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(54) **DIAGNOSTIC AND THERAPEUTIC USE OF VAULT POLYNUCLEOTIDES AND PROTEINS FOR NEURODEGENERATIVE DISEASES**

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(57) **ABSTRACT**

The present invention discloses the differential expression of the minor vault protein ADPRTL1 gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating Alzheimer's disease in a subject, or for determining whether a subject is at increased risk of developing Alzheimer's disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using a gene coding for a vault protein, in particular the gene coding for the minor vault protein ADPRTL1. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

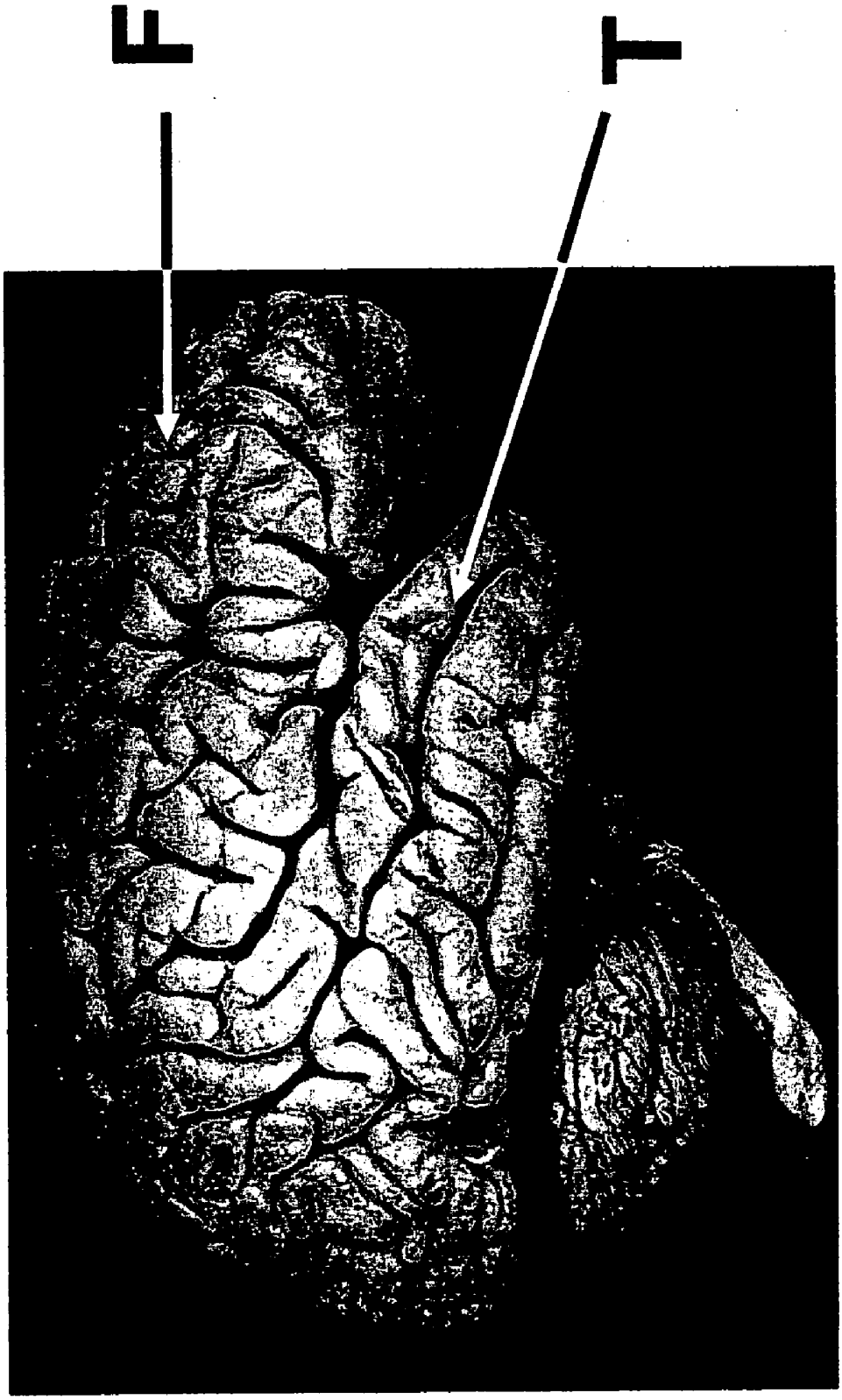

Fig. 1: Identification of genes involved in Alzheimer's Disease pathology

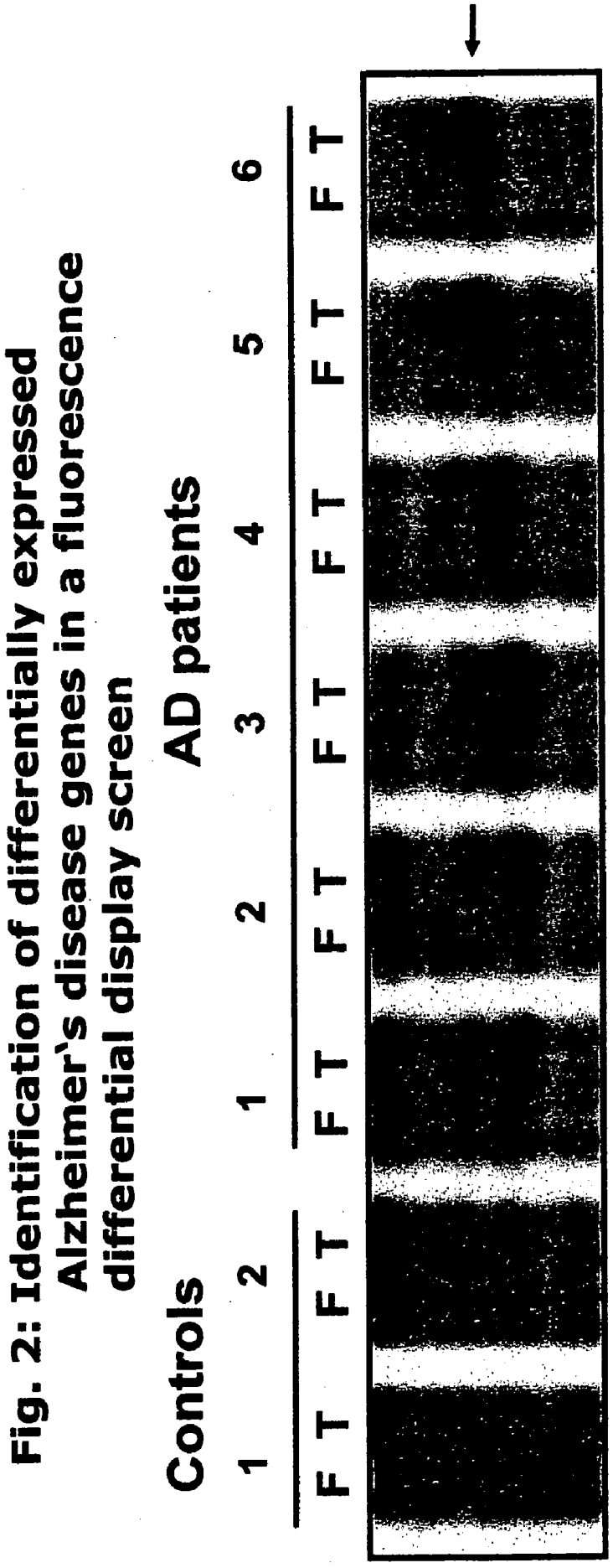

Fig. 2: Identification of differentially expressed Alzheimer's disease genes in a fluorescence differential display screen

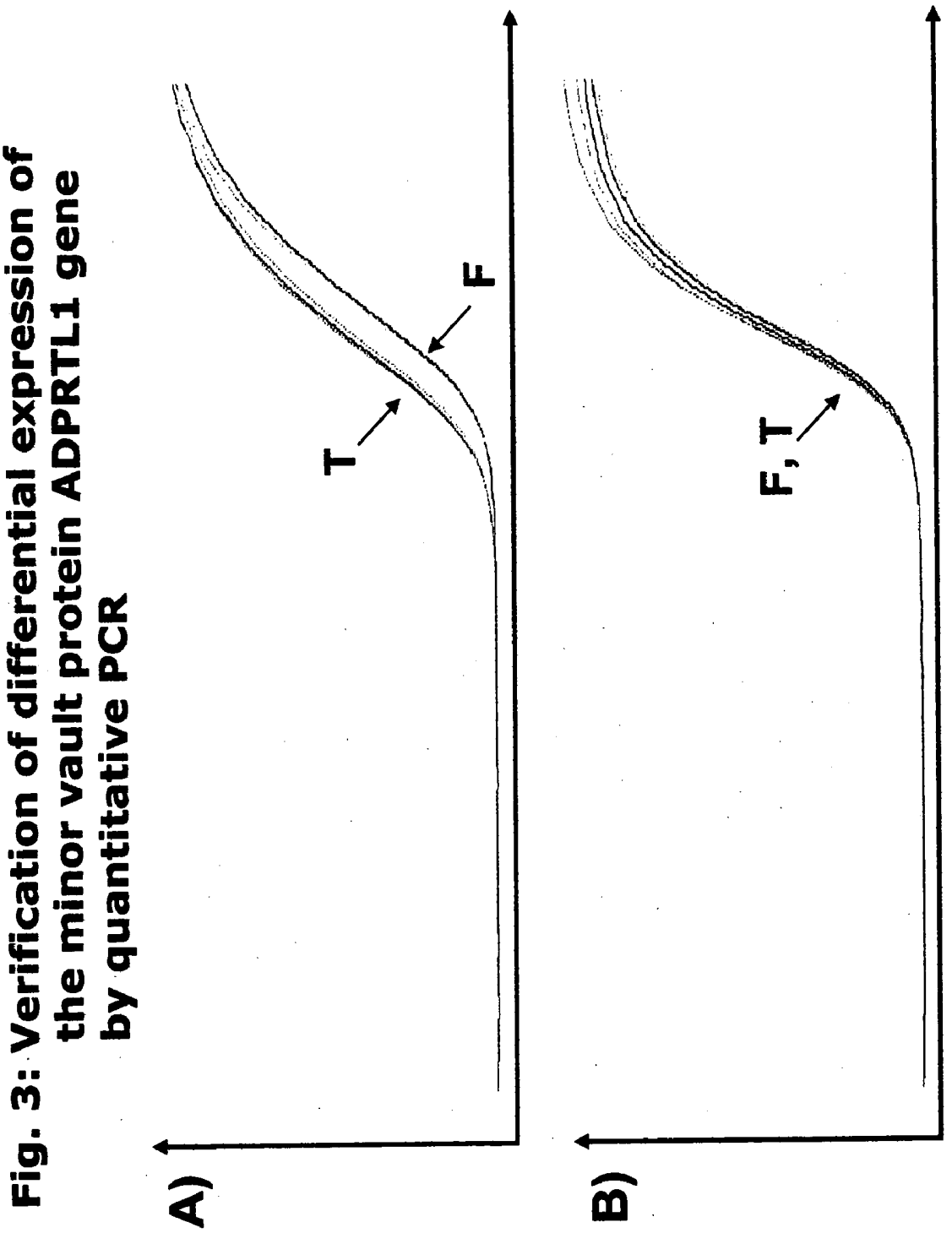

Fig. 3: Verification of differential expression of the minor vault protein ADPRTL1 gene by quantitative PCR

