Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 12180–12184, December 1995
Neurobiology

Identification and expression analysis of a potential familial Alzheimer disease gene on chromosome 1 related to *AD3*

JINHE LI*, JUNLI MA†, AND HUNTINGTON POTTER*‡

*Department of Neurobiology, Harvard Medical School, 220 Longwood Avenue, Boston, MA 02115; and †Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142

Communicated by Edward A. Kravitz, Harvard Medical School, Boston, MA, September 19, 1995 (received for review August 16, 1995)

ABSTRACT **The inheritance of much early-onset Alzheimer disease (AD) has been linked to a dominant-acting locus on chromosome 14. Recently, the gene likely responsible for this genetic linkage has been identified and termed *AD3*. Five mutations have been found in *AD3* that segregate with the disease phenotype in seven AD families and are not present in unaffected individuals. Here we report the existence of a gene encoding a seven transmembrane domain protein very similar to that encoded by *AD3* in structure and sequence. This gene is located on chromosome 1, is expressed in a variety of tissues, including brain, and is predicted to harbor mutations causing nonchromosome 14 familial AD. The presence of several S/TPXX DNA binding motifs in both the AD3 protein and the AD3-like protein/AD4 protein suggests a possible role in intracellular signaling and gene expression or in linking chromatin to the nuclear membrane. Ways in which mutations in either gene could lead to AD are discussed.**

Alzheimer disease (AD) is a neurodegenerative disorder of the central nervous system characterized by progressive deficits in memory and cognition and by certain neuropathological lesions—extracellular amyloid plaques and intracellular neurofibrillary tangles—that serve to define the disease at autopsy (1). Of the two AD lesions, amyloid deposition is the most definitive, since neurofibrillary tangles also occur in other neurodegenerative disorders and are a common feature of dying neurons.

The major component of the AD amyloid deposits is an ≈42-amino acid peptide, termed Aβ, which is derived by proteolytic cleavage from the much larger amyloid precursor protein (APP) (2, 3). In addition to Aβ, AD amyloid deposits also contain several other proteins, in particular antichymotrypsin (ACT) (4–8) and apolipoprotein E (apoE) (9, 10). These amyloid-associated proteins have been shown to bind to two specific regions of the Aβ peptide *in vitro* and to promote its polymerization into neurotoxic amyloid filaments (11–18).

Despite a uniformity in clinical and neuropathological features, AD appears to be genetically heterogeneous. At least 20% of cases are due to the inheritance of one of several autosomal dominant mutations located on several different chromosomes (19–24). Another 30% of cases reflect the activity of a few known genetic risk factors (13, 25–27), and the remainder are apparently sporadic and under no identifiable environmental or genetic influence.

Thus far, four genes have been identified in which nucleotide changes correlate with the inheritance of familial AD. The gene encoding the APP resides on chromosome 21 and can carry mutations in several positions that cause early-onset inherited AD with virtually 100% penetrance (19). These mutations have been shown to affect the synthesis, the processing, or the amyloidogenicity of the Aβ peptide (28–31). Mutations in the APP gene (now termed *AD1*) account for 2–3% of early-onset AD and <1% of all cases (32, 33).

The E4 allele of the apoE gene, located on chromosome 19 and termed *AD2*, has been linked by genetic and epidemiological studies to the late-onset (>65 years of age) form of familial AD (13, 25, 26). The apoE4 protein binds more strongly to the Aβ peptide *in vitro* than do the nonpathogenic E2 and E3 isoforms (13) and is a strong promoter ("pathological chaperone") of amyloid filament formation *in vitro* (10, 15–17).

The protease inhibitor ACT is associated with the AD amyloid deposits *in vivo* and, like apoE, binds to the Aβ peptide *in vitro* and promotes its polymerization into amyloid filaments (4–8, 11, 12, 15). Preliminary linkage analysis of the ACT gene on chromosome 14 and familial AD was weakly positive (H.P., unpublished results) and helped prompt further genetic investigation of AD and chromosome 14. Position q24 of chromosome 14 was first identified by Schellenberg *et al.* (20) and was rapidly confirmed by others (21–23) as harboring a mutation causing >70% of early-onset AD. Recently, the gene likely most responsible for the chromosome 14 linkage to early-onset AD has been identified and termed *AD3* (24). Five mutations in *AD3* segregate with the disease phenotype in seven AD families, while being absent from normal individuals. *AD3* encodes a membrane protein whose function in AD and normal physiology is unknown and will be discussed below.

