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Monogenetic determinants of Alzheimer's disease: *APP* mutations

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Abstract. Mutations within exons 16 and 17 of the β-amyloid precursor protein (APP) gene were the first known cause of familial Alzheimer's disease. These mutations are rare and have been reported in a handful of families exhibiting autosomal dominant inheritance of Alzheimer's disease with age of onset around 50 years. In vitro and in vivo studies have demonstrated that each of these mutations alters proteolytic processing of APP, resulting in an increase in the production of Aβ42, a highly fibrillogenic peptide, that spontaneously aggregates and deposits in the brain. Transgenic mice carrying a mutant human APP gene also show age-dependent β-amyloid (Aβ) deposition in the brain. The rate of deposition in these mice can be modified by apolipoprotein E expression.

Key words. APP; APOE; Aβ42; proteolytic cleavage; age of onset.

Mutations in the *APP* gene that cause familial AD

A small number of pedigrees have been described in which Alzheimer's disease (AD) is inherited as an autosomal dominant trait with age-dependent penetrance [1]. The age of onset in these families is usually below 65 years. A genetic linkage strategy has been successfully used in such families to identify mutations causing familial AD (FAD). The first mutation to be linked to FAD was reported in 1991 [2]. This was a missense mutation (valine-to-isoleucine at codon 717 – V717I) within the gene (*APP*) coding for the β-amyloid precursor protein (APP) in a family with disease onset in the mid-fifties. The V717I mutation has since been observed in 16 families from both Europe and Japan [3–9]. Haplotype analysis has demonstrated that these families are unrelated to one another, indicating that the V717I mutation in each of these families was an independent mutational event [9]. Four other mutations have been reported within exons 16 and 17 of the *APP* gene that also cause FAD (fig. 1). Two of these mutations also occur at codon 717 (in exon 17) but result in valine-to-glycine (V7171G) and valine-to-phenylalanine (V717F) substitutions, while a third mutation is in the adjacent codon and results in an isoleucine-to-valine (I716V) substitution [10–12]. A double mutation in exon 16 in a large Swedish kindred results in amino acid substitutions in codons 670 and 671 (KM670/671NL) [13]. With the exception of the V717I mutation, each mutation has been described in a single kindred.

APP mutations and cerebral haemorrhage

Two other mutations have been reported within the *APP* gene that are associated with a different disease phenotype. The APP693 (E693Q) mutation was the first *APP* mutation to be linked to disease. It is the causative mutation for hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D), a rare autosomal dominant disorder characterized by recurrent cerebral haemorrhage beginning in the fifth decade of life [14]. The cerebral haemorrhages are ultimately fatal. Despite the difference in clinical presentation the pathological phenotype of HCHWA-D shares similarities with AD. An insoluble proteolytic product of APP metabolism, Aβ, is deposited in the cerebral blood vessel walls (amyloid angiopathy) and as diffuse plaques

within the brain parenchyma in both disorders. However, neuritic plaques and neurofibrillary tangles are not seen in HCHWA-D. A second mutation at the adjacent codon APP692 (A692G) is associated with a mixed phenotype of AD or cerebral haemorrhage with amyloidosis (CHWA) [15]. It is not presently known what factor(s) influence the development of AD vs. CHWA in individuals carrying the APP692 mutation.

Since the identification of the first mutation many FAD cases have been screened for mutations within exons 16 and 17 of the *APP* gene [16–19]. However, far fewer cases have actually been screened for mutations elsewhere within the *APP* gene [20]. With this caveat, it would appear that mutations within the *APP* gene are a rare cause of FAD, accounting for no more than 5–10% of cases.

