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RICHARD W. WIEKING
CLERK U.S. DISTRICT COURT
NORTHERN DISTRICT OF CALIFORNIA

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

ALZHEIMER'S INSTITUTE OF AMERICA, Plaintiff, v. COMENTIS, INC., and OKLAHOMA MEDICAL RESEARCH FOUNDATION, Defendants.	Case No. CV 09 2772 **DEMAND FOR JURY TRIAL** **COMPLAINT FOR DAMAGES AND INFRINGEMENT** VRW

PLAINTIFF'S COMPLAINT

COMES NOW Plaintiff Alzheimer's Institute of America ("AIA"), by and through its attorneys, and for its Complaint against CoMentis, Inc. ("CoMentis") and the Oklahoma Medical Research Foundation ("OMRF") (collectively, "Defendants"), states as follows:

Nature of the Action

1. This Complaint seeks a judgment finding that Defendants have infringed and continue to infringe upon AIA's U.S. Patent Nos. 5,455,169 (the "'169 Patent"), 5,795,963 (the "'963 Patent"), and 6,818,448 (the "'448 Patent") (collectively, the "Patents-in-Suit").

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The Patents-in-Suit cover a wide range of Alzheimer's Disease-related technology, including, but not limited to: nucleic acids coding for the Swedish mutation; vectors, cell lines, and transgenic mice expressing the Swedish mutation containing nucleic acids; and polypeptides encoded by the Swedish mutation containing nucleic acids. The claimed technology providing important insights and tools for Alzheimer's Disease research, including the ability to screen for potential Alzheimer's Disease drug candidates, such as, for example, beta-secretase inhibitors. True and accurate copies of the Patents-in-Suit are attached hereto, respectively, as **Exhibits A, B,** and **C.**

Jurisdiction and Venue

2. This Court has subject matter jurisdiction over this Complaint pursuant to 28 U.S.C. §§ 1331 and 1338(a), and under the patent laws of the United States, 35 U.S.C. § 1, *et seq.*

3. Venue is proper in this judicial district under the provisions of 28 U.S.C. §§ 1391(b) and 1400(b).

Intradistrict Assignment

4. This action is excepted from intradistrict assignment because it is an intellectual property matter. Civil L.R. 3-5(b) and 3-2(c).

The Parties

5. AIA is a corporation organized and existing under the laws of Florida and having its principal place of business at 7837 Parallel Parkway, Kansas City, KS, 66112. AIA is the owner of the Patents-in-Suit.

6. CoMentis is a corporation organized and existing under the laws of Delaware and having its corporate headquarters at 280 Utah Avenue, Suite 275, South San Francisco, CA, 94080. Upon information and belief, CoMentis has committed acts of patent infringement in this district.

7. OMRF is an Oklahoma nonprofit biomedical research institution having its principal place of business at 825 N.E. 13th Street, Oklahoma City, OK, 73104. Upon

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information and belief, OMRF has committed acts of patent infringement and has engaged in substantial contacts with CoMentis in this district.

Facts

8. On or about April 25 2000, AIA contacted OMRF concerning its use of the technology claimed in the '169 Patent. Specifically, AIA asserted that a publication in the *Proceedings of the National Academy of Sciences*, Vol. 97, No. 4, pp. 1456-1460 (February 2000), demonstrated OMRF's use of the technology of the '169 Patent in that it describes the transfection of cells with the Swedish mutation of Amyloid Precursor Protein ("APP Swedish"). The referenced publication listed Xinli Lin, Gerald Koelsch, Shilu Wu, Debbie Downs, Azar Dashti, and Jordan Tang as the OMRF researchers responsible for the research. Dr. Tang was listed as lead or corresponding author and was the principal investigator leading the research.

9. On May 17, 2000, OMRF, acting through its counsel, responded that OMRF's use of the nucleic acids claimed in the '169 Patent was non-commercial and limited to one academic research project. OMRF, acting through its counsel, further represented that OMRF "does not intend to use these nucleic acids in any future projects" and "if future research efforts at OMRF should require use of these nucleic acids, then OMRF will contact AIA to discuss obtaining an appropriate license to the '169 Patent."

10. AIA recently learned, however, that OMRF's use of the nucleic acids claimed in the '169 Patent continued. For example, Dr. Tang *et al.* of OMRF published an article in *The Journal of Biological Chemistry*, Vol. 279, No. 36, pp. 37886-37894 (September 2004), which describes the use of: (1) APP Swedish cloned in the mammalian expression vector pcDNA3.1; (2) HEK-293 cells transfected with APP Swedish; (3) and HeLa cells transfected with APP Swedish. In addition, Dr. Tang *et al.* of OMRF published an article in *The FASEB Journal*, Vol. 21, pp. 3184-3196 (October 2007), which describes the use of: (1) cultured CHO cells transfected with APP Swedish; (2) HEK-293 cells transfected with APP Swedish; (3) and HeLa cells transfected with APP Swedish. These papers are but two examples of OMRF's extensive use of the patented technology. Indeed,

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the primary focus of Dr. Tang's research group is the identification of beta-secretase inhibitors for the treatment of Alzheimer's Disease.

11. On information and belief, and contrary to OMRF's representations, OMRF's research was not and is not "non-commercial." In 2001, Dr. Tang founded Zapaq, Inc. ("Zapaq") to commercialize the discoveries made using AIA's patented technology. On information and belief, OMRF licensed technology derived from the unlicensed use of the Patents-in-Suit to Zapaq. On information and belief, Zapaq, Inc. merged with Athenagen, Inc. in August 2006 and was renamed CoMentis.