A common allele of ACT itself has also recently been shown to constitute a genetic risk factor for developing inherited AD (27). The A allele of the ACT gene encodes an Ala residue (in place of Thr) in the signal peptide of ACT and may lead to enhanced secretion of this amyloid-promoting factor or pathological chaperone. The increased risk associated with the A allele of ACT is particularly striking when combined with the E4 allele of the apoE gene: the odds ratio increases from 4.4 for individuals with an apoE4/E4–ACT-T/T genotype to 34 for those with apoE4/E4–ACT-A/A. If it is confirmed, this result, with the amyloid-promoting activity of ACT, will make it reasonable to refer to ACT as "*AD5*."

In this paper we report on a gene on chromosome 1, which is highly homologous to *AD3* and may become "*AD4*"§ if mutations in it are found in families with nonchromosome 14 inherited AD.

MATERIALS AND METHODS

Identification of T03796 and Cloning of the Full-Length cDNA. All nucleotide sequences in the GenBank data base were translated into amino acid sequences in all six reading

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AD, Alzheimer disease; APP, amyloid precursor protein; ACT, antichymotrypsin; apoE, apolipoprotein E; EST, expressed sequence tag; RACE, rapid amplification of cDNA ends; UTR, untranslated region; YAC, yeast artificial chromosome; AD3LP, AD3-like protein.

‡To whom reprint requests should be addressed.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U34349).

```
AD3LP 1 MLTFMASDSE.....EEVCDERTSLMSAESPTPPSCQEGRQGPEDGENTSQWRSQEN.....EEDGEEDPDRYVCSGVPGRPPGLEEELTLKYGAKH
AD3   1 MTELPAPLSYFQNAQMSEDNHLSNTVRSQNDNRERQEHNDRRSLGHPEPLSNGRPQGNSRQVVEQDEEED.....EELTLKYGAKH

 88 VIMLFVPVTLCMIVVVATIKSVRFYTEKNGQLIYTPFTEDTPSVGQRLLNSVLNTLIMISVIVVMTIFLVVLYKYRCYKFIHGWLIMSSLMLLFLFTY
82 VIMLFVPVTLCMVVVVATIKSVSFYTRKDGQLIYTPFTEDTETVGQRALHSILNAAIMISVIVVMTILLVVLYKYRCYKVIHAWLIISSLLLLFFFSF
                                                                                  L               R

186 IYLGEVLKTYNVAMDYPTLLLTVWNFGAVGMVCIHWKGPLVLQQAYLIMISALMALVFIKYLPEWSAWVILGAISVYDLVAVLCPKGPLRMLVETAQE
182 IYLGEVFKTYNVAVDYITVALLIWNFGVVGMISIHWKGPLRLQQAYLIMISALMALVFIKYLPEWTAWLILAVISVYDLVAVLCPKGPLRMLVETAQE
                                                                                  E               V

284 RNEPIFPALIYSSAMVWTVGMAKLDPSSQGAL.....QLPYDPEMEDSYDSFGEPSYPEVFE.....PPLTGYPGEELEEEEES
278 RNETLFPALIYSSTMVWLVNMAEGDPEAQRRVSKNSKYNAESTERESQDTVAENDDGGFSEEWEAQRDSHLGPHRSTPESRAAVQELSSSILAGEDPE
      VV

358 QGGVKLGLGDFIFYSVLVGKAAATGSGDWNTTLACFVAILIGLCLTLLLLAVFKKALPALPISITFGLIFYFSTDRKHSRFIQMN.....  442
376 ERGVKLGLGDFIFYSVLVGKASATASGDWNTTIACFVAILIGLCLTLLLLAIFKKALPALPISITFGLVFYFATDYLVQPFMDQLAFHQFYI  467
                    V                 Y
```

FIG. 1. Derived protein sequence of a cDNA corresponding to the T03796 EST (AD3LP/AD5) compared to the protein sequence of AD3 (24). The two proteins are almost identical over large stretches and both include seven putative transmembrane domains (underlined). Mutations in *AD3* that lead to inherited AD are indicated below the AD3 line. Putative DNA-binding motifs of the form S/TPXX are shown in boldface type in both proteins. The sequence of the AD3LP/STM2/AD*4* gene was derived partly from the T03796 clone and partly from 5′ RACE-derived cDNA clones.

frames and then compared to the published protein sequence (24) of AD3 (S182), by using TBLASTN (National Center for Biotechnology Information server). The expressed sequence tag (EST) sequence (T03796) whose encoded protein was most highly homologous to AD3 was thus identified, and the corresponding cDNA clone was obtained from the Lawrence Livermore National Laboratory (IMAGE Consortium). After we had determined the full sequence of T03796 (see below), additional EST sequences identical to T03796 (GenBank accession nos. R16831 and R05822) were also identified. Because all of these EST clones were partial, 5′ rapid amplification of cDNA ends (RACE) was used to complete the full-length cDNA corresponding to T03796.

