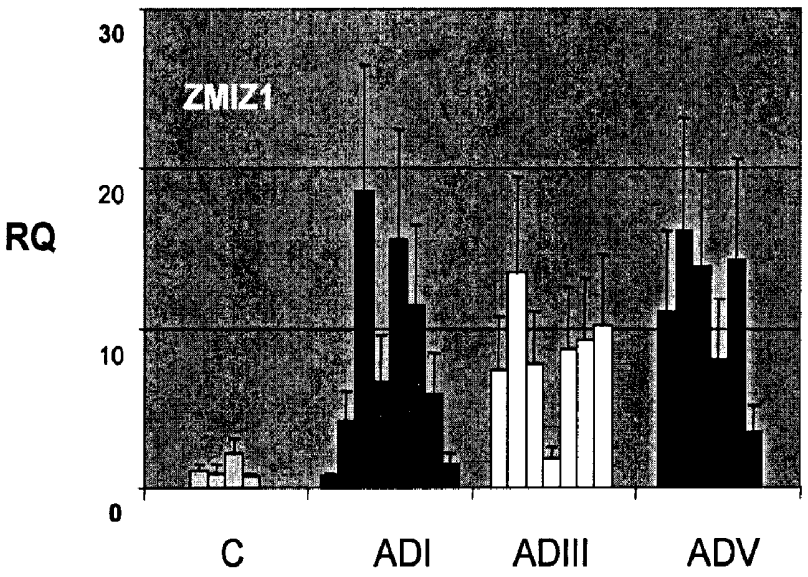


FIGURE 1

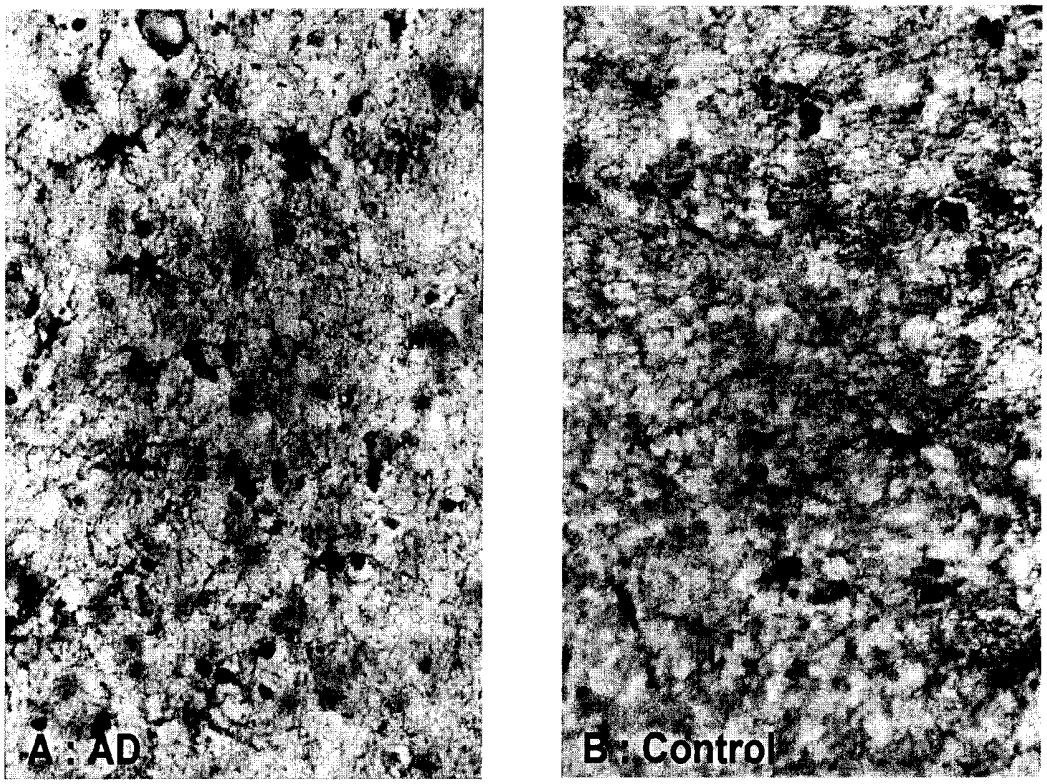


FIGURE 2

METHOD FOR THE DIAGNOSIS AND/OR PROGNOSIS OF ALZHEIMER'S DISEASE

FIELD OF THE INVENTION

[0001] The present invention relates to the health sector, mainly to neurodegenerative diseases. More specifically, the invention is related to procedures for the diagnosis and/or prognosis of Alzheimer's disease.