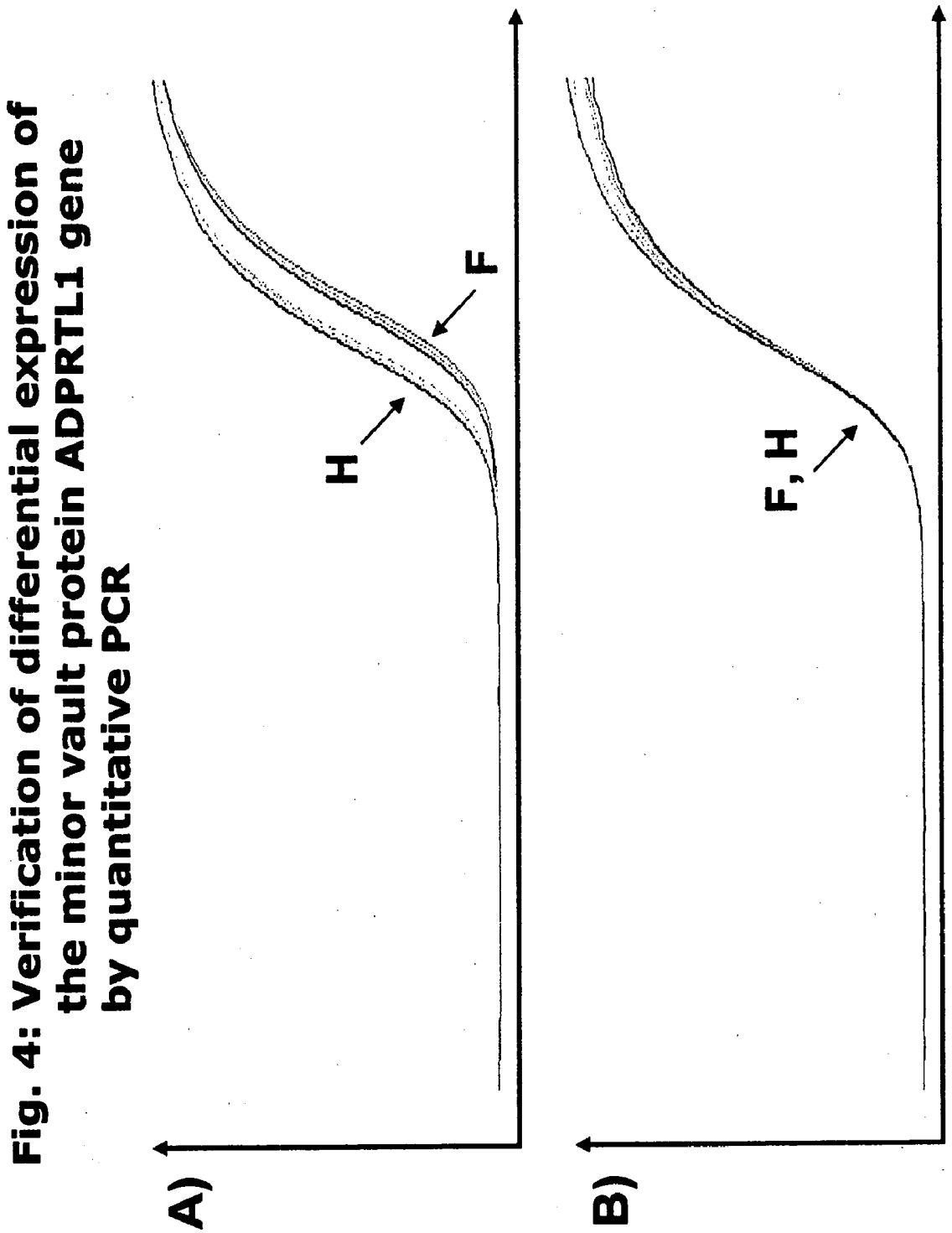

Fig. 4: Verification of differential expression of the minor vault protein ADPRTL1 gene by quantitative PCR

Figure 5: Nucleotide sequence of SEQ ID NO. 1

Length: 35 bp

1 AATCTAGGAA TATTCCCTGG GCTTTTGAGG CAATC

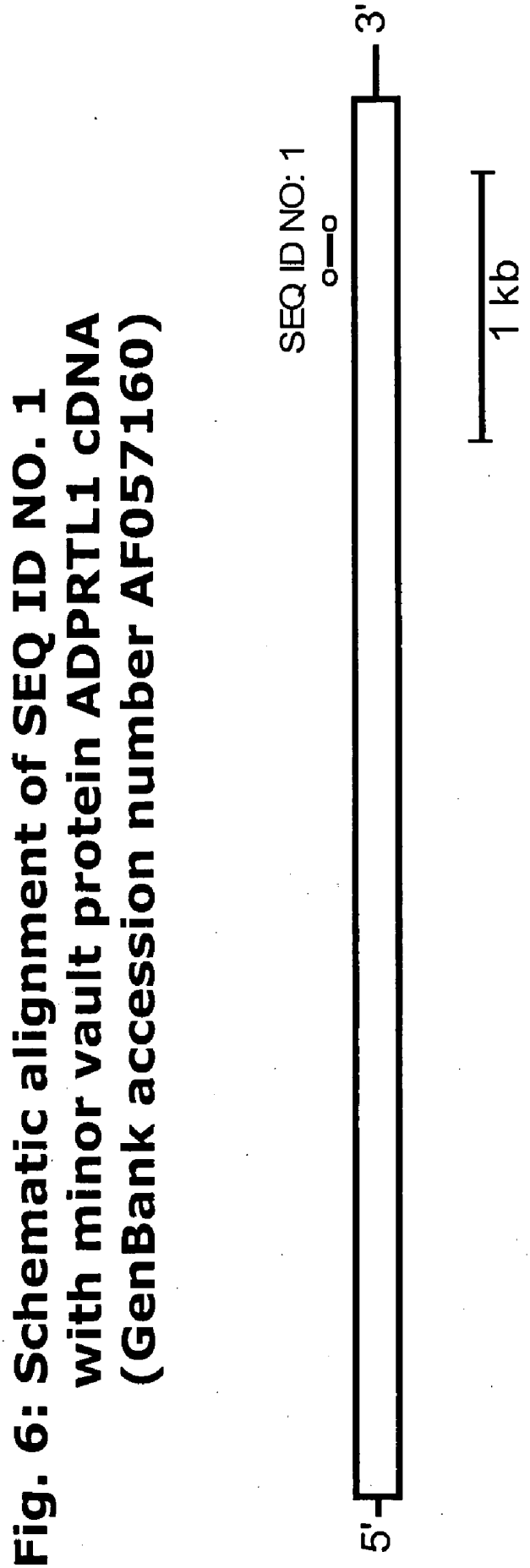

Fig. 6: Schematic alignment of SEQ ID NO. 1 with minor vault protein ADPRTL1 cDNA (GenBank accession number AF057160)

Fig. 7: Alignment of SEQ ID NO.1 with minor vault protein ADPRTL1 cDNA (AF057160)