Sequencing and Analysis of DNA and Encoded Proteins. The T03796 sequence in GenBank consists of 476 nt, with some errors. We resequenced the reported region and then sequenced the rest of T03796 and the 5′ RACE clones for a total of 2.4 kb. DNA sequencing was carried out by the dideoxynucleotide chain-terminating procedure with fluorescent-tag-labeled deoxynucleotides by the Biopolymer Laboratory, Harvard Medical School. Oligonucleotide primers used for sequencing were also synthesized by the Biopolymer Laboratory. Sequences were compared for homology to other sequences in the GenBank, GenEMBL, and dbest data bases, by using FASTA (GCG software package from the Wisconsin Genetics Computer Group) and by BLASTN and BLASTP (National Center for Biotechnology Information server).

Chromosome Localization. Primers in the 3′ untranslated region (UTR) of T03796 were used in a PCR assay of DNA samples from panels of human–rodent somatic cell hybrid cell lines (Mapping panels 1 and 2, Coriell Institute for Medical Research, Camden, NJ). The same primers were also used to establish a yeast artificial chromosome (YAC) contig to link the T03796 [AD3-like protein (AD3LP)] gene to sequence target sites. Primer pairs were 5′-CTTTGTCCATGATGTCCTTG-3′/5′-ATTAAGCCACTTCCCAGCAG-3′ and 5′-ACGGACAGGAAGCACAGCAG-3′/5′-ACACTCTTTGCGCATCTCAG-3′.

Northern Blot Analysis. A single-stranded probe corresponding to the 3′ UTR of AD3LP (T03796) was radiolabeled with [α-^{32}P]dCTP by using an asymmetric PCR from the M13 universal primer. The primer was hybridized to a multitissue Northern (MTN) blot (Clontech) containing 2 μg of poly(A)$^+$ RNA per lane. The blot was hybridized at 42°C and washed at 65°C in 0.1× SSC/0.1% SDS according to manufacturer's instructions. The blot was then exposed to x-ray film overnight with double intensifying screens at −70°C.

RESULTS

Recently, a gene on chromosome 14 (*AD3*) was identified and shown to harbor mutations that cosegregated with inherited AD in several affected families (24). In an attempt to obtain cDNA (or EST) sequences and clones corresponding to *AD3*, and potentially additional related genes, we computer-translated all of the known nucleotide sequences in GenBank in all six reading frames and compared the resulting protein sequences to the published protein sequence of AD3. This search revealed a particular EST (accession no. T03796) whose putative translation product was very similar to that of AD3—an apparent seven transmembrane domain protein. Examination of areas of difference in the two protein sequences indicated that T03796 was likely to be a partial cDNA. We then used 5′ RACE to complete the cloning of the corresponding gene. Fig. 1 shows a comparison of the protein sequences of AD3 and of the gene corresponding to T03796. The two encoded proteins of 442 and 467 amino acids,

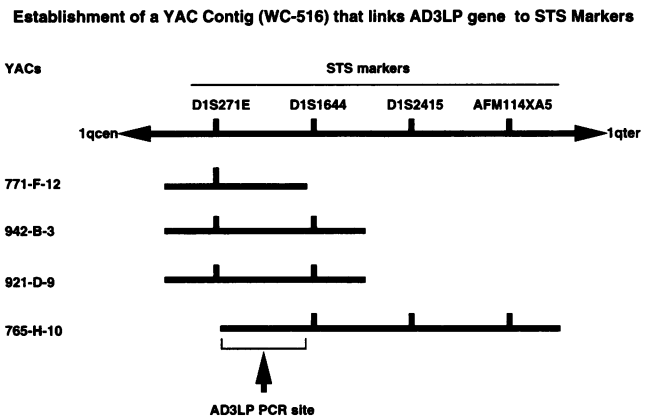

FIG. 2. Chromosome localization of T03796. Human–hamster somatic cell hybrid DNAs were screened by PCR to identify chromosome 1 as the locus of T03796. Further localization was carried out by PCR screening of a YAC contig library. Four YACs mapping to chromosome 1 were so identified and are indicated with the map positions of the sequence target site (STS) sequences that they encompass.

respectively, are almost identical in some regions but completely diverge in others. In particular, the regions between the sixth and seventh transmembrane domains are a different size and sequence in the two proteins. Evidently, the human genome harbors a second gene that is similar to *AD3* at the nucleotide level and encodes a highly homologous protein.

The structural similarity between AD3 and the translation product of the T03796 gene suggests that the two proteins may have related functions. One possibility would be to serve as membrane receptors. Also, examination of the protein sequences of AD3 and T03796 revealed the presence of several DNA-binding motifs of the form S/TPXX (34). These are indicated in Fig. 1. Such motifs suggest that a normal function of these two proteins may involve the control of gene expression or the movement or organization of chromatin.