BACKGROUND OF THE INVENTION

[0002] Alzheimer's disease (AD) is considered the main cause of dementia, this being the fourth cause of death in developed countries (Pappolla M A. *La Neuropatología y la Biología Molecular de la Enfermedad de Alzheimer*. pp. 543-553. *Neuropatología. Diagnosis and Clinical Medicine*. Cruz-Sánchez FF. Ed. Edimsa. 2000.). It is defined as a central nervous system neurodegenerative condition and is characterised by a progressive deterioration of higher cerebral functions.

[0003] Microscopically, AD is characterised by the presence of senile plaques (diffuse and classic), neurofibrillary tangles, neuropil threads, neuronal degeneration, 1'-Amyloid protein deposits, granulovacuolar degeneration and the presence of Hirano bodies, amongst other pathologies (Cruz-Sánchez F F et al. *Neuropathological Diagnostic Criteria for Brain banking*. Ed. IOS Press. 1995).

[0004] Clinical criteria that are well established in the fourth edition of the diagnostic and statistical manual of the American Psychiatric Association (DSM-IV) are used to diagnose AD. (*Diagnostic criteria from DSM-IV*. Washington D.C.:APA; 1994) or by the National Institute of Neurologic, Communicative Disorders and Stroke—Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA). (Mc Khann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan E M. "*Clinical diagnosis of Alzheimer's Disease: report of the NINCDS ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology* 1984; 34:939-44). Nevertheless, the greatest dilemma for these clinical studies is diagnostic certainty. Although AD is diagnosed by means of several neurological tests, currently the only way to confirm the diagnosis is performing a post-mortem analysis in brain tissue in order to find the existence of neurofibrillary tangles and plaques.

[0005] Different genetic markers have been studied in recent years for application in AD diagnosis, such as:

- **[0006]** the determination of mutations in the amyloid precursor protein (APP) gene, mutations in the presenilin-1 (PSI) and presenilin-2 (PS2) genes, only valid for a reduced number of cases of precocious or family AD (Gil Nécija, Eulogio. *Biological diagnosis. Fourth National Course on Alzheimer's Disease*. Seville, 23-24 Sep. 1999. Ed. Andrómico).
- **[0007]** genetic value of the ApoE genotyping, ascertained only in those cases complying with probable AD clinical criteria; the problem is that it gives a high number of false positives.

[0008] As well as these genetic markers, there are biochemical markers such as:

- **[0009]** the Tau protein: this protein is ascertained by means of neuronal antibodies capable of detecting tau in cerebrospinal fluid, however, tau levels in AD are not related to age, sex, disease development, nor with the degree of dementia, as well as high levels of tau being detected in other pathologies such as meningitis, meningeal infiltrations, frontal lobe dementia and Creutzfeldt-Jacobs disease.
- **[0010]** the β-Amyloid protein: this protein lacks diagnostic utility in sporadic forms of AD (Guimerà A. et al "*Update on the pathology of Alzheimer's disease". Rev Esp Patol* 2002; Vol 35, n° 1:21-48.).

[0011] AD presents a pre-symptomatic stage, without definite clinical symptoms, which may last between 10 and 20 years. There is currently no non-intrusive diagnostic tool available with suitable sensitivity, specificity and predictive value for this disease. Moreover, this disease implies huge social costs, due, among other reasons, to the incapacity by the patients to cope for themselves, this leading to the necessity of a reliable diagnostic procedure in pre-clinical stages that allows preventing the disease, improving the treatment and predicting disease development.

[0012] To this effect, the authors of the present invention have surprisingly found that the ZMIZ1 gene expression level is clearly higher in biological samples from patients with Alzheimer's disease compared to reference values from control samples.