Phenotypic characteristics of AD caused by APP mutations

The age of onset of AD associated with mutation within the *APP* gene varies from the early forties to the early sixties. Anecdotal evidence suggests that variation in age of onset within a family may be attributable to apolipoprotein E (*APOE*) genotype, with *APOE* ε4 carriers having an earlier onset than non-ε4 carriers [21, 22]. Other than age of onset, the disease in these families is not atypical. Studies using positron emission tomography (PET) in *APP* mutation families have demonstrated a decrease in hippocampal volume that is detectable up to a decade before the appearance of clinical symptoms [23]. Several individuals in *APP* mutation families have been reported to suffer prodromal headaches, as have individuals in some presenilin families; the significance of this is presently not understood [24, 25].

The disease in these families is characterized pathologically by severe β-amyloid (Aβ) deposition, particularly Aβ42 deposition [26, 27]. Some, but not all, individuals show significant numbers of cortical Lewy bodies, suggesting that their presence does not define a separate disease entity but is part of the spectrum of phenotypes associated with AD.

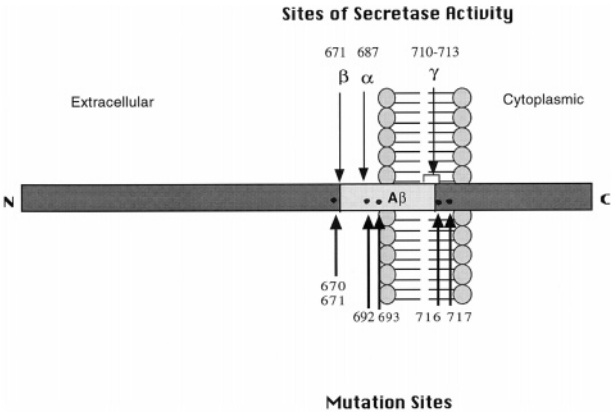

Figure 1. Schematic diagram of APP. The Aβ-coding portion is shown in light grey. Sites of APP mutations and the secretase cleavage sites are given. Site numbering is with reference to the 770 splice variant of APP.

Proteolytic processing of APP

The location of the mutations within APP protein suggested that they may interfere with normal proteolytic processing of APP. The FAD-causing mutations flank the Aβ sequence, while the CHWA mutations occur within the Aβ sequence (fig. 1). Transfection and transgenic experiments were initiated to try to understand the mechanism(s) by which these mutations cause disease. APP is a type I membrane protein that undergoes complex posttranslational modification including glycosylation, phosphorylation, sulphation and proteolytic processing within the secretory pathway. Cleavage by α-secretase occurs at or near the cell surface and results in the release of a large soluble N-terminal fragment, sAPP [28, 29]. The membrane-bound C-terminal fragment is internalized via clathrin-coated vesicles and degraded via the lysosomal system [30]. Cleavage by α-secretase precludes Aβ formation (fig. 1). An alternate processing pathway by activities referred to as β and γ secretases results in the generation of Aβ [31, 32]. Aβ can be generated within the endoplasmic reticulum (ER) but may also be generated at other sites, since soluble Aβ is detected within the ER and in the medium of cultured cells [33–35]. The extracellular pool of Aβ is 90% Aβ40 (40-amino acid peptide) and 10% Aβ42 (with 2 additional amino acids at the C-terminus), whereas almost all of the intracellular Aβ is Aβ42.

Effect of mutations on APP processing

The Swedish mutation was quickly shown to cause a 5–10-fold increase in Aβ production leading to elevated levels of Aβ40 and Aβ42 [36, 37]. The location of the 670/671 mutation, right at the β-secretase cleavage site suggested that the increase in Aβ levels may be due to a preference of the β-secretase for the mutant sequence. However, inhibitor studies suggest that the elevated Aβ production may be due to an alternate β-secretase-like activity [38]. In contrast, the *APP717* and *APP716* mutations have no effect on Aβ40 levels but cause a specific increase in Aβ42 lev-

els [39, 12]. In vitro, Aβ42 has been shown to be far more prone to aggregation than Aβ40, suggesting that a modest change in Aβ42 levels could potentially cause a significant increase in Aβ deposition [40]. Analysis of intracellular and extracellular pools of Aβ suggest that the percentage increase in Aβ42 levels, caused by an *APP717* mutation, is greater in the intracellular compartment, although absolute levels are higher in the extracellular compartment [35]. The significance, if any, of this observation is not clear. The introduction of both the 717 and a 716 mutation into a complementary DNA (cDNA) construct leads to a bigger increase in Aβ42 levels than with either mutation alone [12].