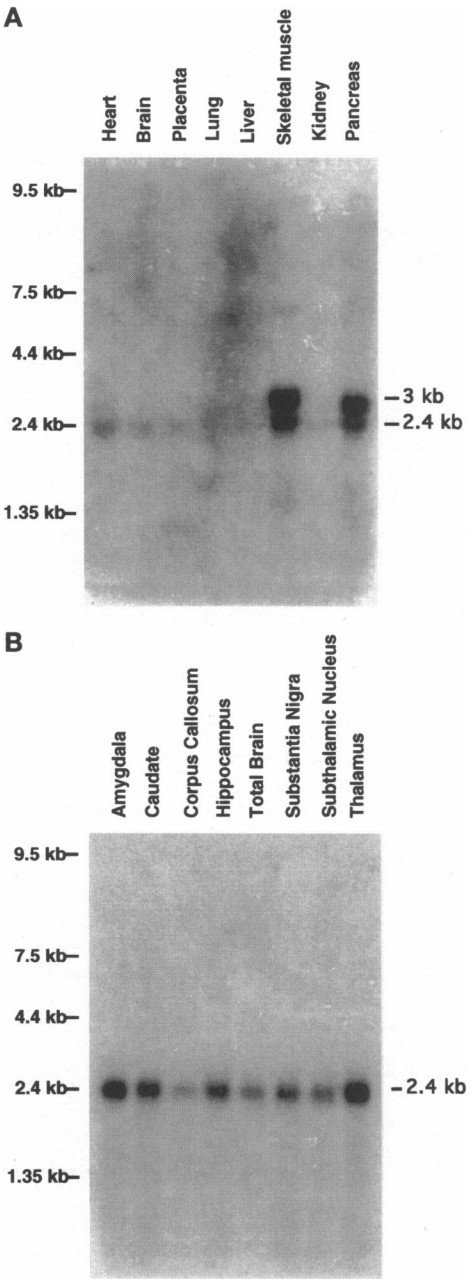

FIG. 3. Northern blot analysis. Two micrograms of poly(A)$^+$ RNA from the indicated tissues was electrophoresed, blotted, and probed with a radiolabeled PCR fragment. Transcripts of 2.4, 3.0, and 7.0 kb were detected that were expressed in different patterns in different tissues. Expression in the brain was relatively uniform in different regions and was restricted primarily to the 2.4-kb transcript.

The final confirmation that T03796 corresponds to a gene related to the chromosome 14 AD gene, *AD3*, was obtained by genomic mapping and Northern blot analysis. For genomic mapping, two pairs of oligonucleotide primers were designed on the basis of the T03796 sequence and used for PCR analysis of genomic DNAs isolated from panels of human–rodent somatic cell hybrid cell lines. Chromosome 1 rather than chromosome 14, where *AD3* resides, was unequivocally identified as the site of the gene corresponding to T03796. Sublocalization with PCR of YAC libraries placed the gene corresponding to T03796 on the long arm of chromosome 1, ≈290 centimorgans from the centromere. The four YACs encompassing T03796 sequences are shown in Fig. 2, with the corresponding sequence target site locations.

Northern blot analysis of various tissues was carried out using a radiolabeled PCR fragment from the 3′ UTR of T03796 as a probe (Fig. 3). All tissues analyzed, including brain, expressed this gene. Three bands corresponding to 7.0 kb, 3.0 kb, and 2.4 kb were observed, with different tissues expressing different levels of the three transcripts. The two transcripts of 7.0 kb and 3.0 kb are similar in size to those corresponding to *AD3* but are not due to cross-reaction because the PCR probe was chosen to be specific for T03796. The pattern of *AD3* gene expression is fairly uniform, with reduced expression in the liver (24). In contrast, T03796 is very highly expressed in skeletal muscle and pancreas, where, unlike in other tissues, the 3.0-kb transcript is particularly prominent. T03796 is expressed uniformly throughout the brain, including