[0013] The ZMIZ1 gene ("zinc finger, MIZ-type containing 1", also called RAI17; Zimp10 or hZIMP10) codes for a protein with the same name expressed in urogenital tissue, including the prostate, testicles and ovaries, as well as in prostate and mammary tumour cell lines. ZMIZ1 belongs to the PIAS-like ("protein inhibitor of activated STAT") protein family and, as other proteins from this family, it has a conserved Miz domain ("msx-interacting zinc finger") through which it interacts with the androgen receptor (AR), suggesting a biological role of ZMIZ1 in androgen signaling (Sharma et al. *hZimp10 is an androgen receptor co-activator and forms a complex with SUMO-1 at replication foci. EMBO J.* 2003 Nov. 17; 22(22): 6101-6114). Said receptor plays a central role in masculine sexual development and in the growth and survival of normal and tumour prostate cells. AR transcriptional activation is regulated by interaction with various co-factors, amongst which is ZMIZ1. A strong intrinsic transactivation domain has been identified on the proline-rich C-terminal end of ZMIZ1. Endogenous proteins AR and ZMIZ1 co-locate in the nuclei of prostate epithelial cells in human tissue samples. ZMIZ1 increases AR transcriptional activity in prostate tumour cells. Moreover, ZMIZ1 co-locates with AR and SUMO1 (small ubiquitin-like modifier 1) in replication foci during S phase, and it is capable of increasing AR SUMOylation in vivo, such that the increase in AR activity by ZMIZ1 depends on receptor SUMOylation. This indicates that ZMIZ1 acts as a co-regulator of AR and modifies its activity in DNA transcription and replication, which suggests that ZMIZ1 and its connection with the SUMO pathway, combined with other modification pathways such as methylation, phosphorylation and acetylation is involved in regulating AR transcriptional activity by modifying chromatin formation during the early and late S phases of the cell cycle. On the other hand, ZMIZ1 has been described to present little or no effect on other nuclear hormonal receptors, such as the glucocorticoid receptor, the estrogen receptor, the β thyroid receptor or the vitamin D receptor.

[0014] Similarly, ZMIZ1 has also been related to TGF-β ("Transforming growth factor β") (Li et al. *The Novel PIAS-like Protein hZimp10 Enhances Smad Transcriptional Activity. J. Biol. Chem.*, VoL 281, Issue 33, 23748-23756, Aug. 18,

2006). The TGF-β factor plays important roles in controlling cell proliferation, differentiation and apoptosis. Smad proteins are substrates of the Type I TGF-β receptor and they are responsible for transducing receptor signals to target genes in the nucleus. PIAS proteins were originally identified as transcriptional co-regulators of the JAK-STAT pathway. It has subsequently been demonstrated that the interconnection between PIAS proteins and other signaling pathways is involved in several cell processes. Particularly, PIAS proteins modulate TGF-β signaling, regulating Smad3 transcriptional activity. It has thus been observed that the expression of exogenous ZMIZ1 increases Smad3 transcriptional activity, which depends on Smad4 and responds to TGF-β induction. Furthermore, suppression of endogenous ZMIZ1 reduces Smad3 transcriptional activity. On the other hand, a protein-protein interaction has been identified between Smad3 and Smad4 with ZMIZ1. The regions responsible for this interaction are the Miz domain of ZMIZ1 and the MH2 domains of Smad3 and Smad4. It has also been demonstrated by immunostaining tests that Smad3, Smad4 and ZMIZ1 co-locate in the cell nucleus. Similarly, it has been demonstrated that Smad3/Smad4-mediated transcription is significantly altered in response to TGF-β induction in ZMIZ1-deficient embryo fibroblasts. All these results indicate that ZMIZ1 interacts with Smad3/Smad4 proteins and increases the transcriptional activity of said protein complex, such that ZMIZ1 plays an important role in regulating the TGF-β/Smad signaling pathway.

[0015] ZMIZ1 is located at cell level both in the cytoplasm and the nucleus in prostate cancer cells (Sharma M, Li X Wang Y, Zarnegar M, Huang C Y, Palvimo J J, Lim B, Sun Z. *hZimp*10 *is an androgen receptor co-activator and forms a complex with SUMO*-1 *at replication foci. EMBO J* 2003 Nov. 17; 22(22):6101-14).

[0016] Nevertheless, there is no evidence to date on the relationship between this gene and Alzheimer's Disease.

[0017] Based on this finding and the requirements of the state of the art, the authors of the present invention have developed a simple and reliable diagnostic and/or prognostic method for AD based on the detection of ZMIZ1 gene expression levels.

[0018] The use of the ZMIZ1 gene as a genetic marker for AD allows establishing an early diagnosis of the disease in pre-clinical stages, as well as a prognosis of the development thereof.

DESCRIPTION OF THE DRAWINGS

[0019] FIG. **1**: Relative quantification (RQ) measured by quantitative PCR of the expression of the ZMIZ1 gene with respect to the beta glucuronidase (GUSB) gene, used as an endogenous control (C). A) hippocampus; B) frontal cortex.