12. On information and belief, CoMentis (formerly Zapaq) and OMRF have collaborated on Alzheimer's Disease research that includes the use of the technology claimed in the Patents-in-Suit. *See*, *e.g.*, Jordan Tang *et al.*, *Bioorg. Med. Chem. Lett.*, Vol. 18, No. 3, pp. 1031-1036 (February 2008); Jordan Tang *et al.*, *Current Alzheimer Research*, Vol. 4, No. 4, pp. 418-422 (2007); Jordan Tang, *et al.*, *Journal of Neurochemistry*, Vol. 89, pp. 1409-1416 (2004); Jordan Tang, *et al.*, *Biochemistry*, Vol. 40, No. 34, pp. 10001-10006 (August 2001).

13. On information and belief, CoMentis has an active Alzehimer's Disease research program and drug development pipeline, including the development of certain drug candidates known as beta-secretase inhibitors. In the course of identifying and developing these drug candidates, CoMentis has made extensive use of the technology claimed in the Patents-in-Suit. For example, several CoMentis patents and patent applications, including U.S. Patent No. 7,504,420, describe, among other things, the use of "Chinese hamster ovary cells that over-express human APP695 with the London and Swedish mutations."

COUNT I

Patent Infringement of U.S. Patent No. 5,455,169

14. AIA incorporates by reference each and every allegation set forth in paragraphs 1 through 13 of its Complaint as if fully set forth and restated herein.

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15. The '169 Patent entitled "Nucleic acids for diagnosing and modeling Alzheimer's disease" was duly and regularly issued on October 3, 1995. AIA is the sole and exclusive owner of the '169 Patent. At all relevant times, the owner of the '169 Patent has complied with 35 U.S.C. § 287(a).

16. Defendants, without the authority or consent of AIA, have been and continue to, upon information and belief, use in the United States, including, but not limited to, in this judicial district, technologies which infringe upon the '169 Patent. Upon information and belief, Defendants' infringement of the '169 Patent is knowing and willful.

17. Defendants' infringement of the '169 Patent has caused and continues to cause irreparable harm and other harm to AIA.

COUNT II

Patent Infringement of U.S. Patent No. 5,795,963

18. AIA incorporates by reference each and every allegation set forth in paragraphs 1 through 17 of its Complaint as if fully set forth and restated herein.

19. The '963 Patent entitled "Amyloid precursor protein in alzheimer's disease" was duly and regularly issued on August 18, 1998. The inventor of the '963 Patent assigned all right, title and interest in, to and under the '963 Patent to AIA. AIA is the sole and exclusive owner of the '963 Patent. At all relevant times, the owner of the '963 Patent has complied with 35 U.S.C. § 287(a).

20. Defendants, without the authority or consent of AIA, have been and continue to, upon information and belief, use technologies in the United States, including, but not limited to, in this judicial district, technologies which infringe upon the '963 Patent. Upon information and belief, Defendants' infringement of the '963 Patent is knowing and willful.

21. Defendants' infringement of the '963 Patent has caused and continues to cause irreparable harm and other harm to AIA.

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COUNT III

Patent Infringement of U.S. Patent No. 6,818,448

22. AIA incorporates by reference each and every allegation set forth in paragraphs 1 through 21 of its Complaint as if fully set forth and restated herein.

23. The '448 Patent entitled "Isolated cell comprising HAPP 670/671 DNAS sequences" was duly and regularly issued on November 16, 2004. The inventor of the '448 Patent assigned all right, title and interest in, to and under the '448 Patent to AIA. AIA is the sole and exclusive owner of the '448 Patent. At all relevant times, the owner of the '448 Patent has complied with 35 U.S.C. § 287(a).

24. Defendants, without the authority or consent of AIA, have been and continue to, upon information and belief, use technologies in the United States, including, but not limited to, in this judicial district, technologies which infringe upon the '448 Patent. Upon information and belief, Defendants' infringement of the '448 Patent is knowing and willful.

25. Defendants' infringement of the '448 Patent has caused and continues to cause irreparable harm and other harm to AIA.

PRAYER

WHEREFORE, Plaintiff Alzheimer's Institute of America respectfully prays that the Court enter judgment in its favor and award the following relief against Defendants:

A. Find that Defendants infringed upon the Patents-in-Suit and will continue to infringe said patents unless enjoined therefrom;

B. Find that the infringement of Defendants upon the Patents-in-Suit was knowing and willful;

C. Enjoin Defendants and their respective officers, directors, employees, agents, licensees, representatives, affiliates, related companies, servants, successors and assigns, and any and all persons acting in privity or in concert with any of them, preliminarily and permanently, from further infringing upon the Patents-in-Suit;

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D. Order that an accounting be made to establish damages arising out of Defendants' infringement of the Patents-in-Suit;

E. Award AIA actual damages pursuant to 35 U.S.C. § 284, in an amount to be determined at trial, as a result of Defendants' infringement upon the Patents-in-Suit;

F. Award AIA treble damages pursuant to 35 U.S.C. § 284 in an amount to be determined at trial, as a result of Defendants' knowing and willful infringement upon the Patents-in-Suit;

G. Award AIA its costs and reasonable attorneys' fees incurred in connection with this action; and

H. Award and grant AIA such other and further relief as the Court deems just and proper under the circumstances.

DEMAND FOR JURY TRIAL

Plaintiff demands a jury trial.