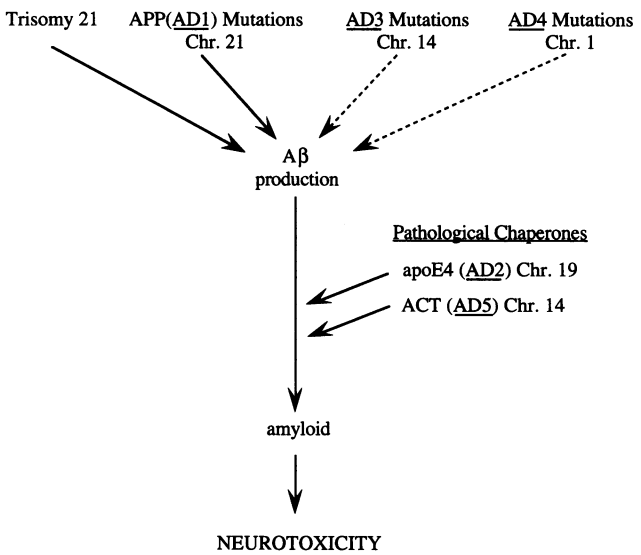

FIG. 4. Genetic mutations implicated in AD. At least 50% of AD cases are caused or strongly influenced by one or more genetic alterations in the affected individuals. In addition to the striking effect of trisomy 21, which leads to AD in virtually 100% of Down syndrome patients (51), five genes (*AD1–5*) have been identified that can harbor point mutations that cause or contribute to AD in otherwise-normal individuals. Mutations in three of these genes (APP, now termed *AD1*) (19), *AD3* (20–24), and AD3LP/STM2/AD4 (reported here and in refs. 52–54) are dominant and lead to AD with 100% penetrance, while inheritance of certain alleles of the apoE (*AD2*) and ACT (*AD5*) genes strongly increases the risk of developing AD (4, 5, 10–18, 26, 27). The points at which these various alterations are currently believed to function in the AD pathogenic pathway are indicated. Trisomy 21 and mutations in *AD1* act by increasing the production or amyloidogenicity of the Aβ peptide (28–31, 36). Recent studies of fibroblast cultures and sera from individuals carrying mutations in *AD3* and STM2/AD4 (55, 56) suggest that these genes may act indirectly to effect the same result, as shown by the dotted lines in the figure. The apoE and ACT proteins function in the next step in the pathway, as amyloid promoting factors or "pathological chaperones."

areas prone to AD pathology. The 2.4-kb transcript is most prominent.

DISCUSSION

Sequence comparison, chromosome localization, and expression analysis have demonstrated the existence of a gene related to *AD3*—the familial AD gene on chromosome 14. In contrast to *AD3*, this gene is located on the long arm of human chromosome 1 and, like *AD3*, is expressed in a wide range of tissues, including various regions of the brain.

The fact that the proteins encoded by *AD3* and its chromosome 1 homologue represented by the T03796 EST clone are nearly identical in certain regions and share an overall structural organization indicates a likely similarity of function. Thus, mutations in this gene, like those found in *AD3*, may similarly lead to some cases of familial AD. In this vein it is noteworthy that each of the five known mutations in *AD3* that lead to AD change amino acids that are identical in the T03796-encoded AD3LP.

Several families with early-onset inherited AD, in particular the Volga Germans, do not show linkage to chromosome 14 (32) and might be predicted instead to harbor mutations in the T03796/AD3LP gene sequence, putatively AD*4* (see *Note*).

Neither AD3 nor STM2/AD4 show sufficient homology to other proteins of known function to allow their roles in normal physiology or AD to be clearly deduced by analogy. However, several sequence motifs can be recognized in both proteins that provide hints as to their possible function. For example, both *AD3* and *STM2/AD4* appear to encode integral membrane proteins with seven transmembrane domains. Many such proteins have been identified and perform a number of physiological functions (35). These include serving as receptors, often coupled to G proteins, acting as ion channels, and functioning as links between the cytoskeleton or nucleoskeleton and the particular membrane in which the protein is localized. Any of these functions could, if changed by mutation, lead to altered expression or processing of APP to yield increased amounts of the amyloidogenic Aβ peptide. For instance, by influencing the intracellular trafficking of membrane-bound vesicles containing the APP protein, AD3 or STM2/AD4 may alter the processing in the Aβ peptide (24).

Interestingly, most of the mutations in *AD3* that cause familial AD reside in or near the transmembrane domains. The other known genetic mutations that contribute to AD are also related to lipid-binding functions of their host proteins: the APP mutations are in a membrane protein, often within the transmembrane segment; apoE4 is a lipid carrier protein involved in membrane recycling; the A allele of the ACT gene involves the membrane-targeting signal sequence of the ACT protein. Perhaps the required intramembrane processing of APP at the γ-secretase site is exquisitely sensitive to changes in both APP and in other membrane-associated proteins, such as apoE, ACT, AD3, and STM2/AD4.

Another protein motif of potential interest, which occurs four times in STM2/AD4 and twice in AD3, is a DNA-binding sequence of the form S/TPXX (34). Some of these sequences are also potential sites of phosphorylation by the $p34^{cdc2}$ kinase involved in cell cycle control, and one such sequence occurs in the identical position in the two proteins. The presence of potential DNA-binding regions in AD3 and STM2/AD4 indicates that these proteins may be involved in the control of gene expression. If mutations in AD3 or STM2/AD4 were to increase expression of APP, it could lead to AD just as overexpression of APP likely underlies the AD neuropathology seen in Down syndrome (36) and some APP transgenic mice (37, 38). Alternatively, changes in the expression of APP processing enzymes might cause AD through the overproduction of amyloidogenic forms of the Aβ peptide. Interestingly, the ACT (AD4) protein also binds DNA and has been found localized in the nuclei of neurons and other cells (4, 6, 39–42).