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   1 TCTAGGAATATTCCCTGGGCTTTTGAGGCAAT 33
     || ||||||||||||||||||||||||||||||
5027 TCCAGGAATATTCCCTGGGCTTTTGAGGCAAT 5058
```

Fig. 8: SEQ ID NO. 2: amino acid sequence of human minor vault protein ADPRTL1

Length: 1724 aa

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   1  MVMGIFANCI FCLKVKYLPQ QQKKKLQTDI KENGGKFSFS LNPQCTHIIL
  51  DNADVLSQYQ LNSIQKNHVH IANPDFIWKS IREKRLLDVK NYDPYKPLDI
 101  TPPPDQKASS SEVKTEGLCP DSATEEEDTV ELTEFGMQNV EIPHLPQDFE
 151  VAKYNTLEKV GMEGGQEAVV VELQCSRDSR DCPFLISSHF LLDDGMETRR
 201  QFAIKKTSED ASEYFENYIE ELKKQGFLLR EHFTPEATQL ASEQLQALLL
 251  EEVMNSSTLS QEVSDLVEMI WAEALGHLEH MLLKPVNRIS LNDVSKAEGI
 301  LLLVKAALKN GETAEQLQKM MTEFYRLIPH KGTMPKEVNL GLLAKKADLC
 351  QLIRDMVNVC ETNLSKPNPP SLAKYRALRC KIEHVEQNTE EFLRVRKEVL
 401  QNHHSKSPVD VLQIFRVGRV NETTEFLSKL GNVRPLLHGS PVQNIVGILC
 451  RGLLLPKVVE DRGVQRTDVG NLGSGIYFSD SLSTSIKYSH PGETDGTRLL
 501  LICDVALGKC MDLHEKDFSL TEAPPGYDSV HGVSQTASVT TDFEDDEFVV
 551  YKTNQVKMKY IIKFSMPGDQ IKDFHPSDHT ELEEYRPEFS NFSKVEDYQL
 601  PDAKTSSSTK AGLQDASGNL VPLEDVHIKG RIIDTVAQVI VFQTYTNKSH
 651  VPIEAKYIFP LDDKAAVCGF EAFINGKHIV GEIKEKEEAQ QEYLEAVTQG
 701  HGAYLMSQDA PDVFTVSVGN LPPKAKVLIK ITYITELSIL GTVGVFFMPA
 751  TVAPWQQDKA LNENLQDTVE KICIKEIGTK QSFSLTMSIE MPYVIEFIFS
 801  DTHELKQKRT DCKAVISTME GSSLDSSGFS LHIGLSAAYL PRMWVEKHPE
 851  KESEACMLVF QPDLDVDLPD LASESEVIIC LDCSSSMEGV TFLQAKQIAL
 901  HALSLVGEKQ KVNIIQFGTG YKELFSYPKH ITSNTAAAEF IMSATPTMGN
 951  TDFWKTLRYL SLLYPARGSR NILLVSDGHL QDESLTLQLV KRSRPHTRLF
1001  ACGIGSTANR HVLRILSQCG AGVFEYFNAK SKHSWRKQIE DQMTRLCSPS
1051  CHSVSVKWQQ LNPDAPEALQ APAQVPSLFR NDRLLVYGFI PHCTQATLCA
1101  LIQEKEFCTM VSTTELQKTT GTMIHKLAAR ALIRDYEDGI LHENETSHEM
1151  KKQTLKSLII KLSKENSLIT QFTSFVAVEK RDENESPFPD IPKVSELIAK
1201  EDVDFLPYMS WQGEPQEAVR NQSLLASSEW PELRLSKRKH RKIPFSKRKM
1251  ELSQPEVSED FEEDGLGVLP AFTSNLERGG VEKLLDLSWT ESCKPTATEP
1301  LFKKVSPWET STSSFFPILA PAVGSYLTPT TRAHSPASLS FASYRQVASF
1351  GSAAPPRQFD ASQFSQGPVP GTCADWIPQS ASCPTGPPQN PPSAPYCGIV
1401  FSGSSLSSAQ SAPLQHPGGF TTRPSAGTFP ELDSPQLHFS LPTDPDPIRG
1451  FGSYHPSAYS PFHFQPSAAS LTANLRLPMA SALPEALCSQ SRTTPVDLCL
1501  LEESVGSLEG SRCPVFAFQS SDTESDELSE VLQDSCFLQI KCDTKDDSIP
1551  CFLEVKEEDE IVCTQHWQDA VPWTELLSLQ TEDGFWKLTP ELGLILNLNT
1601  NGLHSFLKQK GIQSLGVKGR ECLLDLIATM LVLQFIRTRL EKEGIVFKSL
1651  MKMDDPSISR NIPWAFEAIK QASEWVRRTE GQYPSICPRL ELGNDWDSAT
1701  KQLLGLQPIS TVSPLHRVLH YSQG
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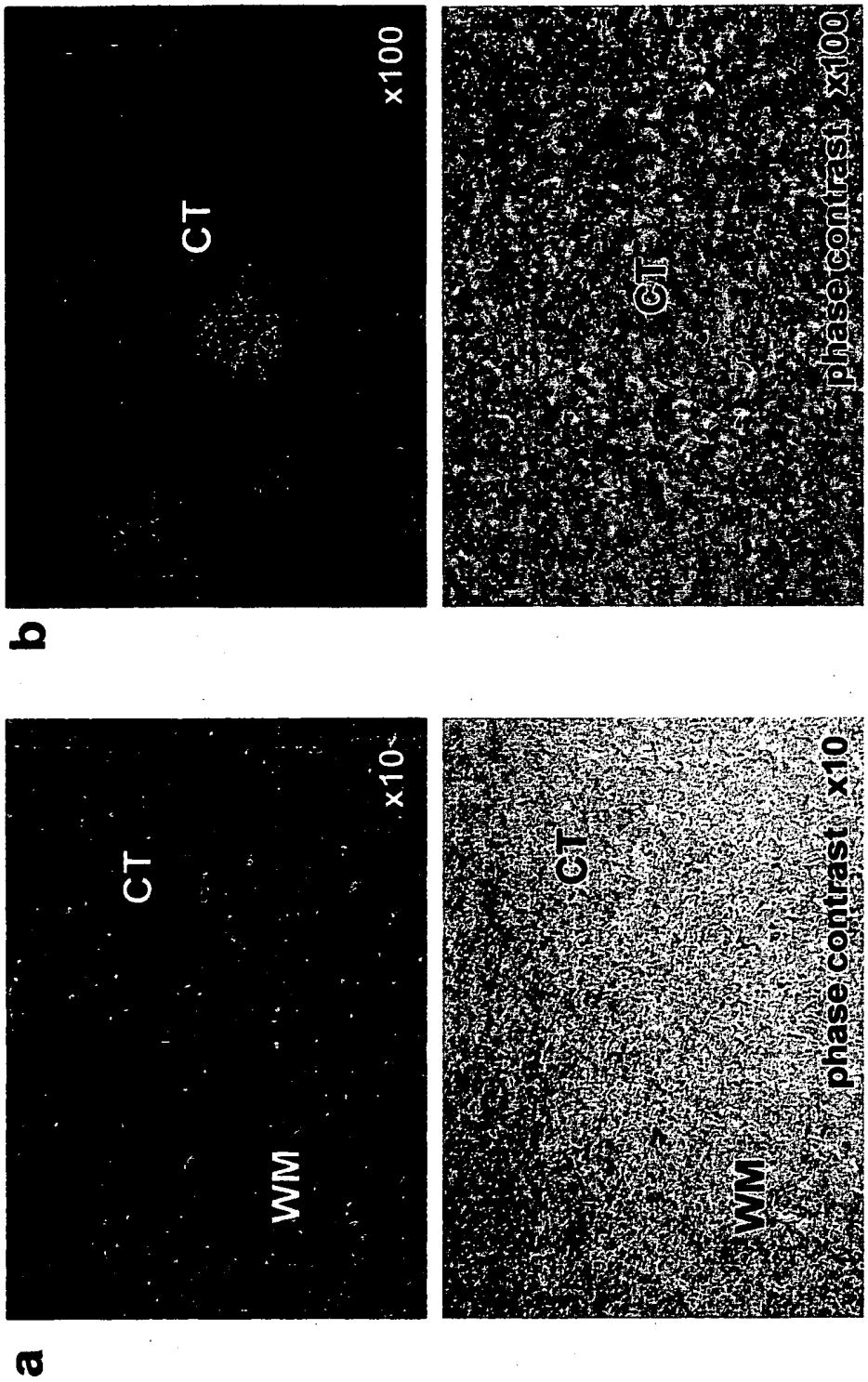


Fig. 9: Images of the human cerebral cortex labeled with anti-ADPRTL1 monoclonal antibody and with DAPI

Table 1 :

sample	Δ (fold) (temporal / frontal cortex)
control C011	0.77
control C012	0.98
control C014	0.93
control C005	1.02
control C008	1.23
patient P012	0.91
patient P016	1.62
patient P010	0.91
patient P011	1.69
patient P014	1.33
patient P017	1.53
patient P019	1.55

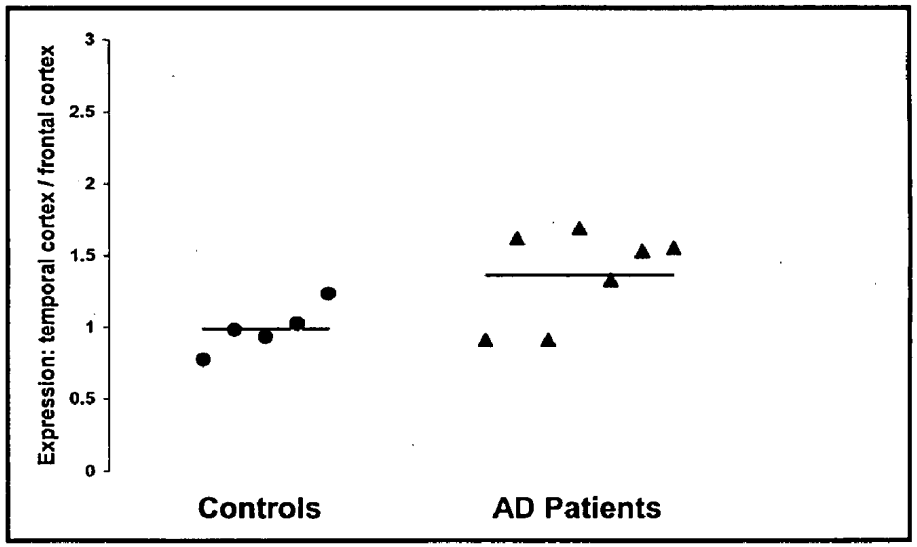
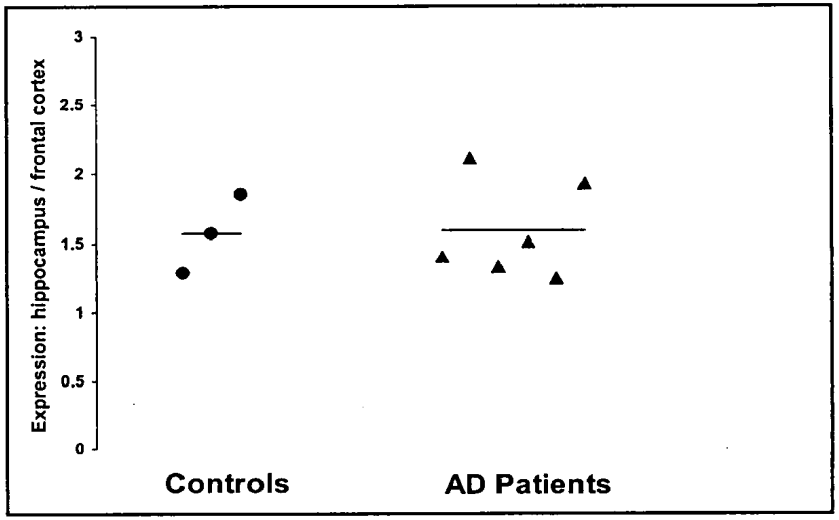


Table 2:

sample	Δ (fold) (hippocampus / frontal cortex)
control C005	1.28
control C008	1.57
control C004	1.86
patient P012	1.40
patient P016	2.11
patient P010	1.32
patient P011	1.51
patient P014	1.24
patient P019	1.93



DIAGNOSTIC AND THERAPEUTIC USE OF VAULT POLYNUCLEOTIDES AND PROTEINS FOR NEURODEGENERATIVE DISEASES

[0001] The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

[0002] Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. AD is the most common neurodegenerative disease, accounting for about 70% of all dementia cases, and it is probably the most devastating age-related neurodegenerative condition affecting about 10% of the population over 65 years of age and up to 45% over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles.

[0003] The amyloid-β (Aβ) protein evolves from the cleavage of the amyloid precursor protein (APP) by different kinds of proteases. The cleavage by the β/γ-secretase leads to the formation of Aβ peptides of different lengths, typically a short more soluble and slow aggregating peptide consisting of 40 amino acids and a longer 42 amino acid peptide, which rapidly aggregates outside the cells, forming the characteristic amyloid plaques (Selkoe, *Physiological Rev* 2001, 81: 741-66; Greenfield et al., *Frontiers Bioscience* 2000, 5: D72-83). Two types of plaques, diffuse plaques and neuritic plaques, can be detected in the brain of AD patients, the latter ones being the classical, most prevalent type. They are primarily found in the cerebral cortex and hippocampus. The neuritic plaques have a diameter of 50 μm to 200 μm and are composed of insoluble fibrillar amyloids, fragments of dead neurons, of microglia and astrocytes, and other components such as neurotransmitters, apolipoprotein E, glycosaminoglycans, α1-antichymotrypsin and others. The generation of toxic Aβ deposits in the brain starts very early in the course of AD, and it is discussed to be a key player for the subsequent destructive processes leading to AD pathology. The other pathological hallmarks of AD are neurofibrillary tangles (NFTs) and abnormal neurites, described as neuropil threads (Braak and Braak, *Acta Neuropathol* 1991, 82: 239-259). NFTs emerge inside neurons and consist of chemically altered tau, which forms paired helical filaments twisted around each other. Along the formation of NFTs, a loss of neurons can be observed. It is discussed that said neuron loss may be due to a damaged microtubule-associated transport system (Johnson and Jenkins, *J Alzheimers Dis* 1996, 1: 38-58; Johnson and Hartigan, *J Alzheimers Dis* 1999, 1: 329-351). The appearance of neurofibrillary tangles and their increasing number correlates well with the clinical severity of AD (Schmitt et al., *Neurology* 2000, 55: 370-376).

[0004] AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. The cognitive disturbances include among other things memory impairment, aphasia, agnosia and the loss of executive functioning. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-92).

[0005] The age of onset of AD may vary within a range of 50 years, with early-onset AD occurring in people younger than 65 years of age, and late-onset of AD occurring in those older than 65 years. About 10% of all AD cases suffer from early-onset AD, with only 1-2% being familial, inherited cases.

[0006] Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon 4 allele of the three different existing alleles (epsilon 2, 3, and 4) of the apolipoprotein E gene (ApoE) (Strittmatter et al., *Proc Natl Acad Sci USA* 1993, 90: 1977-81; Roses, *Ann NY Acad Sci* 1998, 855: 738-43). The polymorphic plasmaprotein ApoE plays a role in the intercellular cholesterol and phospholipid transport by binding low-density lipoprotein receptors, and it seems to play a role in neurite growth and regeneration. Efforts to detect further susceptibility genes and disease-linked polymorphisms, lead to the assumption that specific regions and genes on human chromosomes 10 and 12 may be associated with late-onset AD (Myers et al., *Science* 2000, 290: 2304-5; Bertram et al., *Science* 2000, 290: 2303; Scott et al., *Am J Hum Genet* 2000, 66: 922-32).

[0007] Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP) on chromosome 21, presenilin-1 on chromosome 14, and presenilin-2 on chromosome 1, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The mutations found to date account for only half of the familial AD cases, which is less than 2% of all AD patients. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, agents, compositions, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

[0008] Vaults are barrel-shaped ribonucleoprotein complexes of 13 Mega Dalton molecular weight. They are

composed of three protein species and an untranslated RNA molecule called vault or vRNA. The three vault proteins are named major vault protein (or, alternatively, MVP, LRP, p100), minor vault protein TEP1 (p240), and minor vault protein ADPRTL1 (VPARP, PHP5, p193). The major vault protein is present in 96 copies per vault particle, and the minor vault proteins TEP1 and ADPRTL1 are found in 2 and 8 copies per particle, respectively (Kong et al., *RNA* 2000, 6: 890-900; Scheffer et al., *Curr. Opin. Oncol.* 2000, 12: 550-556). Vaults of nearly identical size and composition have been found in species as diverse as mammals, avians, amphibians, and the slime mold *Dictyostelium discoideum*. In spite of this evolutionary conserved and therefore apparently important function the exact cellular role of vaults is far from being understood. Several studies have implicated vaults in nucleocytoplasmic transport of different substrates including steroid hormone receptors and ribosomal particles (Scheffer et al., *Curr. Opin. Oncol.* 2000, 12: 550-556). Tissue distribution studies of vault components have shown higher levels of vaults in tissues that are chronically exposed to elevated levels of xenobiotics, in metabolically active tissue and in macrophages. An up-regulation of vault expression during the differentiation and maturation of human monocyte-derived dendritic cells, known as xenobiotic scavenging cells, has been observed in vitro (Scheffer et al., *Curr. Opin. Oncol.* 2000, 12: 550-556). Collectively, this argues for a prominent role of vaults in detoxification of tissues (Kickhoefer et al., *J. Cell Biol.* 1999, 146: 917-928; Scheffer et al., *Curr. Opin. Oncol.* 2000, 12: 550-556). Of outstanding medical relevance was the finding that vaults are overexpressed in multidrug-resistant cancer cell lines and expression levels of vault components correlated with the extent of drug resistance, again arguing for a role in transport of xenobiotics and tissue detoxification (Schoeijers et al., *Cancer Res.* 2000, 60: 1104-1110; Siva et al., *Int. J. Cancer* 2001, 92: 195-202; Scheffer et al., *Curr. Opin. Oncol.* 2000, 12: 550-556). In fact, the major vault protein has also been coined LRP, which stands for lung resistance-related protein (Scheper et al., *Cancer Res.* 1993, 53: 1475-1479).

[0009] While the major vault protein is thought to play a more structural role in particle assembly, the minor vault proteins seem to play more active, enzymatic roles. The minor vault protein TEP1 is shared with so called telomerase complexes, other ribonucleoprotein complexes essential for the maintenance of the length of the chromosomal telomeres in dividing cells (Kickhoefer et al., *J. Biol. Chem.* 1999, 274: 32712-32717; Collins, *Curr. Opin. Cell Biol.* 2000, 12: 378-383). However, thus far it has not been demonstrated that vaults have telomerase activity (Kickhoefer et al., *J. Biol. Chem.* 1999, 274: 32712-32717). The other minor vault protein, ADPRTL1, also called p193, offers a number of interesting homologies to well known cellular factors: an amino-terminal BRCT domain shared with proteins active in DNA repair, a poly (ADP-ribose) polymerase domain that may hold a function in cellular differentiation, proliferation, tumor transformation, and recovery from DNA damage, an inter-alpha-trypsin inhibitor domain, a putative nuclear localization signal, and a carboxy-terminal domain for interaction with the major vault protein (Still et al., *Genomics* 1999, 62: 533-536; Kickhoefer et al., *J. Cell Biol.* 1999, 146: 917-928; Jean et al., *FEBS Lett.* 1999, 446: 6-8; Chiarugi, *Trends Pharmacol. Sci.* 2002, 23: 122-129). A patent application featuring purified human ADPRTL1/p193 nucleotide sequences, polypeptide sequences, and variants thereof, for the diagnosis and treatment of multidrug resistant cancer has been put forward (WO 99/62547). The nucleotide sequence of the gene coding for ADPRTL1 was initially determined by Still et al. (*Genomics* 1999, 62:533-536) and deposited in the GenBank database with the accession number AF057160. The ADPRTL1 gene codes for a polypeptide of 1724 amino acids in length with a predicted molecular weight of 193 kDa.

[0010] It is primarily the combination of the BRCT domain with the poly(ADP-ribose) polymerase domain that fuels interest in ADPRTL1. It was found that ADPRTL1 poly(ADP-ribosyl)ates itself and the major vault protein at the expense of nicotineamide adenine dinucleotide (Kickhoefer et al., *J. Cell Biol.* 1999, 146: 917-928). This activity resembles the enzymatic activity of a large family of poly-(ADP-ribose) polymerases or PARPs (Johansson, *Genomics* 1999, 57: 442-445; Chiarugi, *Trends Pharmacol. Sci.* 2002, 23: 122-129). PARPs sense DNA damage and participate in DNA excision repair. Upon binding to DNA strand breaks, PARPs polymerize nicotineamide adenine dinucleotides into branched polymers of ADP-ribose that are transferred to nuclear housekeeping proteins including DNA polymerase I and II, Ca^{2+}—Mg^{2+}-endonuclease, histones, chromatin-binding proteins, and the PARPs themselves (for review, Chiarugi, *Trends Pharmacol. Sci.* 2002, 23: 122-129). These modifications are thought to facilitate the repair process of the DNA. Excessive activation of PARPs may ultimately drive cells into energy crisis due to depletion of nicotineamide adenine dinucleotide pools and eventually elicit cell death. In fact, small-molecule inhibitors of PARPs hold therapeutic promise as anti-apoptotic drugs (Szabo et al., *Trends Pharmacol. Sci.* 1998, 19: 287-298; Pieper et al., *Trends Pharmacol. Sci.* 1999, 20: 171-181).

[0011] Kickhoefer and coworkers analysed the distribution of ADPRTL1/p193 messenger RNA in human tissues and found a prominent transcript in kidney, spleen, and liver but no transcript in brain (Kickhoefer et al., *J. Cell Biol.* 1999, 146: 917-928). In the present invention, using an unbiased and sensitive differential display approach, an ADPRTL1 transcript is detected in human brain samples. Importantly, the present invention discloses an up-regulation of ADPRTL1 transcripts in the inferior temporal lobe of brain samples taken from AD patients relative to frontal cortex samples. No such up-regulation is observed in samples from age-matched healthy controls. To date, no experiments have been described that show a relationship between a differential expression of the ADPRTL1 gene and the pathology of neurodegenerative diseases, particularly AD. Likewise, no experiments have been described that demonstrate a link between the dysregulation of vault gene expression and neurodegenerative disorders. Such a link offers new ways, inter alia, for the diagnosis and treatment of said disorders, in particular AD.

[0012] The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as