Dated: June 22, 2009

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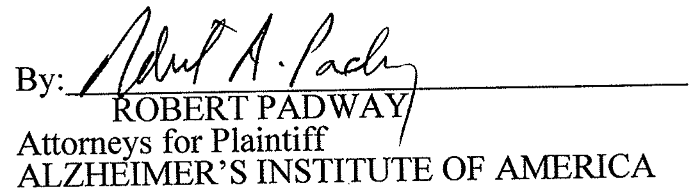

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EXHIBIT A

US005455169A

United States Patent [19]

Mullan

[11] **Patent Number: 5,455,169**

[45] **Date of Patent: Oct. 3, 1995**

[54] **NUCLEIC ACIDS FOR DIAGNOSING AND MODELING ALZHEIMER'S DISEASE**

[75] Inventor: **Michael J. Mullan**, Tampa, Fla.

[73] Assignee: **Alzheimer's Institute of America, Inc.**, Prairie Village, Kans.

[21] Appl. No.: **894,211**

[22] Filed: **Jun. 4, 1992**

[51] **Int. Cl.**[6] **C12N 5/10**; C12N 15/12; C12N 15/85

[52] **U.S. Cl.** **435/240.2**; 435/320.1; 536/23.1; 536/23.5; 536/24.31; 536/24.33

[58] **Field of Search** 435/240.2, 320.1, 435/172.3, 6; 536/23.1, 23.5, 24.31, 24.33

[56] **References Cited**

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5,015,570	5/1991	Scangos et al.	536/24.31
5,218,100	6/1993	Müller-Hill et al.	536/23.5
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5,221,607	6/1993	Cordell et al.	536/23.5

FOREIGN PATENT DOCUMENTS

9207068 4/1992 WIPO .

OTHER PUBLICATIONS

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Kang et al., "The precursor of Alzheimer's disease amyloid A4 . . ." *Nature* 325:733–736, (Feb. 1987).

Yoshikai et al., "Genomic organization of the human amyloid . . . gene", *Gene* 87:257–263 (Mar. 1990).

Murrell et al., "A Mutation in the Amyloid Precursor Protein . . ." *Science* 254:97–99.

Chartier–Harlan et al., "Early–onset Alzheimer's diseased caused by mutations at codon 717 of the β–amyloid precursor protein gene," *Nature* 353:844–847 (1991).

Goate et al., "Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease," *Nature* 349:704–706 (1991).

Levy et al., "Mutation of the Alzheimer's Disease Amyloid Gene in Hereditary Cerebral Hemorrhage, Dutch Type," *Science* 248:1124–1126 (1990).

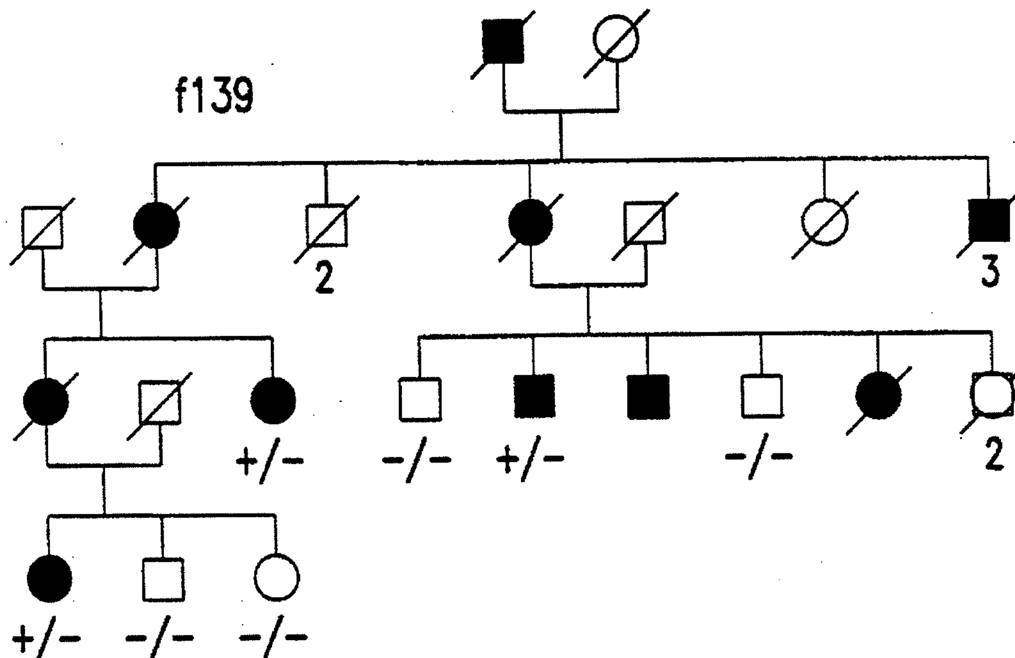
Primary Examiner—Stephen G. Walsh

Attorney, Agent, or Firm—Needle & Rosenberg

[57] **ABSTRACT**

The invention provides an isolated nucleic acid characteristic of human amyloid precursor protein 770 including the nucleotides encoding codon 670 and 671, wherein the nucleic acid encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671. Also provided is a method of diagnosing or predicting a predisposition to Alzheimer's disease, comprising detecting in a sample from a subject the presence of a mutation at a nucleotide position corresponding to codons 670 and/or 671 of amyloid precursor protein or fragment thereof, the presence of the mutation indicating the presence of or a predisposition to Alzheimer's disease.