[0020] FIG. **2**: Specific staining using an anti-ZMIZ1 antibody. Immunohistochemistry of frontal cortex sections. Left: tissue from an Alzheimer's disease patient (A: AD), right: tissue from an individual not suffering Alzheimer's disease (B: control)

OBJECT OF THE INVENTION

[0021] Firstly, the invention relates to a procedure for the diagnosis and/or prognosis of Alzheimer's Disease based on determining ZMIZ1 gene expression level in biological samples.

[0022] Also object of the present invention is a kit for the diagnosis and/or prognosis of AD to carry out the determination of ZMIZ1 gene expression level according to the previous procedure.

DESCRIPTION OF THE INVENTION

[0023] The present invention provides a method for the diagnosis and/or prognosis of Alzheimer's Disease by means of determining ZMIZ1 gene expression level.

[0024] A main aspect of the invention relates to a method for the diagnosis and/or prognosis of Alzheimer's Disease in a subject by determining the ZMIZ1 gene expression level in a biological sample isolated from the subject and by comparing said level with a reference value, wherein the alteration of said level is indicative of Alzheimer's disease and of the stage of said disease.

[0025] Thus, said procedure may be performed with a diagnostic purpose (diagnostic method) and with a prognosis purpose (prognosis method). A diagnostic procedure relates to a method that allows determining genes that are differentially expressed between samples of Alzheimer's disease patients and control samples (from healthy individuals). The prognostic method relates to a method that allows predicting, at least in part, disease development by means of analysing the differential expression of said genes in the different stages of the disease. In this sense a subject who has previously diagnosed with AD might be analyzed to know the progress of the disease.

[0026] The term "subject" used in the present invention relates to a human being.

[0027] The expression "reference value" in the present invention designates mRNA or ZMIZ1 protein levels present in a healthy individual not suffering from AD or other diseases affecting mRNA or ZMIZ1 protein levels.

[0028] According to a particular embodiment of the invention, the biological sample comprises a tissue, preferably said tissue is a tissue homogenate, preferably the tissue homogenate is obtained from nervous tissue cells or peripheral neuroendocrine cells.

[0029] According to another particular embodiment of the invention, the biological sample is a biological fluid, preferably said biological fluid is cerebrospinal fluid, blood, plasma or serum.

[0030] In a particular embodiment of the invention, the determination of ZMIZ1 gene expression level is performed by analysing the amount of RNA or protein coded by said gene or fragments thereof.

[0031] Particularly, the determination of ZMIZ1 gene expression level is performed by image analysis. In preferred embodiments, the image analysis may be carried out from quantification on immunohistochemical images. Examples of quantification methods include, but are not limited to, morphometry, densitometry and fluorescence intensity.

[0032] In a particular embodiment of the invention, the determination of ZMIZ1 gene expression level is carried out by means of an indicator substance that binds specifically to the RNA or the protein coded by said gene.

[0033] In the present invention, the expression "indicator substance" refers to an antibody, a monoclonal antibody, an antibody fragment, an oligonucleotide, a macromolecule, an organic molecule or, in general, any substance that may bind specifically to RNA or the protein coded by the ZMIZ1 gene. Said indicator substance comprises a marker which may be an enzyme, a radioisotope, a dye, a fluorescent compound, a

chemoluminescent compound, a bioluminescent compound, a metal chelate or, generally, any known marker of the state of the art that may be detected by a detection method.

[0034] According to another particular embodiment of the invention, determination of the ZMIZ1 gene expression level is performed by analysing the amount of RNA coded by said gene or fragments thereof.

[0035] In a preferred embodiment of the invention, the analysis of the amount of RNA coded by said gene or fragments thereof is performed by amplification, using oligonucleotides specific to PCR, SDA or any other amplification method for cDNA allowing a quantitative estimation of ZMIZ1 transcript levels.

[0036] In another preferred embodiment of the invention, the analysis of the amount of RNA coded by said gene or fragments thereof is performed by means of DNA biochips made with oligonucleotides deposited by any mechanism known by a person skilled in the art or synthesised in situ by means of photolithography or by means of any other mechanism known by a person skilled in the art.

[0037] In another particular embodiment of the invention, the determination of the expression level for the gene coding for ZMIZ1 is performed by analysing the amount of protein coded by said gene or fragments thereof.

[0038] In a preferred embodiment of the invention, the analysis of the amount of protein coded by said gene or fragments thereof is performed by Western-Blot.

[0039] In another preferred embodiment of the invention, the analysis of the amount of protein coded by said gene or fragments thereof is performed by means of protein chips using specific antibodies against ZMIZ1 or fragments thereof or by protein profiles performed by mass spectrometry or by any other mechanism allowing a quantitative estimate of ZMIZ1 protein levels.