Studies using plasma or fibroblast cultures from individuals carrying *APP* mutations show that similar increases in Aβ levels are detectable in vivo [41]. Longitudinal studies are not yet available in humans to determine how these levels vary during the course of the disease, although it is known that Aβ42 levels are elevated in presymptomatic individuals.

Developing an animal model of Aβ deposition

The development of transgenic mice overexpressing mutant *APP* cDNAs has enabled such longitudinal studies to be performed in a model system. During the last couple of years several transgenic mouse lines have been constructed that develop spontaneous age-dependent Aβ deposition in the brain [42, 43]. Although there are differences in the promoters, the exact cDNA construct and the FAD mutation used, the common theme of these animals is 3–5$\times$ overexpression in neurons of a human *APP* cDNA carrying an FAD mutation. These animals deposit large amounts of Aβ within plaques in the cortex with increasing age, but this is not accompanied by extensive tau pathology or neuronal degeneration. APP synthesis remains relatively constant over time, but soluble Aβ levels increase dramatically with age [44]. The regions of the brain most severely affected by pathology also appear to be those that show the highest Aβ levels, suggesting that there may be a threshold effect. However, the lack of Aβ deposition in unaffected brain regions even in the presence of high Aβ levels (Aβ levels in the thalamus of homozygous animals are equivalent to Aβ levels in the cortex of heterozygous animals) argues that elevated Aβ levels alone are insufficient to cause amyloid deposition [44]. This suggests that there are region-specific factors that either promote or inhibit Aβ deposition. At present, the identity of these factors is unknown. There are several possible explanations for the absence of neuronal cell loss even in the presence of massive Aβ deposition: that Aβ is less toxic to mouse neurons than human neurons, that Aβ fibrillogenesis alone is not sufficient to cause neurodegeneration or that the concurrent overexpression of the neuroprotective secreted APP molecule (sAPP) prevents neurodegeneration even in the context of Aβ overproduction. These mouse models differ fundamentally from the human disease in two ways: FAD mutations do not lead to overexpression of APP (although wild-type APP is overexpressed in Down's syndrome, DS); the temporal and spatial expression pattern differs from that seen in FAD because heterologous promoters are used (the platelet-derived growth factor, prion or Thy-1 promoters).

Recently, these *APP* mice have been bred with mutant presenilin 1 (*PS1*) mice and with *APOE* knockout mice to make doubly transgenic animals [45, 46]. In both cases the phenotype of the *APP* mice was modified by the second transgene. Transgenic mice carrying a mutant *PS1* gene do not show any Aβ deposition, but they do show elevated levels of mouse Aβ42/43 [47–49]. When these animals are bred with an animal carrying a mutant human *APP* gene, the resulting animals have higher levels of human Aβ42/43 and deposit amyloid at an earlier age than do animals transgenic for the mutant human *APP* gene alone [47]. This result is consistent with the presumed mechanism of *PS* mutations based on measurement of Aβ42/43 levels in patients and transfected cell lines [41].

Breeding mutant *APP* mice onto an *APOE* knockout background results in a decrease in Aβ deposition [45]. This decrease in Aβ deposition is dose-dependent, with Aβ deposition being highest in animals with two copies of *APOE* and lowest in animals with no functional copies of *APOE*. Animals having one copy of *APOE* show intermediate levels of Aβ deposition. This result suggests that *APOE* could increase risk for AD by modifying Aβ deposition but does not explain the allelic differences in risk for AD that have been widely observed in human populations. Recent data from our laboratory and that of Dr. Fernando Valdivieso suggest that there are polymorphisms in the promoter of *APOE* in human populations that alter the basal level of *APOE* transcription in astrocytes and also alter the risk for AD [50]. Consistent with the observation in the *APOE* knockout mice, individuals that have higher basal expression levels of *APOE* are at higher risk for AD than those who express APOE at a lower level. Together these pieces of data suggest that variation in the *APOE* gene may alter risk for AD by modifying the amount of Aβ deposited in the brain. This strongly suggests that in sporadic AD, as in familial AD, Aβ deposition is central to AD pathogenesis.