12 Claims, 1 Drawing Sheet



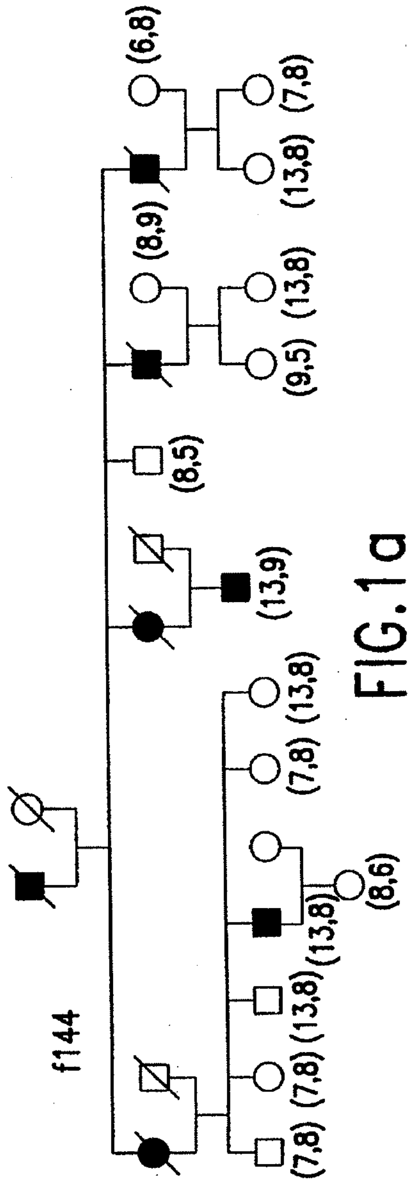


FIG. 1a

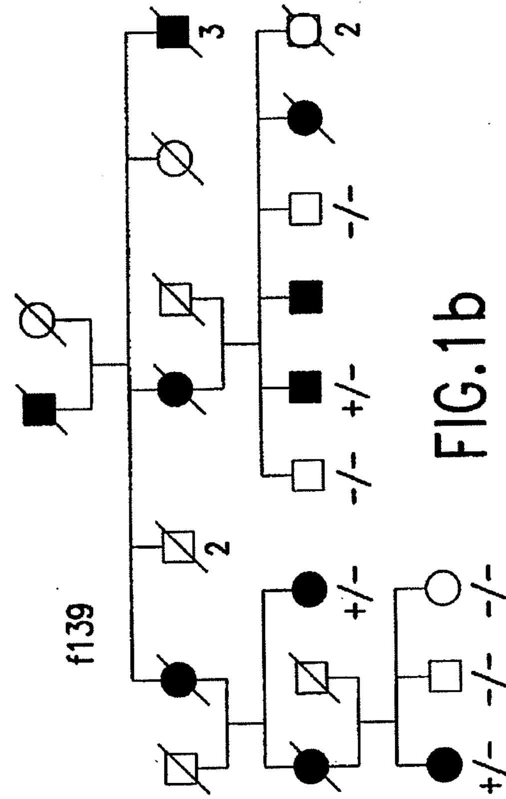


FIG. 1b

NUCLEIC ACIDS FOR DIAGNOSING AND MODELING ALZHEIMER'S DISEASE

BACKGROUND OF THE INVENTION

Alzheimer's disease (AD) is a progressive disease known generally as senile dementia. Broadly speaking, the disease falls into two categories, namely late onset and early onset. Late onset, which occurs in old age (65+ years), may be caused by the natural atrophy of the brain occurring at a faster rate and to a more severe degree than normal. Early onset AD is much more infrequent but shows a pathologically identical dementia with diffuse brain atrophy which develops well before the senile period, i.e., between the ages of 35 and 60 years. There is evidence that one form of this type of AD shows a tendency to run in families and is therefore known as familial Alzheimer's disease (FAD).

In both types of AD the pathology is the same but the abnormalities tend to be more severe and more widespread in cases beginning at an earlier age. The disease i s characterized by two types of lesions in the brain: senile plaques and neurofibrillary tangles.

Senile plaques are areas of disorganized neuropil up to 150 mm across with extracellular amyloid deposits at the center. Neurofibrillary tangles are intracellular deposits of amyloid protein consisting of two filaments twisted about each other in pairs.

The major protein subunit, β-amyloid protein (also referred to in the art as amyloid β protein (AβP) and A4) of the amyloid filaments of both the neurofibrillary tangle and the senile plaque, is a highly aggregating small polypeptide of approximate relative molecular mass 4,500. This protein is a cleavage product of a much larger precursor protein called amyloid precursor protein (APP).

The sequence of the deposited β-amyloid protein in particular brain regions is one of the main pathologic characteristics of AD. The β-amyloid protein is an approximately 4 kD protein (39 to 42 amino acids) which is derived, as an internal cleavage product, from one or more isoforms of a larger APP. There are at least five distinct isoforms of APP: 563, 695, 714, 751, and 770 amino acids, respectively (Wirak et al. (1991) *Science* 253:323). These isoforms of APP are generated by alternative splicing of primary transcripts of the APP gene, which is located on human chromosome 21. It is known that the APP isoforms are glycosylated transmembrane proteins (Goldgaber et al. (1987) *Science* 235:877), and that two of the isoforms APP751 and APP770, have a protease inhibitor domain that is homologous to the Kunitz type of serine protease inhibitors. The β-amyloid protein segment comprises approximately half of the transmembrane domain and approximately the first 28 amino acids of the extra cellular domain of an APP isoform.