[0040] In another preferred embodiment of the invention, the analysis of the amount of protein coded by said gene or fragments thereof is performed by immunohistochemical techniques.

[0041] In another preferred embodiment of the invention, the analysis of the amount of protein coded by ZMIZ1 or fragments thereof is performed by incubation with a specific antibody, such as for example a purified rabbit polyclonal antibody against ZMIZ1 protein (rabbit polyclonal RA117 antibody (c-term), #AP6236a by Abgent) or a purified rabbit polyclonal antibody against a synthetic peptide of ZMIZ1 protein (rabbit polyclonal RAI17 antibody, #PAB-11055, de Orbigen).

[0042] In another preferred embodiment, the analysis of the amount of protein coded by said gene is performed by means of ELISA or any other enzymatic method.

[0043] Another main aspect of the invention is a kit for the diagnosis and/or prognosis of Alzheimer's Disease comprising the reagents necessary for carrying out the determination of ZMIZ1 gene expression level. The Kit allows carrying out the method according to the invention that has just been described.

[0044] In a particular embodiment of the invention, the diagnosis and/or prognosis kit for AD comprises the reagents necessary for determining the ZMIZ1 gene expression level by means of image analysis.

[0045] In another particular embodiment, the reagents necessary to determine the ZMIZ1 gene expression level comprise a composition comprising an indicator substance that binds specifically to the RNA or the protein coded by said gene, where said indicator substance is marked with a detectable marker and a physiologically acceptable carrier liquid.

[0046] In a particular embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of RNA coded by the ZMIZ1 gene and/or fragments thereof.

[0047] In a preferred embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of RNA coded by the ZMIZ1 gene and/or fragments thereof by amplification.

[0048] In another preferred embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of RNA coded by the ZMIZ1 gene and/or fragments thereof by DNA biochips.

[0049] In another particular embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of protein coded by the ZMIZ1 gene and/or fragments thereof.

[0050] In a preferred embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of protein coded by the ZMIZ1 gene and/or fragments thereof by Western Blot.

[0051] In another preferred embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of protein coded by the ZMIZ1 gene and/or fragments thereof by protein chips.

[0052] In another preferred embodiment of the invention, the diagnosis and/or prognosis kit for Alzheimer's disease comprises the reagents necessary for determining the level of protein coded by the ZMIZ1 gene and/or fragments thereof by immunohistochemical techniques.

[0053] In another preferred embodiment of the invention, the diagnosis and/or prognosis kit for AD comprises the reagents necessary for analysing the amount of protein coded by the ZMIZ1 gene and/or fragments thereof by incubation with a specific antibody.

[0054] Finally, a preferred embodiment of the invention contemplates a diagnosis and/or prognosis kit for AD comprising the reagents necessary for analysing the amount of protein coded by the ZMIZ1 gene and/or fragments thereof by ELISA or any other enzymatic procedure.

[0055] Other aspects of the invention will become evident for a person of average skill in the art.

[0056] The following examples serve to illustrate but not limit the present invention.

EXAMPLES

Example 1

Initial Determination of Genes Differentially Expressed in Samples from Patients with AD

[0057] An experiment was performed on DNA micromatrices in order to identify genes that had differential expression levels between brain tissue samples from Alzheimer patients and controls. Neocortex and hippocampus tissues from several donors in development stages I/II and II/IV were used for the study, compared with normal expression in normal tissue from material extracted from the same areas. Especially, the ZMIZ1 gene was determined as an overexpressed gene in Alzheimer disease patients.

[0058] Brain samples were obtained by autopsy of 12 patients with AD and 6 controls. Informed consent was obtained from the patients or their relatives and the study was approved by the Ethics Committees.

[0059] The time between death and tissue processing was 2-10 hours. Half the brain was cut in 1 cm thick coronal sections and was frozen in dry ice at −80° C. until use.

[0060] For the morphological examination, the brains were fixed by immersion in a 10% formalin buffer during at least 48 hours at 4° C.

[0061] The neuropathological study was carried out in 4 μm paraffin sections without wax of the upper frontal cortex, anterior convolution of the corpus callosum, frontal white matter from the semioval centre, occipital lobe white matter from semioval centre, head of caudate nucleus and nucleus accumbens, nucleus of Meynert, lenticular nucleus, anterior thalamus, medial central thalamus, dorsal thalamus, hippocampus, lower temporal convolution and amygdaloid nucleus, anterior insula, pre- and post-central convolution, calcarine convolution, mesencefalon at the level of the substantia nigra, high protuberance at the level of the cerulen loci, low protuberance, medulla oblongata, spinal chord, spinal ganglions, cerebellar vermis, cerebellar hemisphere and dentate nucleus, optic chiasm and olfactory bulb.