Summary

The observation of mutations within the *APP* gene in familial AD that increase Aβ42 deposition and of normal variants of *APOE* in sporadic AD that increase *APOE* expression (and possibly Aβ deposition) suggests

that modifying Aβ deposition may be a key pathogenic mechanism in all cases of AD.

1 Nee L. E., Polinsky R. J., Eldridge R., Weingartner H., Smallberg S. and Ebert M. (1983) A family with histologically confirmed Alzheimer's disease. Arch. Neurol. **40:** 203–208
2 Goate A., Chartier-Harlin M. C., Mullan M., Brown J., Crawford F., Fidani L. et al. (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature **349:** 704–706
3 Naruse S., Igarashi S., Kobayashi H., Aoki K., Inuzuka T., Kaneko K. et al. (1991) Mis-sense mutation Val → Ile in exon 17 of amyloid precursor protein gene in Japanese familial Alzheimer's disease. Lancet **337:** 978–979
4 Yoshioka K., Miki T., Katsuya T., Ogihara T. and Sakaki Y. (1991) The 717Val → Ile substitution in amyloid precursor protein is associated with familial Alzheimer's disease regardless of ethnic groups. Biochem. Biophys. Res. Commun. **178:** 1141–1146
5 Karlinsky H., Vaula G., Haines J. L., Ridgley J., Bergeron C., Mortilla M. et al. (1992) Molecular and prospective phenotypic characterization of a pedegree with familial Alzheimer's disease and a missense mutation in codon 717 of the β-amyloid precursor protein gene. Neurology **42:** 1445–1453
6 Sorbi S., Nacmias B., Forleo P., Piacentini S., Amaducci, L. and Provincali L. (1993) APP717 and Alzheimer's disease in Italy. Nature Genet. **4:** 10
7 Brooks W. S., Martins R. N., De Voecht J., Nicholson G. A., Schofield P. R., Kwok J. B. et al. (1995) A mutation in codon 717 of the amyloid precursor protein gene in an Australian family with Alzheimer's disease. Neurosci. Lett. **199:** 183–186
8 Sorbi S., Nacmias B., Forleo P., Piacentini S., Latorraca S. and Amaducci L. (1995) Epistatic effect of APP717 mutation and apolipoprotein E genotype in familial Alzheimer's disease. Ann. Neurol. **38:** 124–127
9 Campion D., Brice A., Hannequin D., Charbonnier F., Dubois B., Martin C. et al. (1996) No founder effect in three novel Alzheimer's disease families with APP717 Val → Ile mutation. J. Med. Genet. **33:** 661–664
10 Chartier-Harlin M. C., Crawford F., Houlden H., Warren A., Hughes D., Fidani L. et al. (1991) Early onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. Nature **353:** 844–846
11 Murrell J., Farlow M., Ghetti B. and Benson M. (1991) A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science **254:** 97–99
12 Eckman C. B., Mehta N. D., Crook R., Perez-tur J., Prihar G., Pfeiffer E. et al. (1997) A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of Aβ42/43. Hum. Mol. Genet. **6:** 2087–2090
13 Mullan M., Crawford F., Axelman K., Houlden H., Lilius L., Winblad B. et al. (1992) A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β-amyloid. Nature Genet. **1:** 345–347
14 Levy E., Carman M. D., Fernandez-Madrid I. J., Power M. D., Lieberburg I., van Duinen S. G. et al. (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. Science **248:** 1124–1126
15 Hendriks L., van Duijn C. M., Cras P., Cruts M., Van Hul W., van Harskamp F. et al. (1992) Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the β-amyloid precursor protein gene. Nature Genet. **1:** 218–221
16 Chartier-Harlin M. C., Crawford F., Hamandi K., Mullan M., Goate A., Hardy J. et al. (1991) Screening for the β-amyloid precursor protein mutation (APP: Val → Ile) in extended pedigrees with early onset Alzheimer's disease. Neurosci. Lett. **129:** 134–135