Proteolytic processing of APP in vivo is a normal physiological process. Carboxy-terminal truncated forms of APP695, APP751, and APP770 are present in brain and cerebrospinal fluid (Palmert et al. (1989) *Proc. Natl. Acad. Sci. (U.S.A.)* 86:6338; Weidemann et al. (1989) *Cell* 57:115) and result from cleavage of the APP isoform at a constitutive cleavage site within the β-amyloid protein peptide domain of an APP isoform (Esch et al. (1990) *Science* 248:1122). Normal proteolytic cleavage at the constitutive cleavage site yields a large (approximately 100 kD) soluble, N-terminal fragment that contains the protease inhibitor domain in some isoforms, and a 9 kD membrane-bound, C-terminal fragment that includes most of the β-amyloid protein domain.

Generation of pathogenic β-amyloid protein appears to be the result of aberrant proteolytic processing of APP, such that normal cleavage at the constitutive site within the β-amyloid protein domain does not occur, but rather cleavage occurs at two specific sites which flank the β-amyloid protein domain. One of these aberrant cleavage sites is in the transmembrane domain and the other aberrant cleavage site is located approximately at the N-terminus of the first 28 amino acids of the extra cellular domain. Such aberrant proteolytic cleavage produces the β-amyloid protein polypeptide which is prone to forming dense amyloidogenic aggregates that are resistant to proteolytic degradation and removal. The resultant β-amyloid protein aggregates presumably are involved in the formation of the abundant amyloid plaques and cerebrovascular amyloid that are the neuropathological hallmarks of AD. However, the exact aberrant cleavage sites are not always precise; β-amyloid molecules isolated from the brain of a patient with AD show N- and C- terminal heterogeneity. Therefore, the aberrant cleavage pathway may involve either sequence-specific proteolysis followed by exopeptidase activity (creating end-heterogeneity), or may not be sequence-specific.

The APP gene is known to be located on human chromosome 21. A locus segregating with FAD has been mapped to chromosome 21 (St. George Hyslop et al. (1987) *Science* 235:885) close to the APP gene. Recombinants between the APP gene and the AD locus have been previously reported (Schellenberg et al. (1988) *Science* 241:1507). The data appeared to exclude the APP gene as the site of any mutation that might cause FAD (Van Broekhoven et al. (1987) *Nature* 329:153; Tanzi et al. (1987) *Nature* 329:156).

Recombinant DNA technology provides several techniques for analyzing genes to locate possible mutations. For example, the polymerase chain reaction (john Bell (1989) *Immunology Today* 10:351–355) may be used to amplify specific sequences using intronic primers, which can then be analyzed by direct sequencing.

Using such techniques, a single base substitution, a C to T transition at base pair 2149, has been found in part of the sequence of the APP gene in some cases of FAD. This base pair transition leads to an amino acid substitution, i.e., valine to isoleucine at amino acid 717 (APP770) (see Yoshikai et al. (1990) *Gene* 87:257), close to the C-terminus of the β-amyloid protein. This suggests that some cases of AD are caused by mutation in the APP gene, specifically mutations that change codon 717 such that it encodes an amino acid other than valine.

A second APP allelic variant wherein glycine is substituted for valine at codon 717 is now identified, and is so closely linked to the AD phenotype as to indicate that allelic variants at codon 717 of the APP gene, particularly those encoding an amino acid other than valine, and more particularly those encoding an isoleucine, glycine, or phenylalanine, are pathogenic and/or pathognomonic alleles (Chartier-Harlin et al. (1991) *Nature* 353:844).

Proteolysis on either side of the β-amyloid protein region of APP may be enhanced or qualitatively altered by the specific mutations at codon 717, increasing the rate of β-amyloid deposition and aggregation. Such codon 717 mutations may increase β-amyloid formation by providing a poorer substrate for the main proteolytic pathway (cleavage at the constitutive site) or a better substrate for a competing, alternative cleavage pathway (at aberrant cleavage sites).

There are several disease states which give rise to progressive intellectual deterioration closely resembling the dementia associated with AD for which treatment is avail-

able. A further diagnostic test for AD would therefore provide a valuable tool in the diagnosis and treatment of these other conditions, by way of being able to exclude AD.

Also important is the development of experimental models of AD that can be used to define further the underlying biochemical events involved in AD pathogenesis. Such models could be employed to screen for agents that alter the degenerative course of AD. For example, a model system of AD could be used to screen for environmental factors that induce or accelerate the pathogenesis of AD. In contradistinction, an experimental model could be used to screen for agents that inhibit, prevent, or reverse the progression of AD. Such models could be employed to develop pharmaceuticals that are effective in preventing, arresting, or reversing AD.

The present invention provides the discovery of additional heretofore unknown mutations in β-amyloid protein. These mutations can be utilized advantageously to detect, treat and screen in subjects and model systems.