[0062] The sections were stained with hematoxylin and eosin, Luxol Fast Blue by the Kluver Barrera procedure and for glial fibre acid protein immunohistochemistry, CD 68 and tomato lectin for microglia, β-Amyloid, pan-tau, specifically phosphorylated tau at Thr181, Ser202, Ser214, Ser262, Ser396 and Ser422 and αB-crystallin, α-sinuclein and ubiquitin.

[0063] AD stages were established according to amyloid load and according to neurofibrillary pathology following the Braak and Braak classification:

Stage A: initial deposits in the basal neocortex

Stage B: deposits extended to the association areas of the neocortex

Stage C: strong deposits throughout the entire cortex

I-II: neurofibrillary pathology stages in transentorhinal region

III-IV: limbic region

VI: neocortical region

[0064] Having prepared the samples, both ZMIZ1 mRNA and protein mRNA were determined and biochemical, immunohistochemical and microscopic studies were performed. The samples of control and diseased brains were processed in parallel. The results from these studies demonstrate that there is an increase in ZMIZ1 and protein mRNA expression levels in the cerebral cortex in early stages of AD. ZMIZ1 is located in the cytoplasm of neurons and astrocytes.

Example 2

mRNA Isolation and Confirmation of Results by cDNA Synthesis and TaqMan PCR

[0065] Quantitative PCR with specific probes is the technique usually used as a reference for validating changes in gene expression detected by oligonucleotide micromatrices. 20 independent tissue samples were used for the validation, from the entorhinal area (8 controls, 5 ADI and 7 ADIII), 19 hippocampus samples (6 controls, 7 ADI and 6 ADIII) and 28 neocortex samples (7 controls, 8 ADI, 7 ADIII, 6 ADV).

[0066] Total RNA was isolated using Trizol Reagent® (Life Technologies) followed by RNeasy Protect Mini Kit (Qiagen). Frozen human cerebral tissues were homogenised directly in 1 ml of Trizol per 100 mg of tissue. Total RNA was extracted following the protocol suggested by the supplier. The purified Total RNA was then resuspended in 100 μl of RNase-free water. mRNA was purified following the RNeasy Protect Mini Kit protocol with minimal modifications. Treatment with DNase was dismissed due to the elimination of genomic DNA during extraction with Trizol. The concentration of each sample was measured at A_{260}, and RNA integrity was verified by formaldehyde-agarose gel electrophoresis and by bioanalyzer analysis.

[0067] The samples selected for the analysis were chosen very strictly regarding RNA quality. Degradation is a parameter which clearly influences obtaining a reliable result or otherwise a result that hardly allows quantifying the expression level.

[0068] The High-Capacity cDNA Archive Kit (Ref 4322171) by Applied Biosystems was used for cDNA synthesis. Calibration curves were obtained starting from 2.5 μg of RNA which were passed to cDNA and for the remaining samples the synthesis was performed starting from 1 μg of total RNA. For each 100 μl of reverse transcription reaction, the human RNA was mixed with the master mix provided by the retailer containing: random hexamers, $MgCl_2$, 500 μM each of dATP, dTTP, dCTP and dGTP, 0.4 U/μl of RNase inhibitor and 1.25 U/μl of transcriptase in the appropriate buffer. Reactions were carried out at 25° C. for 10 minutes in order to maximise bonding between the template RNA and the primer, followed by 120 minutes at 37° C. and after by incubation for 5 minutes at 95° C. in order to deactivate the reverse transcriptase.

[0069] TaqMan low density Arrays-Microfluidic Cards by Applied Biosystems were used to validate 20 genes that were differentially expressed in the DNA micromatrices performed with postmortem cerebral tissue samples from patients with Alzheimer's disease. The endogenous controls incorporated in the Microfluidic Cards were the GUS (β-glucuronidase) and 18S (18S ribosomal subunit) genes.

[0070] The TaqMan probe (Applied Biosystems) binds to the template DNA strand between the direct and reverse primers. The probe contains a fluorescent molecule and another molecule capable of screening the first molecule's fluorescence if it is close enough. If there is a specific reaction, the probe is degraded by the Taq polymerase during amplification, releasing the fluorescent molecule, which separates from its screening molecule thus emitting fluorescence. The amount of fluorescence produced will therefore be proportional to the amount of product accumulated.