17 Crawford F., Hardy J., Mullan M., Goate A., Hughes D., Fidani L. et al. (1991) Sequencing of exons 16 and 17 of the beta-amyloid precursor protein gene in 14 families with early onset Alzheimer's disease fails to reveal mutations in the beta-amyloid sequence. Neurosci. Lett. **133:** 1–2
18 Schellenberg G. D., Anderson L., O'dahl S., Wisman E. M., Sandovnick A. D., Ball M. J. et al. (1991) APP717, APP693 and PRIP gene mutations are rare in Alzheimer disease. Am. J. Hum. Genet. **49:** 511–517
19 Tanzi R., Vaula G., Romano D. M., Mortilla M., Huang T. L., Tupler R. G. et al. (1992) Assessment of amyloid beta-protein pecursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. Am. J. Hum. Genet. **51:** 273–282
20 Fidani L., Rooke K., Chartier-Harlin M. C., Hughes D., Tanzi R., Mullan M. et al. (1992) Screening for mutations in the open reading frame and promoter of the β-amyloid precursor protein gene in familial Alzheimer's disease: identification of a further family with APP717 Val → Ile. Hum. Mol. Genet. **1:** 165–168
21 Hardy J. and Mullan M. (1992) In search of the soluble. Nature **359:** 268–269
22 St. George-Hyslop P., Crapper-McLachlan D., Tsud T., Rogaev E., Karlinsky H., Lippa C. et al. (1994) Alzheimer's disease and possible gene interaction. Science **263:** 441–580
23 Fox N., Warrington E. K., Freeborough P. A., Hartikainen P., Kennedy A. M., Stevens J. M. et al. (1996) Presymptomatic hippocampal atrophy in Alzheimer's disease. A longitudinal MRI study. Brain **119:** 2001–2007
24 Mullan M., Tsuji S., Miki T., Katsuya T., Naruse S., Kaneko K. et al. (1993) Clinical comparison of Alzheimer's disease in pedigrees with codon 717 Val-Ile mutation in the amyloid precursor protein gene. Neurobiol. Aging **14:** 407–419
25 Lopera F., Ardilla A., Martinez A., Madrigal L., Arango-Viana J. C., Lemere C. A. et al. (1997) Clinical features of early onset Alzheimer's disease in a large kindred with an E280A presenilin mutation. JAMA **277:** 793–799
26 Iwatsubo T., Odaka A., Suzuki N., Mizusawa H., Nukina N. and Ihara Y. (1994) Visualization of Aβ42(43) and Aβ40 in senile plaques with end-specific Aβ monoclonals: evidence that an initially deposited species is Aβ42(43). Neuron **13:** 45–53
27 Lemere C. A., Lopera F., Kosik K. S., Lendon C. L., Ossa J., Saido T. C. et al. (1996) The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. Nature Med. **2:** 1146–1150
28 Esch F. S., Keim P. S., Beattie E. C., Blacher R. W., Culwell A. R., Ottersdorf T. et al. (1990) Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science **248:** 1122–1124
29 Sisodia S., Koo E., Beyreuther K., Unterbeck A. and Price D. (1990) Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. Science **248:** 492–495
30 Haass C., Koo E., Mellon A., Hung A. and Selkoe D. (1992) Targeting of cell-surface β-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. Nature **357:** 500–503
31 Haass C., Schlossmacher M. G., Hung A. Y., Vigo-Pelfrey C., Mellon A., Ostaszewski B. L. et al. (1992) Amyloid β-peptide is produced by cultured cells during normal metabolism. Nature **359:** 322–325
32 Seubert P., Vigo-Pelfrey C., Esch F., Lee M., Dorey H., Davis D. et al. (1992) Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids. Nature **359:** 325–327
33 Cooke D., Froman M., Sung J., Leight S., Kolson D., Iwatsubo T. et al. (1997) Alzheimer's Aβ(1–42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. Nature Med. **3:** 1021–1023
34 Hartmann T., Bieger S. C., Bruhl B., Tiernari P. J., Ida N., Allsop D. et al. (1997) Distinct sites of intracellular production of Alzheimer's disease Aβ40/42 amyloid peptides. Nature Med. **3:** 1016–1020
35 Wild-Bode C., Yamazaki T., Capell A., Leimer U., Steiner H., Ihara Y. et al. (1997) Intracellular generation and accumulation of amyloid-β-peptide terminating at amino acid 42. J. Biol. Chem. **272:** 16085–16088