SUMMARY OF THE INVENTION

The invention provides an isolated nucleic acid characteristic of human amyloid precursor protein 770 including the nucleotides encoding codon 670 and 671, wherein the nucleic acid encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671. Also provided is a method of diagnosing or predicting a predisposition to AD, comprising detecting in a sample from a subject the presence of a mutation at a nucleotide position corresponding to codons 670 and/or 671 of amyloid precursor protein or fragment thereof, the presence of the mutation indicating the presence of or a predisposition to AD.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1*a*** and **1*b*** illustrate two related pedigrees in which early onset AD is apparently inherited as an autosomal dominant disorder. ± refer to presence/absence of mutation. GT12 alleles are in parentheses. The average age of onset in this family is 55 years. Black symbols denote affected individuals and oblique lines indicate deceased individuals. Females are denoted by circles and males by squares. For f139, samples were available from the 7 individuals whose genotypes (presence or absence of the mutation) are illustrated, and for f144 samples were available from 15 individuals whose genotypes at GT12 are shown.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides an isolated nucleic acid characteristic of human amyloid precursor protein including the nucleotides encoding codon 670 and 671 of human amyloid precursor protein 770, wherein the nucleic acid encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671. In one embodiment, the nucleic acid encodes asparagine at codon 670 and leucine at codon 671.

The invention also provides an isolated nucleic acid complementary to the nucleic acid of the invention. The isolated nucleic acid can be labeled with a detectable moiety. The isolated nucleic acid can encode, for example, β-amyloid protein and the entire human amyloid precursor protein 770. In addition, the isolated nucleic acid can further encode an amino acid other than valine at codon 717. The invention also provides polypeptides encoded by these nucleic acids.

Further, the invention provides an antibody specifically reactive with the polypeptides of the invention. Thus, antibodies which react with the unique epitope created by the amino acid other than lysine at codon 670 and/or other than methionine at position 671 are provided.

Vectors comprising the nucleic acids of the invention are also provided. These vectors can be placed in a host capable of expressing the characteristic portion of human amyloid precursor protein.

The invention also provides a method of diagnosing or predicting a predisposition to AD. The method comprises detecting in a sample from a subject the presence of a mutation in a human amyloid precursor protein at a nucleotide position corresponding to codons 670 and/or 671 of amyloid precursor protein 770 or fragment thereof, the presence of the mutation indicating the presence of or a predisposition to AD. In one embodiment, the sequence mutation is a nucleotide substitution, wherein codon 670 encodes asparagine and/or codon 671 encodes leucine. As discussed below in greater detail, the mutation can be detected by many methods. For example, the detecting step can comprise combining a nucleotide probe capable of selectively hybridizing to a nucleic acid containing the mutation with a nucleic acid in the sample and detecting the presence of hybridization. Additionally, the detecting step can comprise amplifying the nucleotides of the mutation and detecting the presence of the mutation in the amplified product. Further, the detecting step can comprise selectively amplifying the nucleotides of the mutation and detecting the presence of amplification. Finally, the detecting step can comprise detecting the loss of a restriction fragment length created by an MboII enzyme digest of the nucleotides of the mutation.

The invention also provides a transgenic non-human animal containing, in a germ or somatic cell, the mutated nucleic acid of the invention, wherein the animal expresses a human amyloid precursor protein or fragment thereof which encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671. Preferably, the animal expresses neuropathological characteristics of AD. These animals can also have a mutation at position 717 wherein the animal expresses a human amyloid precursor protein or fragment thereof which encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671 and an amino acid other than valine at codon 717.

The invention also provides a host containing the nucleic acid of claim **1**, which host expresses a human amyloid precursor protein or fragment thereof which encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671. Preferably, the host is an immortalized cell line. This host can also contain the mutation at codon 717 whereby the host expresses a human amyloid precursor protein or fragment thereof which encodes an amino acid other than lysine at codon 670 and/or an amino acid other than methionine at codon 671 and an amino acid other than valine at codon 717. The invention also provides a method of screening for an agent capable of treating AD. The method comprises contacting these transgenic animals or host cell lines with the agent and monitoring the expression, processing or deposition of amyloid precursor protein or fragments thereof.

As used herein, "isolated" means free of at least some of the contaminants associated with the nucleic acid or polypeptides occurring in a natural environment.

As used here, "nucleic acid characteristic of human amyloid precursor protein" refers to a nucleic acid which has sufficient nucleotides surrounding the codons at position 670 and 671 of human amyloid precursor protein 770 to distinguish the nucleic acid from nucleic acids encoding non-related proteins. The specific length of the nucleic acid is a matter of routine choice based on the desired function of the sequence. For example, if one is making probes to detect the mutation in either codon 670 and/or 671, the length of the mucleic acid will be smaller, but must be long enough to prevent hybridization to the desired background sequences. However, if the desired hybridization is to a nucleic acid which has been amplified, background hybridization is less of a concern and a smaller probe can be used. In general, such a probe will be between 10 and 100 nucleotides, especially between 10 and 40 nucleotides in length.

