Epilepsia, **45**(7):751–756, 2004
Blackwell Publishing, Inc.

CA1 Hippocampal Neuronal Loss in Familial Alzheimer's Disease Presenilin-1 E280A Mutation Is Related to Epilepsy

*Carlos Velez-Pardo, ‡Jon I. Arellano, *Patricia Cardona-Gomez, *Marlene Jimenez Del Rio, †Francisco Lopera, and ‡Javier De Felipe

*School of Medicine, Internal Medicine, Neuroscience Research Program, University of Antioquia, and †Service of Neurology and Neurosciences Program, University Hospital San Vicente de Paul, Medellin, Colombia; and ‡Instituto Cajal (CSIC), Madrid, Spain

Summary: *Purpose:* Alzheimer disease (AD) and epilepsy are brain disorders frequently associated with neuronal cell loss in mesial temporal lobe structures, but presenting different patterns of damage. Recently it was proposed that a causal relation may exist between AD pathology and the appearance of epilepsy in some cases with AD. This study aimed to determine the neuronal loss in CA1 hippocampal region from patients bearing the presenilin-1 [E280A] mutation (PS1[E280A]) associated with seizures.

Methods: Coronal sections from the hippocampal formation (anterior one third) from controls (n = 5) and familial AD (FAD; n = 8) patients were stained by using thionin and thioflavine-S staining to evaluate the number of neurons in the CA1 field, β-plaques, and neurofibrillary tangles, respectively.

Results: Two distinct patterns of neuronal loss in the CA1 field of FAD patients were found: (a) diffuse–patchy neuronal loss (three FAD nonepilepsy patients) characterized by both a general decrease of neurons and the presence of multiple, small regions devoid of neurons; and (b) sclerotic-like neuronal loss (five FAD epilepsy patients) similar to that found typically in the CA1 field of epilepsy patients with hippocampal sclerosis.

Conclusions: This investigation shows for the first time CA1 neuronal depopulation in a subpopulation of patients (five of eight) bearing the PS1[E280A] mutation with epileptic seizures, indicating a relation between hippocampal neuronal loss and epileptic seizures in FAD patients. **Key Words:** Hippocampal region—Familial Alzheimer disease—Presenilin-1 E280A mutation—Epilepsy—Loss.

Alzheimer disease (AD) and epilepsy are two common brain disorders affecting up to 20 million and fifty million people worldwide, respectively (1,2). AD is a progressive degenerative disease characterized by the accumulation of amyloid β plaques, neurofibrillary tangles (NFTs) and extensive neuronal cell loss in limbic and association cortices (3). Epilepsy is a neurologic disorder often related to a variety of structural alterations of the neocortex and/or hippocampal formation. However, the specific ways in which these structural alterations result in seizures are still under debate (4). It has been clearly established that AD can be caused by autosomal dominant mutations to three separate genes: amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2) (5). Familial AD (FAD) accounts for ~4 to 8% of all AD cases, with mutations to PS1 the most frequent (http:www.alzforum.org/members/index.html; http://molgen-www.uia.ac.be/ADMutations) and is responsible for causing early-onset FAD, with a mean onset of disease before 65 years. The largest FAD kindred so far reported has a point mutation in codon 280 that results in a glutamic acid–to-alanine substitution in PS1 (6), characterized in Antioquia, Colombia (7–9). Furthermore, it has been demonstrated that PS1[E280A] increases deposition of amyloid β (Aβ[1-42/43]) (10) by altering the proteolytic processing of AβPP at the C-terminus of Aβ via a presenilin/γ-secretase mechanism (reviewed in refs. 11,12). Consequently, amyloidocentric theories of AD propose that Aβ deposition triggers a neurotoxic cascade causing cell death and AD (13). The hippocampal formation is particularly vulnerable to neuronal loss in all AD, particularly the pyramidal neurons of the cornu ammonis sector 1 (CA1, reviewed in ref. 14). Accordingly, Bobinski et al. (15) found a profound loss of neurons (86%) in the CA1 sector, and it also appears to show a unique relation with clinical measures of AD (16).