used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. "Dysregulation" shall mean an upregulation or downregulation of gene expression. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "ORF" is an acronym for "open reading frame" and refers to a nucleic acid sequence that does not possess a stop codon in at least one reading frame and therefore can potentially be translated into a sequence of amino acids. "Regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids, natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or anorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or "primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. As used herein, "homolog or homology" is a term used in the art to describe the relatedness of a nucleotide or peptide sequence to another nucleotide or peptide sequence, which is determined by the degree of identity and/or similarity between said sequences compared. The term "variant" as used herein refers to any polypeptide or protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention. Furthermore, the term "variant" shall include any shorter or longer version of a polypeptide or protein. "Variants" shall also comprise a sequence that has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of the vault protein. "Variants" of a protein molecule include, for example, proteins with conservative amino acid substitutions in highly conservative regions. "Proteins and polypeptides" of the present invention include variants, fragments and chemical derivatives of the protein comprising the amino acid sequence of SEQ ID NO. 2. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants. The term "isolated" as used herein is considered to refer to molecules that are removed from their natural environment, i.e. isolated from a cell or from a living organism in which they normally occur, and that are separated or essentially purified from the coexisting components with which they are found to be associated in nature. This notion further means that the sequences encoding such molecules can be linked by the hand of man to polynucleotides, to which they are not linked in their natural state, and that such molecules can be produced by recombinant and/or synthetic means. Even if for said purposes those sequences may be introduced into living or non-living organisms by methods known to those skilled in the art, and even if those sequences are still present in said organisms, they are still considered to be isolated. In the present invention, the terms "risk", "susceptibility", and "predisposition" are tantamount and are used with respect to the probability of developing a neurodegenerative disease, preferably Alzheimer's disease.

[0013] The term 'AD' shall mean Alzheimer's disease. "AD-type neuropathology" as used herein refers to neuropathological, neurophysiological, histopathological and clinical hallmarks as described in the instant invention and as commonly known from state-of-the-art literature (see:

Iqbal, Swaab, Winblad and Wisniewski, *Alzheimer's Disease and Related Disorders* (*Etiology, Pathogenesis and Therapeutics*), Wiley & Sons, New York, Weinheim, Toronto, 1999; Scinto and Daffner, *Early Diagnosis of Alzheimer's Disease*, Humana Press, Totowa, N.J., 2000; Mayeux and Christen, *Epidemiology of Alzheimer's Disease: From Gene to Prevention*, Springer Press, Berlin, Heidelberg, New York, 1999; Younkin, Tanzi and Christen, *Presenilins and Alzheimer's Disease*, Springer Press, Berlin, Heidelberg, New York, 1998). Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, argyrophilic grain dementia and other tauopathies, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, age-related macular degeneration, narcolepsy, motor neuron diseases, prion diseases, traumatic nerve injury and repair, and multiple sclerosis.

[0014] In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

[0015] The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences, or fragments, or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and/or variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects. Whether an amplification results in a specific nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimer's disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

[0016] In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

[0017] In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

[0018] In a preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said gene coding for the vault protein is the gene coding for the minor vault protein particularly minor vault protein ADPRTL1, also termed p193. And it is preferred that said vault protein is a minor vault protein, particularly minor vault protein ADPRTL1, also called p193, SEQ ID NO. 2.

[0019] In a further preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said neurodegenerative disease or disorder is Alzheimer's disease, and said subjects suffer from Alzheimer's disease.

[0020] The present invention discloses the differential expression and regulation of the minor vault protein ADPRTL1 gene in specific brain regions of AD patients. Consequently, the minor vault protein ADPRTL1 gene and its corresponding translation products may have a causative role in the regional selective neuronal degeneration typically observed in AD. Alternatively, the minor vault protein ADPRTL1 may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular AD. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

[0021] It is particularly preferred that said sample to be analyzed and determined is selected from the group comprising brain tissue or other tissues or body cells. The sample can also comprise cerebrospinal fluid or other body fluids including saliva, urine, serum plasma, or mucus. Preferably, the methods of diagnosis, prognosis, monitoring the progression or evaluating a treatment for a neurodegenerative disease, according to the instant invention, can be practiced ex corpore, and such methods preferably relate to samples,

for instance, body fluids or cells, removed, collected, or isolated from a subject or patient.

[0022] In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

[0023] In preferred embodiments, an alteration in the level and/or activity of a transcription product of the gene coding for ADPRTL1 and/or a translation product of the gene coding for ADPRTL1 in a sample cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly AD.

[0024] In preferred embodiments, measurement of the level of transcription products of a gene coding for a vault protein is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based micro-array technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, Mass., 2000). An example of an immunoassay is the detection and measurement of enzyme activity as disclosed and described in the patent application WO 02/14543.

[0025] Furthermore, a level and/or an activity of a translation product of a gene coding for a vault protein and/or a fragment, or derivative, or variant of said translation product, and/or the level of activity of said translation product, and/or a fragment, or derivative, or variant thereof, can be detected using an immunoassay, an activity assay, and/or a binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999 and Edwards R, *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford; England, 1999). All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, Mass., 2000).

[0026] In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of a gene coding for a vault protein, and/or of (ii) a translation product of a gene coding for a vault protein, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

[0027] In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular AD, in a subject, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular AD, said kit comprising:

[0028] (a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for a vault protein (ii) reagents that selectively detect a translation product of a gene coding for a vault protein; and

[0029] (b) instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of a subject to develop such a disease by

[0030] detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of a gene coding for a vault protein, in a sample from said subject; and

[0031] diagnosing or prognosticating a neurodegenerative disease, in particular AD, or determining the propensity or predisposition of said subject to develop such a disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular AD, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular AD. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular AD in a subject, as well as monitoring success or failure of therapeutic treatment for such a disease of said subject.

[0032] In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly

affect a level, or an activity, or both said level and said activity, of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence of a translation product of a gene coding for a vault protein, or a fragment, or derivative, or a variant thereof. An agent for treating or preventing a neurodegenerative disease, in particular AD, according to the instant invention, may also consist of a nucleotide, an oligonucleotide, or a polynucleotide. Said oligonucleotide or polynucleotide may comprise a nucleotide sequence of the gene coding for a vault protein, either in sense orientation or in antisense orientation.

[0033] In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

[0034] In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514; the contents of which are incorporated herein by reference). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against transcripts of a gene coding for a vault protein, particularly the minor vault protein ADPRTL1. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligo-deoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed ex vivo with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy. A recently developed method of regulating the intracellular expression of genes by the use of double-stranded RNA, known variously as RNA interference (RNAi), can be another effective approach for nucleic acid therapy (Hannon, *Nature* 2002, 418: 244-251).

[0035] In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection or liposomal mediated transfection (see Mc Celland and Pardee, *Expression Genetics: Accelerated and High-Throughput Methods*, Eaton Publishing, Natick, Mass., 1999).

[0036] In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular AD, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated in vitro to insert a DNA segment encoding said therapeutic protein, said subject cells expressing in vivo in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells in vitro by a viral vector, in particular a retroviral vector.

[0037] Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson D A, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the

germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

[0038] In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an AD-type neuropathology.

[0039] In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii).

[0040] In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

[0041] In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for use in a pharmaceutical composition.

[0042] In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular AD.

[0043] In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

[0044] In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for a vault protein, or a fragment, or a derivative thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular AD. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Jackson and Abbott, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England, 1999). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular AD. Such an animal may be useful for screening, testing and validating compounds, agents and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease. In preferred embodiments, said recombinant, non-human animal comprises a non-native gene sequence coding for a vault protein, in particular the non-native minor vault protein ADPRTL1 gene sequence, or a fragment thereof.

[0045] In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

[0046] In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular AD, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for a vault protein, and/or (ii) a transcription product of a gene coding for a vault protein, and/or (iii) a translation product of a gene coding for a vault protein, and/or (iv) a fragment, or derivative, or variant of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed symptoms of said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level

of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

[0047] In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses a gene coding for a vault protein, or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional regulatory element which is not the native vault protein gene transcriptional control regulatory element.

[0048] In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

[0049] In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and a vault protein, or a fragment, or derivative, or variant thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said vault protein, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said vault protein, or said fragment, or derivative, or variant thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said vault protein, or with said fragment, or derivative, or variant thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said vault protein, or said fragment, or derivative, or variant thereof. Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of a gene coding for a vault protein, or a fragment, or derivative, or variant thereof. One example of a fluorescent binding assay, in this case based on the use of carrier particles, is disclosed and described in patent application WO 00/52451. A further example is the competitive assay method as described in patent WO 02/01226. Preferred signal detection methods for screening assays of the instant invention are described in the following patent applications: WO 96/13744, WO 98/16814, WO 98/23942, WO 99/17086, WO 99/34195, WO 00/66985, WO 01/59436, WO 01/59416.

[0050] In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of a gene coding for a vault protein by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

[0051] In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a vault protein, or to a fragment, or derivative, or variant thereof. Said screening assay comprises (i) adding a liquid suspension of said vault protein, or a fragment, or derivative, or variant thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said vault protein, or said fragment, or derivative, or variant thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said vault protein, or with said fragment, or derivative, or variant thereof, and (v) determining the degree of binding by one or more of said compounds to said vault protein, or said fragment, or derivative, or variant thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a vault protein, or a fragment, or derivative, or variant thereof.