Likewise, polypeptides encoded by the nucleic acids of the invention can be variable depending on the desired function of the polypeptide. While smaller fragments can work, generally to be useful, e.g., immunogenic, the polypeptide must be of at least 8 amino acids and not more than 10,000 amino acids. This polypeptide contains an amino acid internal sequence (listed in order from amino- to carboxy-terminal direction):

-Ser-Glu-Val-X-X-Asp-Ala-Glu- [SEQ ID NO1]

where the X at position 4 is any of the twenty conventional amino acids except lysine, particularly asparagine, and where the X at position 5 is any of the twenty conventional amino acids except methionine, particularly leucine. The amino and carboxy ends of this core sequence can include any number of additional amino acids from the APP sequence. Thus, "fragment" can be a truncated APP isoform or a modified APP isoform (as by amino acid substitutions, deletions, or additions). This definition recognizes that APP is a single gene that undergoes alternative splicing to generate several isoforms that are designated by the total number of amino acids in each. Thus, treatment includes various alternatively spliced exons resulting in isoforms of 770, 751, 714, 695, 563 and 365 amino acids.

As used here, "codon 670 and/or codon 671" refers to the codons (i.e., the tri-nucleotide sequence) that encode the 670th and 671st amino acid positions in APP770, or the amino acid position in an APP fragment that corresponds to the 670th or 671st positions in APP770. For example, a 670 residue long fragment that is produced by truncating APP770 by removing the 100 N-terminal amino acids has its 570th amino acid position corresponding to codon 670. In fact, as used herein, codon 670 refers to the codon that encodes the 651st amino acid residue of APP751 and the 595th amino acid residue of APP695. In addition, codon 670 and/or 671 refers to the complementary sequence on the antisense strand.

As used herein, the term "mutant" refers specifically to a mutation at codons 670/671 (as referenced by the amino acid sequence in APP770) of the APP gene, such that codon 670 encodes one of the nineteen amino acids that are not lysine (i.e., glycine, methionine, alanine, serine, isoleucine, leucine, threonine, proline, histidine, cysteine, tyrosine, phenylalanine, glutamic acid, tryptophan, arginine, aspartic acid, asparagine, valine, and glutamine), but preferably asparagine, and such that codon 671 encodes one of the nineteen amino acids that are not methionine (i.e., glycine, lysine, alanine, serine, isoleucine, leucine, threonine, proline, histidine, cysteine, tyrosine, phenylalanine, glutamic acid, tryptophan, arginine, aspartic acid, asparagine, valine, and glutamine), but preferably leucine. Thus, a mutant APP770 polypeptide is an APP770 polypeptide that has an amino acid residue at posit on 670 that is not lysine and or an amino acid residue at position 671 that is not methionine. Other mutant APP isoforms comprise a non-lysine amino acid at the amino acid residue position that corresponds to codon 670 (i.e., that is encoded by codon 670) or a non-methionine amino acid at the amino acid residue position that corresponds to codon 671 (i.e., that is encoded by codon 671). Similarly, a mutant APP allele or a variant APP codon 670 or 671 allele is an APP allele that encodes a non-lysine amino acid at the amino acid residue position that corresponds to codon 670 (i.e., that is encoded by codon 670) or encodes a non-methionine amino acid at the amino acid residue position that corresponds to codon 671 (i.e., that is encoded by codon 671).

It is apparent to one of skill in the art that nucleotide substitutions, deletions, and additions may be incorporated into the polynucleotides of the invention. However, such nucleotide substitutions, deletions, and additions should not substantially disrupt the ability of the polynucleotide to hybridize to one of the polynucleotide sequences under hybridization conditions that are sufficiently stringent to result in specific hybridization.

"Specific hybridization" is defined herein as the formation of hybrids between a probe nucleic acid (e.g., a nucleic acid which may include substitutions, deletions, and/or additions) and a specific target nucleic acid (e.g., a nucleic acid having the sequence), wherein the probe preferentially hybridizes to the specific target such that, for example, a band corresponding to a variant APP allele or restriction fragment thereof can be identified on a Southern blot, whereas a corresponding wild-type APP allele (i.e., one that encodes lysine at codon 670 and methionine at codon 671) is not identified or can be discriminated from a variant APP allele on the basis of signal intensity. Hybridization probes capable of specific hybridization to detect a single-base mismatch may be designed according to methods known in the art and described in Maniatis et al. (1989) *Molecular Cloning: A Laboratory Manual,* 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; Berger and Kimmel (1987) "Guide to Molecular Cloning Techniques," *Methods in Enzymology,* Volume 152, Academic Press, Inc., San Diego, Calif.; Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437; Kwok et al. (1990) *Nucleic Acids Res.* 18:999; and Miyada et al. (1987) *Methods Enzymol.* 154:94.

The vectors for expressing the polypeptides of the invention require that the nucleic acid be "operably linked." A nucleic acid is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequences. Operably linked means that the DNA sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian) cells or tissues. Agents are evaluated for potential biological activity by inclusion in screening assays described herein below.

As used here, the terms "label" or "labeled" refer to incorporation of a radio labeled nucleotide or by enzymatic or fluorescent marker. DNA or RNA are typically labeled by incorporation of a radio-labeled nucleotide (H3, C14, S35, P32) or a biotinylated nucleotide that can be detected by marked avidin (e.g., avidin containing a fluorescent marker

or enzymatic activity) or digoxygeninylated nucleotide that can be detected by marked specific antibody.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may be alternatively employed.