Epilepsy is a chronic disorder characterized by occurrence of spontaneous seizures lasting from a few seconds to several minutes (30 s to 1–7 min). As classified by the International League Against Epilepsy (ILAE, 17), epilepsy

Accepted March 11, 2004.
Address correspondence and reprint requests to Dr. C. Velez-Pardo, School of Medicine, Internal Medicine, Neuroscience Research Program, University of Antioquia (UdeA), Calle 62 #52-72, Medellin, Colombia. E-mail: cvelezp@quimbaya.udea.edu.co

is broadly divided into (a) partial seizures, in which abnormal electrical discharge originates from one specific area of the brain and does not interfere with consciousness, and (b) generalized epilepsies, in which the whole brain is involved and loss of consciousness occurs (reviewed in ref. 18). Genetic (or idiopathic) and symptomatic seizures (considered to be acquired) have been implicated as etiologic entities of epilepsy (19), but the relation with structural abnormalities has not yet been elucidated. Recently it was proposed that a causal relation may exist between AD pathology and the development and maintenance of some cases of partial status epilepticus (20). We report here, for the first time, that CA1 neuronal depopulation, in a pattern similar to that found in patients with hippocampal sclerosis (21–23), is found in a subpopulation of patients bearing the PS1[E280A] mutation with epileptic seizures, indicating a possible relation between hippocampal neuronal loss and the epileptic seizures in FAD patients. We discuss the possible link between PS1[E280A] mutation, Aβ1-42 generation, cell death, and the development of epilepsy.

MATERIALS AND METHODS

Human postmortem tissue

Human brain material was obtained from the University of Antioquia Brain Bank (coordinator, Dr. F. Lopera). Hippocampal tissue blocks were taken from five autopsy cases who had no history of primary neurologic or psychiatric disorders (cases 42, 43, m11, m12, and m13; age at death, 50, 57, 23, 49, and 63 years, respectively) and from eight patients with diagnosed and neuropathologically confirmed FAD, according to CERAD criteria (24). The mean age at FAD disease onset was 50.9 $\pm$ 7.0 years (n = 8; range, 39–62 years), the duration of clinically symptomatic disease was 9.4 $\pm$ 2.5 years (range, 7–14 years), and the age at death was 60.3 $\pm$ 8.2 years (range, 47–74 years). Six cases were members of "family B," previously reported in Lopera et al. (9). In five of the eight patients (cases 29, 40, 46, 53, and 27), epilepsy developed, characterized by generalized tonic–clonic seizures, lasting from 2 to 10 min and with daily or weekly frequency. The onset of seizures was at age 60 $\pm$ 8.7 years (n = 5; range, 50–73 years), and the duration of seizures, 2.2 $\pm$ 1.3 years (n = 5; range, 1–4 years).

Thionin and thioflavine-S staining

Tissue blocks were obtained from the hippocampal formation (anterior one third) from both control and FAD patients. The blocks were first immersed in 0.1 *M* phosphate buffer (PB) at 4°C overnight, and then fixed in 10% formaldehyde for 48 h. Thereafter, coronal sections (50 μm thick) from the blocks were obtained with a Vibratome and collected in PB. Sections were then stained with thionin. To estimate the number of neurons in the CA1 field, cells were counted by using 10 optical dissectors (25) per case, in 50-μm Nissl-stained sections. Optical dissections were performed by using a $\times$40 objective on a surface of 19,200 μm^2 with a depth of 20 μm, rendering a study volume of 384,000 μm^3 per optical dissector. Nucleoli were counted to obtain an estimation of the number of neurons. In parallel, sections were stained with 0.1% thioflavine-S to visualize β plaques and NFTs. Aβ plaques and NFTs were evaluated under a fluorescence microscope, with a semiquantitative method in which 10 random $\times$ 40 microscopic fields were scored according to Velez-Pardo et al. (26).

Statistical analysis

Comparisons between groups were performed with the Kruskal–Wallis test. Comparisons between controls and FAD groups were performed with the Mann–Whitney *U* test. Statistical significance was set at $p < 0.05$.