[0052] In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of a gene coding for a vault protein by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

[0053] In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

[0054] The present invention features a protein molecule shown in SEQ ID NO. 2, said protein molecule being a translation product of the gene coding for a vault protein, in particular the minor vault protein ADPRTL1, or a fragment, or derivative, or variant thereof, for use as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

[0055] The present invention further features a protein molecule shown in SEQ ID NO. 2, said protein molecule being a translation product of the gene coding for a vault protein, in particular the minor vault protein ADPRTL1, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

[0056] In all types of assays disclosed herein it is preferred to study a vault protein. It is particularly preferred to conduct screening assays with the minor vault protein ADPRTL1.

[0057] The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of a gene coding for a vault protein, in particular the minor vault protein ADPRTL1, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation

product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof (see Dubel and Breitling, *Recombinant Antibodies*, Wiley-Liss, New York, N.Y., 1999). Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods, based on state-in-the-art techniques (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999 and Edwards R., *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford, England, 1999) such as enzyme-immuno assays (e.g. enzyme-linked immunosorbent assay, ELISA), radioimmuno assays, chemoluminescence-immuno assays, Western-blot, immunoprecipitation and antibody microarrays. These methods involve the detection of translation products of a gene coding for a vault protein, in particular the minor vault protein ADPRTL1.

[0058] In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to AD. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in U.S. Pat. No. 6,150,173.

[0059] Other features and advantages of the invention will be apparent from the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

[0060] **FIG. 1** depicts the brain regions with selective vulnerability to neuronal loss and degeneration in AD. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in AD (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in AD. Brain tissues from the frontal cortex (F), the temporal cortex (T) and the hippocampus (H) of AD patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p. 4).

[0061] **FIG. 2** discloses the initial identification of the differential expression of the human gene coding for minor vault protein ADPRTL1 in a fluorescence differential display screen. The figure shows a clipping of a large preparative fluorescent differential display gel. PCR products from the frontal cortex (F) and the temporal cortex (T) of two healthy control subjects and six AD patients were loaded in duplicate onto a denaturing polyacrylamide gel (from left to right). PCR products were obtained by amplification of the individual cDNAs with the corresponding one-base-anchor oligonucleotide and the specific Cy3 labelled random primers. The arrow indicates the migration position where significant differences in intensity of the signals for human minor vault protein ADPRTL1 transcript derived from frontal cortex as compared to the signals derived from the temporal cortex of AD patients exist. The differential expression reflects an up-regulation of human minor vault protein ADPRTL1 gene transcription in the temporal cortex compared to the frontal cortex of AD patients. Comparing the signals derived from temporal cortex and frontal cortex of healthy non-AD control subjects with each other, no difference in signal intensity, i.e. no altered expression level can be detected.

[0062] **FIGS. 3 and 4** illustrate the verification of the differential expression of the minor vault protein ADPRTL1 gene in AD brain tissues by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and the temporal cortex (T) of AD patients (**FIG. 3***a*) and samples from the frontal cortex (F) and the hippocampus (H) of AD patients (**FIG. 4***a*) was performed by the LightCycler rapid thermal cycling technique. Likewise, samples of healthy, age-matched control individuals were compared (**FIG. 3***b* for frontal cortex and temporal cortex, **FIG. 4***b* for frontal cortex and hippocampus). The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for the ribosomal protein S9, cyclophilin B, the transferrin receptor, GAPDH, and beta-actin. The figures depict the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of the minor vault protein ADPRTL1 cDNA from both, the frontal and temporal cortices of a normal control individual, and from the frontal cortex and hippocampus of a normal control individual, respectively, during the exponential phase of the reaction are juxtaposed (**FIGS. 3***b* and **4***b*, arrowheads), whereas in AD (**FIGS. 3***a* and **4***a*, arrowheads) there is a significant separation of the corresponding curves, indicating an up-regulation of the minor vault protein ADPRTL1 mRNA expression in temporal cortex relative to frontal cortex in the respective analyzed brain regions.

[0063] **FIG. 5** depicts SEQ ID NO. 1, the nucleotide sequence of the 35 bp minor vault protein ADPRTL1 cDNA fragment, identified and obtained by fluorescence differential display and subsequent cloning.

[0064] **FIG. 6** charts the schematic alignment of SEQ ID NO. 1 to the nucleotide sequence of the minor vault protein

ADPRTL1 cDNA (GenBank accession number AF057160). The open rectangle represents the minor vault protein ADPRTL1 open reading frame, thin bars represent the 5' and 3' untranslated regions (UTRs).

[0065] **FIG. 7** outlines the sequence alignment of SEQ ID NO. 1 to the nucleotide sequence of the minor vault protein ADPRTL1 cDNA (GenBank accession number AF057160).

[0066] **FIG. 8** discloses SEQ ID NO. 2, the amino acid sequence of the minor vault protein ADPRTL1 (GenBank accession number Q9UKK3). The full-length human minor vault protein ADPRTL1 comprises 1724 amino acids.

[0067] **FIG. 9** depicts human cerebral cortex labeled with anti-ADPRTL1 mouse monoclonal antibodies (green signals). Immunoreactivity of ADPRTL1 was detected in the pre-central cortex (CT) as well as in the white matter (WM) (**FIG. 9*a***, low magnification). The cortex showed punctate immunoreactive signals of ADPRTL1 which were detected in both neuronal and glial cytoplasm (**FIG. 9*b***, high magnification). The white matter exhibited immunopositive signals in the cytoplasm of many glial cells. Blue signals indicate nuclei stained with DAPI.

[0068] Table 1 lists the gene expression levels in the temporal cortex relative to the frontal cortex for the minor vault protein ADPRTL1 gene in seven AD patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P017, P019 (0.91 to 1.69 fold) and five healthy, age-matched control individuals, herein identified by internal reference numbers C005, C008, C011, C012, C014 (0.77 to 1.23 fold). The scatter diagram points up the single values and the mean average value (indicated by a solid line) of the temporal to frontal cortex regulation ratios in control samples (dots) and in AD patient samples (triangles), respectively.

[0069] Table 2 lists the minor vault protein ADPRTL1 gene expression levels in the hippocampus relative to the frontal cortex in six Alzheimer's disease patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P019 (1.24 to 2.11 fold) and three healthy, age-matched control individuals, herein identified by internal reference numbers C004, C005, C008 (1.28 to 1.86 fold). The scatter diagram points up the single values and the mean average value (indicated by a solid line) of the hippocampus to frontal cortex regulation ratios in control samples (dots) and in AD patient samples (triangles), respectively.

EXAMPLE I

(i) Brain Tissue Dissection from Patients with AD:

[0070] Brain tissues from AD patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see **FIG. 1**) and stored at −80° C. until RNA extractions were performed.

(ii) Isolation of Total mRNA:

[0071] Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were utilised to generate a melting curve with the LightCycler technology as described in the supplied protocol by the manufacturer (Roche).

(iii) cDNA Synthesis and Identification of Differentially Expressed Genes by Fluorescence Differential Display (FDD):

[0072] In order to identify changes in gene expression in different tissues we employed a modified and improved differential display (DD) screening method. The original DD screening method is known to those skilled in the art (Liang and Pardee, *Science* 1995, 267:1186-7). This technique compares two populations of RNA and provides clones of genes that are expressed in one population but not in the other. Several samples can be analyzed simultaneously and both up- and down-regulated genes can be identified in the same experiment. By adjusting and refining several steps in the DD method as well as modifying technical parameters, e.g. increasing redundancy, evaluating optimized reagents and conditions for reverse transcription of total RNA, optimizing polymerase chain reactions (PCR) and separation of the products thereof, a technique was developed which allows for highly reproducible and sensitive results. The applied and improved DD technique was described in detail by von der Kammer et al. (*Nucleic Acids Research* 1999, 27: 2211-2218). A set of 64 specifically designed random primers were developed (standard set) to achieve a statistically comprehensive analysis of all possible RNA species. Further, the method was modified to generate a preparative DD slab-gel technique, based on the use of fluorescently labelled primers. In the present invention, RNA populations from carefully selected post-mortem brain tissues (frontal and temporal cortex) of AD patients and age-matched control subjects were compared.

[0073] As starting material for the DD analysis we used total RNA, extracted as described above (ii). Equal amounts of 0.05 μg RNA each were transcribed into cDNA in 20 μl reactions containing 0.5 mM each dNTP, 1 μl Sensiscript Reverse Transcriptase and 1× RT buffer (Qiagen), 10 U RNase inhibitor (Qiagen) and 1 μM of either one-base-anchor oligonucleotides $HT_{11}A$, $HT_{11}G$ or $HT_{11}C$ (Liang et al., *Nucleic Acids Research* 1994, 22: 5763-5764; Zhao et al., *Biotechniques* 1995, 18: 842-850). Reverse transcription was performed for 60 min at 37° C. with a final denaturation step at 93° C. for 5 min. 2 μl of the obtained cDNA each was subjected to a polymerase chain reaction (PCR) employing the corresponding one-base-anchor oligonucleotide (1 μM) along with either one of the Cy3 labelled random DD primers (1 μM), 1× GeneAmp PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$ (Applied Biosystems), 2 μM dNTP-Mix (dATP, dGTP, dCTP, dTTP Amersham Pharmacia Biotech), 5% DMSO (Sigma), 1 U AmpliTaq DNA Polymerase (Applied Biosystems) in a 20 μl final volume. PCR conditions were set as follows: one round at 94° C. for 30 sec for denaturing, cooling 1° C./sec down to 40° C., 40° C. for 4 min for low-stringency annealing of primer, heating 1° C./sec up to 72° C., 72° C. for 1 min for extension. This round was followed by 39 high-stringency cycles: 94° C. for 30 sec, cooling 1° C./sec down to 60° C., 60° C. for 2 min, heating 1° C./sec up to 72° C., 72° C. for 1 min. One final

step at 72° C. for 5 min was added to the last cycle (PCR cycler: Multi Cycler PTC 200, MJ Research). 8 μl DNA loading buffer were added to the 20 μl PCR product preparation, denatured for 5 min and kept on ice until loading onto a gel. 3.5 μl each were separated on 0.4 mm thick, 6%-polyacrylamide (Long Ranger)/7 M urea sequencing gels in a slab-gel system (Hitachi Genetic Systems) at 2000 V, 60 W, 30 mA, for 1 h 40 min. Following completion of the electrophoresis, gels were scanned with a FMBIO II fluorescence-scanner (Hitachi Genetic Systems), using the appropriate FMBIO II Analysis 8.0 software. A full-scale picture was printed, differentially expressed bands marked, excised from the gel, transferred into 1.5 ml containers, overlayed with 200 μl sterile water and kept at −20° C. until extraction.

[0074] Elution and reamplification of DD products: The differential bands were extracted from the gel by boiling in 200 μl H_2O for 10 min, cooling down on ice and precipitation from the supernatant fluids by using ethanol (Merck) and glycogen/sodium acetate (Merck) at −20° C. over night, and subsequent centrifugation at 13.000 rpm for 25 min at 4° C. Pellets were washed twice in ice-cold ethanol (80%), resuspended in 10 mM Tris pH 8.3 (Merck) and dialysed against 10% glycerol (Merck) for 1 h at room temperature on a 0.025 μm VSWP membrane (Millipore). The obtained preparations were used as templates for reamplification by 15 high-stringency cycles in 25-μl PCR mixtures containing the corresponding primer pairs as used for the DD PCR (see above) under identical conditions, with the exception of the initial round at 94° C. for 5 min, followed by 15 cycles of: 94° C. for 45 sec, 60° C. for 45 sec, ramp 1° C./sec to 70° C. for 45 sec, and one final step at 72° C. for 5 min.

[0075] Cloning and sequencing of DD products: Re-amplified cDNAs were analyzed with the DNA LabChip® system (Agilent 2100 Bioanalyzer, Agilent Technologies) and ligated into the pCR-Blunt II-TOPO vector and transformed into *E. coli* Top10F' cells (Zero Blunt TOPO PCR Cloning Kit, Invitrogen) according to the manufacturer's instructions. Cloned cDNA fragments were sequenced by commercially available sequencing facilities. The result of one such FDD experiment for the human minor vault protein ADPRTL1 gene is shown in **FIG. 2**.

(iv) Confirmation of Differential Expression by Quantitative RT-PCR:

[0076] Positive corroboration of differential expression of the human minor vault protein ADPRTL1 gene was performed using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint readout. The ratios of human minor vault protein ADPRTL1 cDNA from the temporal cortex and frontal cortex, and from the hippocampus and frontal cortex, respectively, were determined (relative quantification).

[0077] First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the human minor vault protein ADPRTL1 gene:

```
5'-GATGCTGTGCCTTGGACAGAA-3'
and

5'-TGGTGTAAGTTTCCAGAAGCCA-3'.
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[0078] PCR amplification (95° C. and 1 sec, 56° C. and 5 sec, and 72° C. and 5 sec) was performed in a volume of 20 μl containing LightCycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 μM primers, 2 μl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and, depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 82° C. with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 66 bp for the minor vault protein ADPRTL1 gene was observed in the electropherogram of the sample. In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTTTGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87° C. with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85° C. with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87° C. with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83° C. with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83° C. with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

[0079] For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for minor vault protein ADPRTL1 and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from temporal

cortex and frontal cortex, and from hippocampus and frontal cortex, respectively, were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10\hat{}((C_t \text{ value} - \text{intercept})/\text{slope})\ [\text{ng total brain cDNA}]$$

[0080] The values for temporal and frontal cortex cDNAs, and the values for hippocampus and frontal cortex cDNAs of the minor vault protein ADPRTL1, respectively, were normalized to cyclophilin B, and the ratios were calculated using the following formulas:

$$\text{Ratio} = \frac{\textit{ADPRTL1 temporal [ng]} / \textit{cyclophilin B temporal [ng]}}{\textit{ADPRTL1 frontal [ng]} / \textit{cyclophilin B frontal [ng]}}$$

$$\text{Ratio} = \frac{\textit{ADPRTL1 hippcampus [ng]} / \textit{cyclophilin B hippocampus [ng]}}{\textit{ADPRTL1 frontal [ng]} / \textit{cyclophilin B frontal [ng]}}$$

[0081] In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios, and of the hippocampal to frontal ratios, respectively, of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for the minor vault protein ADPRTL1 to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the respective ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of such quantitative RT-PCR analysis for the minor vault protein ADPRTL1 gene are shown in **FIGS. 3 and 4**.

(v) Immunohistochemistry:

[0082] For immunofluorescence staining of ADPRTL1 in human brain, frozen sections were prepared from post-mortem pre-central gyrus of a donor person (Cryostat Leica CM3050S) and fixed in 4% paraformaldehyde at room temperature for 20 min. After washing in PBS, the sections were pre-incubated with 20 mM glycine in PBS for 10 min and treated with 6 N guanidine-HCL in 50 mM Tris-HCL, pH 7.5 for 10 min. Afterwards, the sections were incubated with blocking buffer (10% normal goat serum, 0.2% Triton X-100 in PBS) for 30 min, and then incubated with anti-ADPRTL1 mouse monoclonal antibodies (1:20 diluted in blocking buffer; clone p193-6 from Chemicon International, Hofheim/Ts, Germany) overnight at 4° C. After rinsing three times in 0.1% Triton X-100/PBS, the sections were incubated with FITC-conjugated goat anti-mouse IgG (1:150 diluted in 1% BSA/PBS) for 2 hours at room temperature, and then again washed in PBS. Staining of the nuclei was performed by incubation of the sections with 5 μM DAPI in PBS for 3 min (blue signal). In order to block the autofluoresence of lipofuscin in human brain, the sections were treated with 1% Sudan Black B in 70% ethanol for 2-10 min at room temperature and sequentially dipped in 70% ethanol, destilled water, and PBS. The sections were coverslipped by 'Vectrashield mounting medium' (Vector Laboratories, Burlingame, Calif.) and observed under an inverted microscope (IX81, Olympus Optical). The digital images were captured with the appropriate software (AnalySiS, Olympus Optical).