[0071] The TaqMan PCR tests for ZMIZ1 and the internal controls were performed in duplicate on cDNA samples on the multifluidic cards. Parallel tests were performed for each sample using β-actin and GUS primers and probes for standardisation. The reaction was carried out using the following parameters: 50° C. for 2 minutes, 95° C. for 10 minutes and 40 cycles at 95° C. for 15 seconds and 60° C. for 1 minute. Standard curves were prepared for ZMIZ1 and for each internal control using serial dilutions of control samples of human RNA. Finally, the TaqMan PCR data was captured using Sequence Detector Software (SDS version 1.9; Applied Biosystems).

[0072] It must be taken into account that in order to perform a relative quantification, the expression of a particular gene in a sample is standardised with respect to an endogenous gene of invariable expression. The lines obtained for the endog-

enous gene and the genes to be studied when representing the threshold cycle (Ct) with respect to the amount of cDNA used. ABI 7700 measures fluorescence accumulation by the PCR products by continuous monitoring. The detection threshold is fixed after the reaction. The detection threshold is an arbitrary value manually assigned to a level above the baseline in the exponential PCR phase in which there is no limiting element. The Ct value establishes the point at which sample amplification crosses the detection threshold. The levels of the internal controls were used to normalise ZMIZ1 mRNA values, which did not vary in the pathological samples with respect to the controls and were also similar between the different pathologies. In order to apply the relative quantification procedure comparing Cts (delta-delta Ct procedure) it is necessary that the lines obtained from the gene to be studied and from the gene used as an endogenous gene are parallel. The relative amount will be defined by the following formula:

$$2^{-\Delta\Delta Ct}$$

[0073] The increase in ZMIZ1 mRNA levels was confirmed by means of TaqMan PCR tests with the control brain samples and with AD (FIG. **1**).

[0074] The results showed a clear relative increase of ZMIZ1 mRNA levels in the frontal cortex and the hippocampus when compared to the controls, even in early states which lack the associated clinical symptoms.

Example 3

Immunohistochemistry

[0075] The 5 μm thick sections without wax from the frontal cortex, hippocampus and frontal cortex were processed for immunohistochemistry following the streptavidin-biotin peroxidase (LSAB) procedure. After incubating with methanol and H_2O_2 in PBS and normal serum, the sections were incubated with rabbit polyclonal anti-human ZMIZ1 antibody: rabbit polyclonal RA117 antibody (c-term), AbGent (AP6236a) in a 1:250 dilution. After incubation with the primary antibody, the sections were incubated with LASB for 15 minutes at room temperature. The peroxidase reaction was visualised with diaminobenzidine and H_2O_2. Immunostaining control included omitting the primary antibody. No signal was obtained following incubation exclusively with the secondary antibody. The sections were slightly stained with hematoxilin.

[0076] ZMIZ1 immunoreactivity characterised by small cytoplasm granules was mainly located in astrocytes. Expression was clearly greater in tissue from patients of Alzheimer's disease (FIG. **2**A than in control samples (FIG. **2**B).

1. A method for the diagnosis and/or prognosis of Alzheimer's Disease (AD) comprising:

determining the expression level of the ZMIZ1 gene in a biological sample isolated from a subject, and

comparing said expression level with a reference value,

in which the alteration of said level is indicative of AD and/or the stage of said disease.

2. A method for the diagnosis or prognosis of AD according to claim **1**, in which the biological sample comprises a tissue.

3. A method for the diagnosis and/or prognosis of AD according to claim **2**, in which the tissue is a homogenate.

4. A method for the diagnosis and/or prognosis of AD according to claim **3**, in which the tissue homogenate is obtained from nervous tissue cells or from peripheral neuroendocrine cells.

5. A method for the diagnosis and/or prognosis of AD according to claim **1**, in which the biological sample comprises a biological fluid.

6. A method for the diagnosis and/or prognosis of AD according to claim **5**, in which the biological fluid is cerebrospinal fluid, blood, plasma or serum.

7. A method for the diagnosis and/or prognosis of AD according to claim **1**, in which the determination of the ZMIZ1 gene expression levels performed by analysing the amount of RNA or protein coded by said gene or fragments thereof.

8. A method for the diagnosis and/or prognosis of AD according to claim **7**, in which the in accordance with gene expression level is determined by image analysis.