Their DNA-binding motifs also suggest a possible role for AD3 and STM2/AD4 in linking chromatin to the nuclear membrane during the cell cycle. Several nuclear membrane proteins appear to have such a function and at least two—the lamin B receptor and *NDC1*—have seven or eight transmembrane domains and several S/TPXX DNA-binding motifs (43–46). The lamin B receptor does, in fact, bind DNA (46) and, when mutant, *NDC1* leads to aberrant chromosome segregation (45). The seven transmembrane protein that is most homologous to AD3 (*Caenorhabditis elegans* spe-4) is also located in an organelle double-membrane (like the nuclear double-membrane) and is required for correct spermatid formation and segregation during meiosis (24, 47). Examination of the spe-4 sequence reveals three S/TPXX DNA-binding motifs. AD3 and STM2/AD4 may share with these structurally similar membrane proteins responsibility for the correct organization and segregation of chromosomes.

A potential chromatin-binding function for AD3 and STM2/AD4 is of interest in the light of growing evidence that chromosome nondisjunction and trisomy 21 may play a role in AD (48–51). The most recent support of such a model is our finding that AD and Down syndrome individuals share, in addition to a similar dementia and neuropathology, a marked hypersensitivity to the pupil-dilating effect of cholinergic antagonists such as tropicamide (50). Moreover, when fluorescence *in situ* hybridization was used to study fibroblasts from AD and control individuals, the AD cells were found to have an increase in trisomy 21 (ref. 51; L. Geller and H. P., unpublished results). If the AD3 and STM2/AD4 AD-related proteins are indeed present in the nuclear membrane and involved in the organization of chromatin, then mutations in these genes could lead to chromosome nondisjunction, trisomy 21, and AD neuropathology.

AD results from a series of steps—a pathogenic pathway—that leads to amyloid formation and neurodegeneration in key areas of the brain involved in cognition and memory. Biochemical studies and the identification of several genes in which nucleotide changes can lead to AD have provided much information about this pathway (ref. 51 and Fig. 4). The future elucidation of the normal and pathological functions of the proteins encoded by *AD3*, and its relative, STM2/AD*4*, reported herein and in refs. 52–54, should provide additional insights and may aid in the rational design of AD therapies.

Note. While this paper was being reviewed, Schellenberg and Levy-Lahad and colleagues (52, 53) and St. George-Hyslop and colleagues (54) published important related studies. The first (52) showed linkage of a 15-centimorgan region of chromosome 1 to the inheritance of AD in the Volga German pedigrees. The closest linked marker in that study, *D1S479*, is adjacent to the small region of chromosome 1 to which we have localized the T03796 sequence. In the second (53) and third (54) reports, the similarity of a differently spliced transcript of the T03796 gene [variously termed STM2, for the second seven transmembrane gene associated with AD or PS2, for presenilin 2] to *AD3* is presented with a single point mutation in the gene that is present in affected individuals in five of seven Volga German families. The combined results are most consistent with the T03796 gene being responsible for the chromosome 1 linkage and the disease phenotype in these nonchromosome 14 families. Thus T03796/AD3LP/STM2/PS2 is the fourth gene (*AD4*) to be causally associated with inherited AD.

Note Added in Proof. Recently, the proposition discussed here that *AD3* and *AD4* are involved in intracellular signaling and control of gene expression and/or chromosome localization and segregation has received support from the genetic studies of Levitan and Greenwald (57), who identified the *Caenorhabditis elegans* homologue of *AD3* and *AD4* (termed *sel-12*) by virtue of its ability to suppress or enhance the activity of *lin-12*, a member of the Notch family of cell surface receptors.

We are grateful to Ricardo Moreno and Lewis Albert Andres for assistance and to Gerard Schellenberg for helpful discussion. The work was supported by National Institutes of Health Grants AG08084 and AG09665, and a grant by the Freudenberger family. J. L. is supported by a training grant from the National Institute of Aging.