36 Citron M., Oltersdorf T., Haass C., McConlogue L., Hung A. Y., Seubert P. et al. (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature **360:** 672–674
37 Cai X.-D., Golde T. E. and Younkin S. G. (1993) Release of excess amyloid β protein from a mutant amyloid β protein precursor. Science **259:** 514–516
38 Citron M., Diehl T., Gordon G., Biere A., Seubert P. and Selkoe D. (1996) Evidence that the 42- and 40-amino acid forms of amyloid β protein are generated from the β-amyloid precursor protein by different protease activities. Proc. Natl. Acad. Sci. USA **93:** 13170–13175
39 Suzuki N., Cheung T. T., Cai X. D., Odaka A., Otvos L. Jr., Eckman C. et al. (1994) An increased percentage of long amyloid β protein secreted by familial amyloid β protein precursor (βAPP717) mutants. Science **264:** 1336–1340
40 Jarrett J., Berger E. and Lansbury P. (1993) The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry **32:** 4693–4697
41 Scheuner D., Eckman C., Jensen M., Song X., Citron M., Suzuki N. et al. (1996) Secreted amyloid-β-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature Med. **2:** 864–870
42 Games D., Adams D., Alessandrini R., Barbour R., Berthelette P., Blackwell C. et al. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. Nature **373:** 523–527
43 Hsiao K., Chapman P., Nilsen S., Eckman C., Harigaya Y., Younkin S. et al. (1996) Correlative memory deficits, Aβ elevation and amyloid plaques in transgenic mice. Science **274:** 99–102
44 Johnson-Wood K., Lee M., Motter R., Hu K., Gordon G., Barbour R. et al. (1997) Amyloid precursor protein processing and Aβ42 deposition in a transgenic mouse model of Alzheimer's disease. Proc. Natl. Acad. Sci. USA **94:** 1550–1555
45 Bales K. R., Verina T., Dodel R. C., Du Y., Altstiel L., Bender M. et al. (1997) Lack of apolipoprotein E dramatically reduces amyloid β-peptide deposition. Nature Genet. **17:** 263–264
46 Borchelt D. R., Ratovitski T., van Lare J., Lee M. K., Gonzales V., Jenkins N. A. et al. (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron **19:** 939–945
47 Borchelt D. R., Thinakaran G., Eckman C. B., Lee M. K., Davenport F., Ratovitsky T. et al. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1–42/1–40 ratio in vitro and in vivo. Neuron **17:** 1005–1013
48 Duff K., Eckman C., Hutton M., Perez-Tur J., Younkin S. and Hardy J. (1996) Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. Nature **383:** 710–713
49 Citron M., Westaway D., Xia W., Carlson G., Diehl T., Levesque G. et al. (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. Nature Med. **3:** 67–72
50 Bullido M. J., Artiga M. J., Recuero M., Sastre I., Garcia M. A., Aldudo J. et al. (1998) A polymorphism in the regulatory region of APOE associated with risk for Alzheimer's dementia. Nature Genet. **18:** 69–71