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 14

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<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: ADPRTL1 cDNA fragment

<400> SEQUENCE: 1

aatctaggaa tattccctgg gcttttgagg caatc 35

<210> SEQ ID NO 2
<211> LENGTH: 1724
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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20 25 30

Asn Gly Gly Lys Phe Ser Phe Ser Leu Asn Pro Gln Cys Thr His Ile
35 40 45

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Ile Leu Asp Asn Ala Asp Val Leu Ser Gln Tyr Gln Leu Asn Ser Ile
     50                  55                  60

Gln Lys Asn His Val His Ile Ala Asn Pro Asp Phe Ile Trp Lys Ser
 65                  70                  75                  80

Ile Arg Glu Lys Arg Leu Leu Asp Val Lys Asn Tyr Asp Pro Tyr Lys
                 85                  90                  95

Pro Leu Asp Ile Thr Pro Pro Pro Asp Gln Lys Ala Ser Ser Ser Glu
            100                 105                 110

Val Lys Thr Glu Gly Leu Cys Pro Asp Ser Ala Thr Glu Glu Glu Asp
        115                 120                 125

Thr Val Glu Leu Thr Glu Phe Gly Met Gln Asn Val Glu Ile Pro His
    130                 135                 140

Leu Pro Gln Asp Phe Glu Val Ala Lys Tyr Asn Thr Leu Glu Lys Val
145                 150                 155                 160

Gly Met Glu Gly Gly Gln Glu Ala Val Val Val Glu Leu Gln Cys Ser
                165                 170                 175

Arg Asp Ser Arg Asp Cys Pro Phe Leu Ile Ser Ser His Phe Leu Leu
            180                 185                 190

Asp Asp Gly Met Glu Thr Arg Arg Gln Phe Ala Ile Lys Lys Thr Ser
        195                 200                 205

Glu Asp Ala Ser Glu Tyr Phe Glu Asn Tyr Ile Glu Glu Leu Lys Lys
    210                 215                 220

Gln Gly Phe Leu Leu Arg Glu His Phe Thr Pro Glu Ala Thr Gln Leu
225                 230                 235                 240

Ala Ser Glu Gln Leu Gln Ala Leu Leu Leu Glu Glu Val Met Asn Ser
                245                 250                 255

Ser Thr Leu Ser Gln Glu Val Ser Asp Leu Val Glu Met Ile Trp Ala
            260                 265                 270

Glu Ala Leu Gly His Leu Glu His Met Leu Leu Lys Pro Val Asn Arg
        275                 280                 285

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

Lys Ala Ala Leu Lys Asn Gly Glu Thr Ala Glu Gln Leu Gln Lys Met
305                 310                 315                 320

Met Thr Glu Phe Tyr Arg Leu Ile Pro His Lys Gly Thr Met Pro Lys
                325                 330                 335

Glu Val Asn Leu Gly Leu Leu Ala Lys Lys Ala Asp Leu Cys Gln Leu
            340                 345                 350

Ile Arg Asp Met Val Asn Val Cys Glu Thr Asn Leu Ser Lys Pro Asn
        355                 360                 365

Pro Pro Ser Leu Ala Lys Tyr Arg Ala Leu Arg Cys Lys Ile Glu His
    370                 375                 380

Val Glu Gln Asn Thr Glu Glu Phe Leu Arg Val Arg Lys Glu Val Leu
385                 390                 395                 400

Gln Asn His His Ser Lys Ser Pro Val Asp Val Leu Gln Ile Phe Arg
                405                 410                 415

Val Gly Arg Val Asn Glu Thr Thr Glu Phe Leu Ser Lys Leu Gly Asn
            420                 425                 430

Val Arg Pro Leu Leu His Gly Ser Pro Val Gln Asn Ile Val Gly Ile
        435                 440                 445
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Leu Cys Arg Gly Leu Leu Leu Pro Lys Val Val Glu Asp Arg Gly Val
    450                 455                 460

Gln Arg Thr Asp Val Gly Asn Leu Gly Ser Gly Ile Tyr Phe Ser Asp
465                 470                 475                 480

Ser Leu Ser Thr Ser Ile Lys Tyr Ser His Pro Gly Glu Thr Asp Gly
                485                 490                 495

Thr Arg Leu Leu Leu Ile Cys Asp Val Ala Leu Gly Lys Cys Met Asp
            500                 505                 510

Leu His Glu Lys Asp Phe Ser Leu Thr Glu Ala Pro Pro Gly Tyr Asp
        515                 520                 525

Ser Val His Gly Val Ser Gln Thr Ala Ser Val Thr Thr Asp Phe Glu
    530                 535                 540

Asp Asp Glu Phe Val Val Tyr Lys Thr Asn Gln Val Lys Met Lys Tyr
545                 550                 555                 560

Ile Ile Lys Phe Ser Met Pro Gly Asp Gln Ile Lys Asp Phe His Pro
                565                 570                 575

Ser Asp His Thr Glu Leu Glu Glu Tyr Arg Pro Glu Phe Ser Asn Phe
            580                 585                 590

Ser Lys Val Glu Asp Tyr Gln Leu Pro Asp Ala Lys Thr Ser Ser Ser
        595                 600                 605

Thr Lys Ala Gly Leu Gln Asp Ala Ser Gly Asn Leu Val Pro Leu Glu
    610                 615                 620

Asp Val His Ile Lys Gly Arg Ile Ile Asp Thr Val Ala Gln Val Ile
625                 630                 635                 640

Val Phe Gln Thr Tyr Thr Asn Lys Ser His Val Pro Ile Glu Ala Lys
                645                 650                 655

Tyr Ile Phe Pro Leu Asp Asp Lys Ala Ala Val Cys Gly Phe Glu Ala
            660                 665                 670

Phe Ile Asn Gly Lys His Ile Val Gly Glu Ile Lys Glu Lys Glu Glu
        675                 680                 685

Ala Gln Gln Glu Tyr Leu Glu Ala Val Thr Gln Gly His Gly Ala Tyr
    690                 695                 700

Leu Met Ser Gln Asp Ala Pro Asp Val Phe Thr Val Ser Val Gly Asn
705                 710                 715                 720

Leu Pro Pro Lys Ala Lys Val Leu Ile Lys Ile Thr Tyr Ile Thr Glu
                725                 730                 735

Leu Ser Ile Leu Gly Thr Val Gly Val Phe Phe Met Pro Ala Thr Val
            740                 745                 750

Ala Pro Trp Gln Gln Asp Lys Ala Leu Asn Glu Asn Leu Gln Asp Thr
        755                 760                 765

Val Glu Lys Ile Cys Ile Lys Glu Ile Gly Thr Lys Gln Ser Phe Ser
    770                 775                 780

Leu Thr Met Ser Ile Glu Met Pro Tyr Val Ile Glu Phe Ile Phe Ser
785                 790                 795                 800

Asp Thr His Glu Leu Lys Gln Lys Arg Thr Asp Cys Lys Ala Val Ile
                805                 810                 815

Ser Thr Met Glu Gly Ser Ser Leu Asp Ser Ser Gly Phe Ser Leu His
            820                 825                 830

Ile Gly Leu Ser Ala Ala Tyr Leu Pro Arg Met Trp Val Glu Lys His
        835                 840                 845

Pro Glu Lys Glu Ser Glu Ala Cys Met Leu Val Phe Gln Pro Asp Leu
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    850                 855                 860

Asp Val Asp Leu Pro Asp Leu Ala Ser Glu Ser Glu Val Ile Ile Cys
865                 870                 875                 880

Leu Asp Cys Ser Ser Ser Met Glu Gly Val Thr Phe Leu Gln Ala Lys
                885                 890                 895

Gln Ile Ala Leu His Ala Leu Ser Leu Val Gly Glu Lys Gln Lys Val
            900                 905                 910

Asn Ile Ile Gln Phe Gly Thr Gly Tyr Lys Glu Leu Phe Ser Tyr Pro
        915                 920                 925

Lys His Ile Thr Ser Asn Thr Ala Ala Ala Glu Phe Ile Met Ser Ala
    930                 935                 940

Thr Pro Thr Met Gly Asn Thr Asp Phe Trp Lys Thr Leu Arg Tyr Leu
945                 950                 955                 960

Ser Leu Leu Tyr Pro Ala Arg Gly Ser Arg Asn Ile Leu Leu Val Ser
                965                 970                 975

Asp Gly His Leu Gln Asp Glu Ser Leu Thr Leu Gln Leu Val Lys Arg
            980                 985                 990

Ser Arg Pro His Thr Arg Leu Phe Ala Cys Gly Ile Gly Ser Thr Ala
        995                 1000                1005

Asn Arg His Val Leu Arg Ile Leu Ser Gln Cys Gly Ala Gly Val Phe
    1010                1015                1020

Glu Tyr Phe Asn Ala Lys Ser Lys His Ser Trp Arg Lys Gln Ile Glu
1025                1030                1035                1040

Asp Gln Met Thr Arg Leu Cys Ser Pro Ser Cys His Ser Val Ser Val
                1045                1050                1055

Lys Trp Gln Gln Leu Asn Pro Asp Ala Pro Glu Ala Leu Gln Ala Pro
            1060                1065                1070

Ala Gln Val Pro Ser Leu Phe Arg Asn Asp Arg Leu Leu Val Tyr Gly
        1075                1080                1085

Phe Ile Pro His Cys Thr Gln Ala Thr Leu Cys Ala Leu Ile Gln Glu
    1090                1095                1100

Lys Glu Phe Cys Thr Met Val Ser Thr Thr Glu Leu Gln Lys Thr Thr
1105                1110                1115                1120

Gly Thr Met Ile His Lys Leu Ala Ala Arg Ala Leu Ile Arg Asp Tyr
                1125                1130                1135

Glu Asp Gly Ile Leu His Glu Asn Glu Thr Ser His Glu Met Lys Lys
            1140                1145                1150

Gln Thr Leu Lys Ser Leu Ile Ile Lys Leu Ser Lys Glu Asn Ser Leu
        1155                1160                1165

Ile Thr Gln Phe Thr Ser Phe Val Ala Val Glu Lys Arg Asp Glu Asn
    1170                1175                1180

Glu Ser Pro Phe Pro Asp Ile Pro Lys Val Ser Glu Leu Ile Ala Lys
1185                1190                1195                1200

Glu Asp Val Asp Phe Leu Pro Tyr Met Ser Trp Gln Gly Glu Pro Gln
                1205                1210                1215