1. Terry, R. D. & Katzman, R. (1983) *Ann. Neurol.* **14,** 497–506.
2. Muller-Hill, B. & Beyreuther, K. (1989) *Annu. Rev. Biochem.* **58,** 287–307.
3. Selkoe, D. J. (1994) *Annu. Rev. Cell Biol.* **10,** 373–403.
4. Abraham, C. R., Selkoe, D. J. & Potter, H. (1988) *Cell* **52,** 487–501.
5. Abraham, C. R., Selkoe, D. J., Potter, H., Price, D. C. & Cork, L. C. (1989) *Neuroscience* **32,** 715–720.
6. Abraham, C. R., Shirahama, T. & Potter, H. (1990) *Neurobiol. Aging* **11,** 123–129.
7. Rozemuller, J. M., Stam, F. C. & Eikelenboom, P. (1990) *Neurosci. Lett.* **119,** 75–78.
8. Rozemuller, J. M., Abbink, J. J., Kamp, A. M., Stam, F. C., Hack, C. E. & Eikelenboom, P. (1991) *Acta Neuropathol.* **82,** 200–207.
9. Namba, Y., Tomonaga, M., Kawasaki, H., Otomon, E. & Ikeda, K. (1991) *Brain Res.* **541,** 163–166.
10. Wisniewski, T. & Frangione, B. (1992) *Neurosci. Lett.* **135,** 235–238.
11. Potter, H., Abraham, C. R. & Dressler, D. (1991) in *Alzheimer's Disease: Basic Mechanisms, Diagnosis, and Therapeutic Strategies*, eds. Iqbal, K., MacLachlan, D. R. C., Winblad, B. & Wisniewski, H. M. (Wiley, New York), pp. 275–279.
12. Potter, H., Nelson, R. B., Das, S., Siman, R., Kayyali, U. S. & Dressler, D. (1992) *Ann. N.Y. Acad. Sci.* **674,** 161–173.
13. Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G. S. & Roses, A. D. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1977–1981.
14. Wisniewski, T., Golabek, A., Matsubara, E., Ghiso, J. & Frangione, B. (1993) *Biochem. Biophys. Res. Commun.* **192,** 359–365.
15. Ma, J., Yee, A., Brewer, H. B., Das, S. & Potter, H. (1994) *Nature (London)* **372,** 92–94.
16. Sanan, D. A., Weisgraber, K. H., Russell, S. J., Mahley, R. W., Huang, D., Saunders, D. A., Schmechel, D., Wisniewski, T., Frangione, B., Roses, A. D. & Strittmatter, W. J. (1994) *J. Clin. Invest.* **94,** 860–869.
17. Wisniewski, T., Castaño, E. M., Golabek, A., Vogel, T. & Frangione, B. (1994) *Am. J. Pathol.* **145,** 1030–1035.
18. Evans, K. C., Berger, E. P., Cho, C.-G., Weisgraber, K. H. & Lansbury, P. T. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 763–767.
19. Ashall, F. & Goate, A. M. (1994) *Trends Biochem. Sci.* **19,** 42–46.
20. Schellenberg, G. D., Bird, T. D., Wijsman, E. M., Orr, H. T., Anderson, L., Nemens, E., White, J. A., Bonnycastle, L., Weber, J. L., Alonso, M. E., Potter, H., Heston, L. L. & Martin, G. M. (1992) *Science* **258,** 668–671.
21. St. George-Hyslop, P., Haines, J., Rogaev, E., Mortilla, M., Vaula, G., *et al.* (1992) *Nat. Genet.* **2,** 330–334.
22. Van Broeckhoven, C., Backhovens, H., Cruts, M., De Winter, G., Bruyland, M., Cras, P. & Martin, J.-J. (1992) *Nat. Genet.* **2,** 335–339.
23. Mullan, M., Houlden, H., Windelspecht, M., Fidani, L., Lombardi, C., Diaz, P., Crook, R., Hardy, J., Duff, K. & Crawford, F. (1992) *Nat. Genet.* **2,** 340–343.
24. Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., *et al.* (1995) *Nature (London)* **375,** 754–760.
25. Pericak-Vance, M. A., Bebout, J. L., Gaskell, P. C., Yamaoka, L. H., Hung, W.-Y., Alberts, M. J., Walker, A. P., Bartlett, R. J., Haynes, C. A., Welsh, K. A., Earl, N. A., Heyman, A., Clark, C. M. & Roses, A. D. (1991) *Am. J. Hum. Genet.* **48,** 1034–1050.
26. Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L. & Pericak-Vance, M. A. (1993) *Science* **261,** 921–923.
27. Kamboh, M. I., Sanghera, D. K., Ferrell, R. E. & DeKosky, S. T. (1995) *Nat. Genet.* **10,** 486–488.
28. Wisniewski, T., Ghiso, J. & Frangione, B. (1991) *Biochem. Biophys. Res. Commun.* **179,** 1247–1254.
29. Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I. & Selkoe, D. J. (1992) *Nature (London)* **360,** 672–674.
30. Cai, X.-D., Golde, T. E. & Younkin, S. G. (1993) *Science* **259,** 514–516.
31. Suzuki, N., Cheung, T. T., Cai, M.-D., Odaka, A., Otvos, L., Eckman, C., Golde, T. E. & Younkin, S. G. (1994) *Science* **264,** 1336–1340.
32. Schellenberg, G. D., Pericak-Vance, M. A., Wijsman, E. M., Morre, D. K., Gaskell, P. C., Yamaoka, L. A., Bebout, J. L., Anderson, L., Welsh, K. A., Clark, C. M., Martin, G. M., Roses, A. D. & Bird, T. D. (1991) *Am. J. Hum. Genet.* **48,** 563–583.
33. Tanzi, R. E., Vaula, G., Romano, D. M., Mortilla, M., Huang, T., *et al.* (1992) *Am. J. Hum. Genet.* **51,** 273–282.
34. Suzuki, M. (1989) *J. Mol. Biol.* **207,** 61–84.
35. Dohlman, H. G., Thorner, J., Caron, G. & Lefkowitz, R. J. (1991) *Annu. Rev. Biochem.* **60,** 653–688.
36. Neve, R. L., Finch, E. A. & Dawes, L. R. (1988) *Neuron* **1,** 669–677.
37. Quon, D., Wang, Y., Catalano, R., Scardina, J. M., Murakami, K. & Cordell, B. (1991) *Nature (London)* **352,** 239–241.
38. Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., *et al.* (1995) *Nature (London)* **373,** 523–527.
39. Katsunuma, T., Tsuda, M., Kusumi, T., Ohkuda, T., Mitomi, T., Nakasaki, H., Tajima, T., Yokoyama, S., Kamiguchi, H., Kobayashi, K. & Shinoda, H. (1980) *Biochem. Biophys. Res. Commun.* **93,** 552–557.
40. Takada, S., Tsuda, M., Fujinami, M., Yamamura, M., Mitomi, T. & Kasunuma, T. (1986) *Cancer Res.* **46,** 3688–3691.
41. Tahara, E., Ito, H., Taniyama, K., Yokozaki, H. & Haifa, J. (1984) *Hum. Pathol.* **15,** 957–964.
42. Naidoo, N., Cooperman, B. S., Wang, Z., Liu, X. & Rubin, H. (1995) *J. Biol. Chem.* **270,** 14548–14555.
43. Worman, H. J., Evans, C. D. & Blobel, G. (1990) *J. Cell Biol.* **111,** 1535–1542.
44. Foisner, R. & Gerace, L. (1993) *Cell* **73,** 1267–1279.
45. Winey, M., Hoyt, M. A., Chan, C., Goetsch, L., Botstein, D. & Byers, B. (1993) *J. Cell Biol.* **122,** 743–751.
46. Ye, Q. & Worman, H. J. (1994) *J. Biol. Chem.* **269,** 11306–11311.
47. L'Hernault, S. W. & Arduengo, P. M. (1992) *J. Cell Biol.* **119,** 55–68.
48. Potter, H. (1991) *Am. J. Hum. Genet.* **48,** 1192–1200.
49. Schupf, N., Kapell, D., Lee, J. H., Ottman, R. & Mayeux, R. (1994) *Lancet* **344,** 353–356.
50. Scinto, L. F. M., Daffner, K. R., Dressler, D., Ransil, B., Rentz, D., Weintraub, S., Mesulam, M. M. & Potter, H. (1994) *Science* **266,** 1051–1054.
51. Potter, H., Ma, J., Das, S., Geller, L. N., Benjamin, M., Kayyali, U. S. & Dressler, D. (1995) in *Research Advances in Alzheimer's Disease and Related Disorders*, eds. Iqbal, K., Mortimer, J. A., Winblad, B. & Wisniewski, W. H. (Wiley, New York), pp. 643–654.
52. Levy-Lahad, E., Wijsman, E. M., Nemens, E., Anderson, L., Goddard, K. A. B., Weber, J. L., Bird, T. D. & Schellenberg, G. D. (1995) *Science* **269,** 970–973.
53. Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D. M., Oshima, J., *et al.* (1995) *Science* **269,** 973–977.
54. Rogaev, E. I., Sherrington, R., Rogaeva, E. A., Levesque, G., Ikeda, M., *et al.* (1995) *Nature (London)* **376,** 775–778.
55. Scheuner, D., Bird, T., Citron, M., Lannfelt, L., Schellenberg, G., Selkoe, D., Viitanen, M. & Younkin, S. G. (1995) *Soc. Neurosci. Abstr.* **21,** 1500.
56. Song, X.-H., Suzuki, N., Bird, T., Peskind, E., Schellenberg, G. & Younkin, S. G. (1995) *Soc. Neurosci. Abstr.* **21,** 1501.
57. Levitan, D. & Greenwald, I. (1995) *Nature (London)* **377,** 351–354.