Detection of Mutant Codon 670/671 APP Alleles

F139 and F144 are two large Swedish families which originate from the same county. They were specifically ascertained for genetic analysis. We tested F144 for linkage between AD and GT12 (FIG. 1*a*). A lod value of 2.34 with no recombination was obtained between GT12 and AD. Linkage analysis of 1000 simulated pedigrees (Weeks et al. (1990) *Am. J. Hum. Genet.* 47:A204 (Suppl.)) (of identical structure to F144) showed that this value was likely to occur less than one time in 100 if the marker and the disease were unlinked. We therefore sequenced exons 16 and 17 of the APP gene as these encode β-amyloid protein. Two base pair transversions (G→T and A→C) were observed in affected individuals in both families in exon 16 at codons 670 and 671 (APP 770 transcript). These changes predict lysine to asparagine and methionine to leucine substitutions in the intact protein. In addition, the codon 670 mutation causes the loss of an MboII recognition site. Using this, we screened all available members of both F144 and F139 by PCR amplification of exon 16 and digestion with this enzyme. All affected members of both families had lost the MboII cut site. The presence of the mutation was confirmed by direct sequencing of four individuals. This mutation is linked to the disease in these families with a lod score of 4.36 with no recombination. Taken with the fact that we could not detect this variant in 50 normal chromosomes, this demonstrates that this mutation is pathogenic of the dementia in these families.

Therefore, the method of the invention involves identifying a genetic alteration at amino acids 670 or 671 or both which may cause the consensus lysine or methionine (respectively) to be changed. This will generally be performed on specimens removed from the subject.

This mutation differs from previous mutations causing AD in that it is at the N-terminus of deposited β-amyloid protein rather than the C-terminus. The processing of APP has not been fully elucidated but two pathways have been postulated as important (Hardy and Higgins (1992) *Science* 256:184–5). The main proteolytic cleavage by "secretase" normally cuts APP through β-amyloid (Esch et al. (1990) *Science* 248:1122–1124; Anderson et al. (1991) *Neurosci. Letts.* 128:126–128), thus preventing its formation. An alternative endosomal/lysosomal pathway (Estus et al. (1992) *Science* 255:726–728; Golde et al. (1992) *Science* 255:728–730) apparently produces β-amyloid containing fragments and involves cleavage at or close to the site of the codon 670/671 mutation reported here.

APP717 variants have been suggested to produce amyloidogenic fragments by a number of mechanisms. Thus, they have been postulated to inhibit degradation of amyloidogenic fragments of APP directly or to alter cellular addressing of the APP molecule so that it is mismetabolised, or to alter APP expression levels by effecting mRNA stability (Goate et al. (1991) *Nature* 349:704–706; Chartier Harlin et al. (1991) *Nature* 353:844–846; and Hardy and Higgins (1992) *Science* 256:184–5).

If the dementia-causing mutations in APP all have a related action which underlies their pathogenicity, then the mutations reported here (APP670/1) would also be expected to have their effect through one of the above mechanisms. The position of this mutation right at the N-terminal of β-amyloid protein is most consistent with the notion that the AD causing-mutations directly inhibit the degradation of preamyloidogenic fragments. It seems likely that given the relatively late age of onset of disease development in humans with either codon 717 or 670/1 variants, transgenic animals with any of these mutations may not develop significant pathology. An important consequence of this mutation can be its combination with pathogenic codon 717 variants to increase the likelihood of producing Alzheimer-like pathology in transgenic animals.

Therefore, genetic alterations in the APP gene which result in altered degradative properties are particularly important in the application of the invention. There are several methodologies available from recombinant DNA technology which may be used for detecting and identifying a genetic mutation responsible for AD. These include, for example, direct probing, polymerase chain reaction (PCR) methodology, restriction fragment length polymorphism (RFLP) analysis and single stand conformational analysis (SSCA). However, any other known methods or later discovered methods can likewise be used to detect the mutations. Once the location of the 670/1 mutations are known and associated with AD, the methods to detect the mutations are standard in the art. The sequence of various nucleotide probes can be determined from the sequence of APP, especially the sequences surrounding 670/1. The sequence of APP is set forth in Yoshikai et al. (1990) "Genomic organization of the human amyloid beta-protein precursor gene," *Gene* 87:257–263.

Detection of point mutations using direct probing involves the use of oligonucleotide probes which may be prepared synthetically or by nick translation. The DNA probes may be suitably labeled using, for example, a radio label, enzyme label, fluorescent label, biotinavidin label and the like for subsequent visualization in the example of Southern blot hybridization procedure. The labeled probe is reacted with a bound sample DNA, e.g., to a nitrocellulose sheet under conditions such that only fully complementary sequences hybridize. The areas that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling may then be visualized, for example, by autoradiography. The labeled probe is reacted with a DNA sample bound to, for example, nitrocellulose under conditions such that only fully complementary sequences will hybridize. Tetra-alkyl ammonium salts bind selectively to A-T base pairs, thus displacing the dissociation equilibrium and raising the melting temperature. At 3M Me 4NCl this is sufficient to shift the melting temperature to that of G-C pairs. This results in a marked sharpening of the melting profile. The stringency of hybridization is usually 5° C. below the Ti (the irreversible melting temperature of the hybrid formed between the probe and its target sequence) for the given chain length. For 20 mers the recommended hybridization temperature is 58° C. The washing temperatures are unique to the sequence under investigation and need to be optimized for each variant.

Alternative probing techniques, such as ligase chain reaction (LCR), involve the use of mismatch probes, i.e., probes which are fully complementary with the target except at the point of the mutation. The target sequence is then allowed to hybridize both with oligonucleotides which are fully complementary and have oligonucleotides containing a mismatch, under conditions which will distinguish between the