Glu Ala Val Arg Asn Gln Ser Leu Leu Ala Ser Ser Glu Trp Pro Glu
            1220                1225                1230

Leu Arg Leu Ser Lys Arg Lys His Arg Lys Ile Pro Phe Ser Lys Arg
        1235                1240                1245

Lys Met Glu Leu Ser Gln Pro Glu Val Ser Glu Asp Phe Glu Glu Asp
    1250                1255                1260
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Gly Leu Gly Val Leu Pro Ala Phe Thr Ser Asn Leu Glu Arg Gly Gly
1265                1270                1275                1280

Val Glu Lys Leu Leu Asp Leu Ser Trp Thr Glu Ser Cys Lys Pro Thr
                1285                1290                1295

Ala Thr Glu Pro Leu Phe Lys Lys Val Ser Pro Trp Glu Thr Ser Thr
            1300                1305                1310

Ser Ser Phe Phe Pro Ile Leu Ala Pro Ala Val Gly Ser Tyr Leu Thr
        1315                1320                1325

Pro Thr Thr Arg Ala His Ser Pro Ala Ser Leu Ser Phe Ala Ser Tyr
    1330                1335                1340

Arg Gln Val Ala Ser Phe Gly Ser Ala Ala Pro Pro Arg Gln Phe Asp
1345                1350                1355                1360

Ala Ser Gln Phe Ser Gln Gly Pro Val Pro Gly Thr Cys Ala Asp Trp
                1365                1370                1375

Ile Pro Gln Ser Ala Ser Cys Pro Thr Gly Pro Pro Gln Asn Pro Pro
            1380                1385                1390

Ser Ala Pro Tyr Cys Gly Ile Val Phe Ser Gly Ser Ser Leu Ser Ser
        1395                1400                1405

Ala Gln Ser Ala Pro Leu Gln His Pro Gly Gly Phe Thr Thr Arg Pro
    1410                1415                1420

Ser Ala Gly Thr Phe Pro Glu Leu Asp Ser Pro Gln Leu His Phe Ser
1425                1430                1435                1440

Leu Pro Thr Asp Pro Asp Pro Ile Arg Gly Phe Gly Ser Tyr His Pro
                1445                1450                1455

Ser Ala Tyr Ser Pro Phe His Phe Gln Pro Ser Ala Ala Ser Leu Thr
            1460                1465                1470

Ala Asn Leu Arg Leu Pro Met Ala Ser Ala Leu Pro Glu Ala Leu Cys
        1475                1480                1485

Ser Gln Ser Arg Thr Thr Pro Val Asp Leu Cys Leu Leu Glu Glu Ser
    1490                1495                1500

Val Gly Ser Leu Glu Gly Ser Arg Cys Pro Val Phe Ala Phe Gln Ser
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Ser Asp Thr Glu Ser Asp Glu Leu Ser Glu Val Leu Gln Asp Ser Cys
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Phe Leu Gln Ile Lys Cys Asp Thr Lys Asp Asp Ser Ile Pro Cys Phe
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Leu Glu Val Lys Glu Glu Asp Glu Ile Val Cys Thr Gln His Trp Gln
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Asp Ala Val Pro Trp Thr Glu Leu Leu Ser Leu Gln Thr Glu Asp Gly
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Phe Trp Lys Leu Thr Pro Glu Leu Gly Leu Ile Leu Asn Leu Asn Thr
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Asn Gly Leu His Ser Phe Leu Lys Gln Lys Gly Ile Gln Ser Leu Gly
                1605                1610                1615

Val Lys Gly Arg Glu Cys Leu Leu Asp Leu Ile Ala Thr Met Leu Val
            1620                1625                1630

Leu Gln Phe Ile Arg Thr Arg Leu Glu Lys Glu Gly Ile Val Phe Lys
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Ser Leu Met Lys Met Asp Asp Pro Ser Ile Ser Arg Asn Ile Pro Trp
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1. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a fragment, or derivative, or variant of said transcription or translation product

in a sample obtained from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. A method of monitoring the progression of a neurodegenerative disease in a subject, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a fragment, or derivative, or variant of said transcription or translation product in a sample obtained from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby monitoring the progression of said neurodegenerative disease in said subject.

3. A method of evaluating a treatment for a neurodegenerative disease, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample obtained from a subject being treated for said disease and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby evaluating said treatment for said neurodegenerative disease.

4. The method according to claim 1 wherein said neurodegenerative disease is Alzheimer's disease.

5. The method according to claim 1 wherein said sample comprises a cell, or a tissue, or a body fluid, in particular cerebrospinal fluid or blood.

6. The method according to claim 1 wherein said reference value is that of a level and/or an activity of

(i) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a fragment, or derivative, or variant of said transcription or translation product,

in a sample obtained from a subject not suffering from said neurodegenerative disease.

7. The method according to claim 1 wherein an alteration in the level and/or activity of a transcription product of the gene coding for the minor vault protein ADPRTL1 and/or a translation product of a gene coding for the minor vault protein ADPRTL1, and/or a fragment, or derivative, or variant thereof, in a sample cell, or tissue, or body fluid, in particular cerebrospinal fluid, obtained from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

8. A kit for diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop such a disease by:

(i) detecting in a sample obtained from said subject a level, or an activity, or both said level and said activity of a transcription product and/or of a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, compared to a reference value representing a known health status; and said kit comprising:

a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and (ii) reagents that selectively detect a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1.

9. A method of treating or preventing a neurodegenerative disease, in particular AD, in a subject comprising administering to said subject in a therapeutically or prophylactically effective amount an agent or agents which directly or indirectly affect an activity and/or a level of

(i) a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii).

10. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of

(i) a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii).

11. A recombinant, non-human animal comprising a non-native gene sequence coding for a vault protein, the minor vault protein ADPRTL1, or a fragment, or a derivative, or a variant thereof, said animal being obtainable by:

(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii) introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv) transplanting said embryo into a pseudopregnant non-human animal, and

(v) allowing said embryo to develop to term, and

(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing symptoms of a neurodegenerative disease or related diseases or disorders.

12. The animal according to claim 11 wherein said minor vault protein ADPRTL1 is the minor vault protein of SEQ ID NO. 2.

13. Use of the recombinant, non-human animal according to claim 11 for screening, testing, and validating compounds, agents, and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

14. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iv) a fragment, or derivative, or variant of (i) to (iii), said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

15. A method of screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(ii) a transcription product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(iii) a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, and/or

(v) a fragment, or derivative, or variant of (i) to (iii), said method comprising:

(a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect of the substances recited in (i) to (iv);

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) or (iv) in a matched control animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders in respect to the substances recited in (i) to (iv) and to which animal no such test compound has been administered;

(d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in

the test animal indicates that the test compound is a modulator of said diseases or disorders.

16. The method according to claim 15 wherein said test animal and/or said control animal is a recombinant animal which expresses a vault protein, the minor vault protein ADPRTL1 or a fragment, or a derivative, or a variant thereof, under the control of a transcriptional control element which is not the native vault protein gene transcriptional control element.

17. An assay for testing a compound, preferably for screening a plurality of compounds for inhibition of binding between a ligand and a vault protein, the minor vault protein ADPRTL1 or a fragment, or derivative, or a variant thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said vault protein, or a fragment, or a derivative, or a variant thereof, to a plurality of containers;

(ii) adding a compound, preferably a plurality of compounds, to be screened for said inhibition of binding to said plurality of containers;

(iii) adding a detectable ligand, in particular a fluorescently detectable ligand, to said containers;

(iv) incubating the liquid suspension of said vault protein, or said fragment, or derivative, or variant thereof, and said compound, preferably said plurality of compounds, and said ligand;

(v) measuring amounts of detectable ligand or fluorescence associated with said vault protein, or with said fragment, or derivative, or variant thereof; and

(vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said vault protein, or said fragment, or derivative, or variant thereof.

18. An assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a vault protein, the minor vault protein ADPRTL1, or to a fragment, or derivative, or variant thereof, said assay comprising the steps of:

(i) adding a liquid suspension of said vault protein, or a fragment, or derivative, or variant thereof, to a plurality of containers;

(ii) adding a detectable compound, preferably a plurality of detectable compounds, in particular fluorescently detectable compounds, to be screened for said binding to said plurality of containers;

(iii) incubating the liquid suspension of said vault protein, or said fragment, or derivative, or variant thereof, and said compound, preferably said plurality of compounds;

(iv) measuring amounts of detectable compound or fluorescence associated with said vault protein, or with said fragment, or derivative, or variant thereof; and

(v) determining the degree of binding by one or more of said compounds to said vault protein, or said fragment, or derivative, or variant thereof.

19. Use of a protein molecule, said protein molecule being a translation product of the gene coding for a vault protein, the minor vault protein ADPRTL1, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, as a diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

20. Use of a protein molecule, said protein molecule being a translation product of the gene coding for a vault protein, the minor vault protein ADPRTL1, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

21. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of a gene coding for a vault protein, the minor vault protein ADPRTL1, SEQ ID NO. 2, or a fragment, or derivative, or variant thereof, for detecting the pathological state of a cell in a sample obtained from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell, and wherein said pathological state relates to a neurodegenerative disease, in particular Alzheimer's disease.

* * * * *

专利名称(译)	穹窿体多核苷酸和蛋白质用于神经退行性疾病的诊断和治疗用途		
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申请(专利权)人(译)	VON DER KAMMER HEINZ POHLNER JOHANNES		
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摘要(译)

本发明公开了小穹窿蛋白ADPRTL1基因在阿尔茨海默病患者特定脑区的差异表达。基于该发现，本发明提供了一种用于诊断或预测受试者的阿尔茨海默氏病的方法，或用于确定受试者是否具有增加患阿尔茨海默病的风险的方法。此外，本发明提供了使用编码穹窿体蛋白的基因，特别是编码次穹窿蛋白ADPRTL1的基因，治疗或预防阿尔茨海默病和相关神经变性疾病的治疗和预防方法。还公开了筛选神经变性疾病的调节剂的方法。

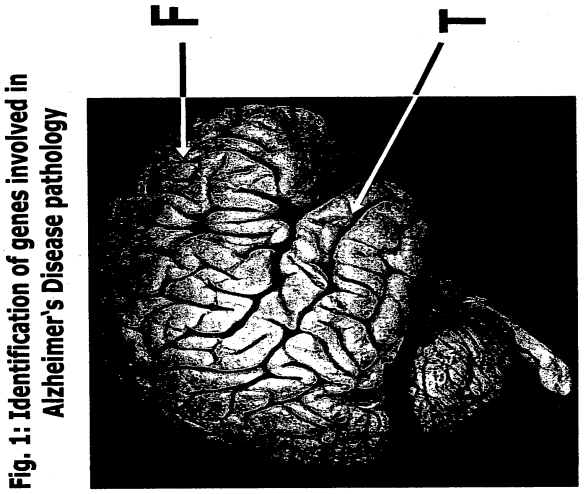

Fig. 1: Identification of genes involved in Alzheimer's Disease pathology