9. A method for the diagnosis and/or prognosis of AD according to claim **7**, in which the determination of the ZMIZ1 gene expression level is performed by means of an indicator substance which binds specifically to the RNA or protein coded by said gene.

10. A method for the diagnosis and/or prognosis of AD according to claim **7**, in which the determination of the ZMIZ1 gene expression level is performed by analysing the amount of RNA coded by said gene or fragments thereof.

11. A method for the diagnosis and/or prognosis of AD according to claim **10**, in which the analysis of the amount of RNA is performed by amplification.

12. A method for the diagnosis and/or prognosis of AD according to claim **10**, in which the analysis of the amount of RNA is performed by DNA biochips.

13. A method for the diagnosis and/or prognosis of AD according to claim **7**, in which the determination of the ZMIZ1 gene expression level is performed by analysing the amount of protein coded by said gene and/or fragments thereof.

14. A method for the diagnosis and/or prognosis of AD according to claim **13**, in which the analysis of the amount of protein is performed by Western Blot.

15. A method for the diagnosis and/or prognosis of AD according to claim **13**, in which the analysis of the amount of protein is performed by protein chips.

16. A method for the diagnosis and/or prognosis of AD according to claim **13**, in which the analysis of the amount of protein is performed by immunohistochemical techniques.

17. A method for the diagnosis and/or prognosis of AD according to claim **13**, in which the analysis of the amount of protein is performed by incubation with a specific antibody.

18. A method for the diagnosis and/or prognosis of AD according to claim **13**, in which the analysis of the amount of protein is performed by ELISA or any other enzymatic procedure.

19. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for determining the ZMIZ1 gene expression level according to the method of claim **1**.

20. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for determining the ZMIZ1 gene expression level by image analysis, according to the method of claim **8**.

21. A Kit for the diagnosis and/or prognosis of AD comprising a composition containing an indicator substance that binds specifically to RNA or to the protein coded by said gene, in which said indicator substance is marked with a detectable marker, and a physiologically acceptable carrier liquid, according to the method of claim **9**.

22. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for the amplification of the RNA coded by the ZMIZ1 gene and/or fragments thereof, according to the method of claim **11**.

23. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for analysing the RNA coded by the ZMIZ1 gene and/or fragments thereof by means of DNA biochips, according to the method of claim **12**.

24. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for analysing the amount of protein coded by the ZMIZ1 gene and/or fragments thereof by Western Blot, according to the method of claim **14**.

25. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for analysing the RNA coded by the ZMIZ1 gene and/or fragments thereof by means of protein chips, according to the method of claim **15**.

26. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for analysing the RNA coded by the ZMIZ1 gene and/or fragments thereof by means of immunohistochemical techniques, according to the method of claim **16**.

27. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for analysing the amount of protein coded by the ZMIZ1 gene and/or fragments thereof by incubation with a specific antibody, according to the method of claim **17**.

28. A Kit for the diagnosis and/or prognosis of AD comprising the reagents necessary for analysing the amount of protein coded by the ZMIZ1 gene and/or fragments thereof by ELISA or any other enzymatic procedure, according to the method of claim **18**.

* * * * *

专利名称(译)	阿尔茨海默病的诊断和/或预后的方法		
公开(公告)号	US20080318220A1	公开(公告)日	2008-12-25
申请号	US11/765669	申请日	2007-06-20
[标]申请(专利权)人(译)	国际泳联BIOTECH.S L波苏埃洛 - ALARCON 奥瑞泽恩基因组学股份有限公司		
申请(专利权)人(译)	国际泳联BIOTECH，S.L.（波苏埃洛 - ALARCON ORYZON基因组学，S.A.		
当前申请(专利权)人(译)	国际泳联的生物技术，S.L. ORYZON基因组学，S.A.		
[标]发明人	ROSELL VIVES ELISABET BARRACHINA CASTILLO MARTA FERRER ABIZANDA ISIDRO MAES TAMARA		
发明人	ROSELL VIVES, ELISABET BARRACHINA CASTILLO, MARTA FERRER ABIZANDA, ISIDRO MAES, TAMARA		
IPC分类号	C12Q1/68 G01N33/53		
CPC分类号	C12Q1/6883 C12Q2600/158 G01N33/6896 G01N2800/2821 C12Q2600/112		
外部链接	Espacenet USPTO		

摘要(译)

本发明涉及通过测定生物样品中的ZMIZ1基因表达水平并将所述水平与参考值进行比较来诊断和/或预测阿尔茨海默病的方法，其中所述水平的改变指示阿尔茨海默病。疾病。