RESULTS

Table 1 shows the clinical and pathological characteristics of PS1[E280A] patients. Neurophatologic assessment of the CA1 field revealed a high density of Aβ plaques (five, >25 AβP/mm^2) and NFTs in all FAD cases, except case 2970, which showed a relatively low density of NFT (one, <5 NFT/mm^2). In all FAD cases, a severe concomitant deposition of AβP and NFTs was observed. Noticeably, the age at onset of seizures in FAD patients (cases 27, 29, 40, 46, 53) occurred 1 to 4 years before death. Moreover, the onset of seizures appeared 6 (three cases) or 1 (two cases) years after the age at AD onset.

At first glance, two distinct patterns of neuronal loss were seen in the CA1 field of FAD patients: diffuse–patchy neuronal loss (Fig. 1B, E, and H) and sclerotic-like neuronal loss (Fig. 1C, F, and I). The former pattern was characterized by both a general decrease of neurons and the presence of multiple, small regions devoid of neurons. This pattern of neuronal loss was found in the three FAD nonepilepsy patients. The sclerotic-like neuronal loss was similar to that found typically in the CA1 field of epilepsy patients with hippocampal sclerosis (22). That is, a large segment of CA1 was practically devoid of neurons. The sclerotic-like damage was found in all FAD epilepsy patients. However, the pattern of neuronal loss in the hilus, CA4, and CA3 fields was in small patches instead of the characteristic loss of neurons in large segments of the epileptic sclerotic hippocampus (27). Quantification of the neuronal loss in FAD patients with and without epilepsy compared with controls (Fig. 1A, D, and G) revealed that neuronal loss was significant greater in both types of patients (Fig. 2; Kruskal–Wallis test, $\chi^2 = 44.181$; $df = 2$; $p = 0.000$). In addition, those patients with epilepsy showed a significantly greater neuronal loss than did those nonepilepsy patients (Fig. 2, Mann–Whitney *U* test, 364.5; $p = 0.000$).

TABLE 1. *Case description of PS1[E280A] familial Alzheimer's disease and control subjects*

Family Group	Case #	Sex	Age at onset (years)	Duration of illness (years)	Age at death (years)	PMDs (hours)	Hippocampus		Age at seizures onset (years)	Frequency of Seizures min (times/week)	Duration of seizures (years)
							AβP	NFT			
B	0212	F	39	8	47	8	5	5	0	n.a.	0
	2970	F	53	9	62	8	5	1	0	n.a.	0
	29	F	62	12	74	7	5	5	73	5–10 min (5/day)	1
	40	F	49	10	59	6	5	5	55	ND (5/day)	4
	46	M	44	8	52	4	5	5	50	ND	2
	53	M	53	7	60	8	5	5	59	ND (3/week)	1
D	3156	F	55	7	62	6	5	5	0	ND	0
This Report	27	F	52	14	66	6	5	5	63	2–3 min (1–2/week)	3
Mean ± SD (n)			50.9 ± 7.0 (8)	9.4 ± 2.5 (8)	60.30 ± 8.2 (8)	6.6 ± 1.4 (8)	n.a.	n.a.	60 ± 8.7 (5)	n.a	2.2 ± 1.3 (5)
Controls	42	M	n.a.	n.a.	50	12	n.a.	n.a.	n.a.	n.a	n.a
	43	M	n.a.	n.a.	57	12	n.a.	n.a.	n.a	n.a	n.a
	m11	M	n.a.	n.a.	23	2	n.a.	n.a.	n.a.	n.a	n.a
	m12	M	n.a.	n.a.	49	3	n.a.	n.a.	n.a	n.a	n.a
	m13	M	n.a.	n.a.	63	3	n.a.	n.a	n.a	n.a	n.a

Proximal cause of death in PS1[E280A] FAD cases was bronchopneumonia, and in control subjects, trauma. Scores of thioflavine-S (Aβ-P)/(NFTs) staining were rated as 0, absent; 1, sparse (<5 AβP-NFT/mm^2); 3, moderate (6–25 AβP-NFTs/mm^2); 5, frequent/severe (>25 AβP-NFTs/mm^2), according to Velez-Pardo et al. (26).

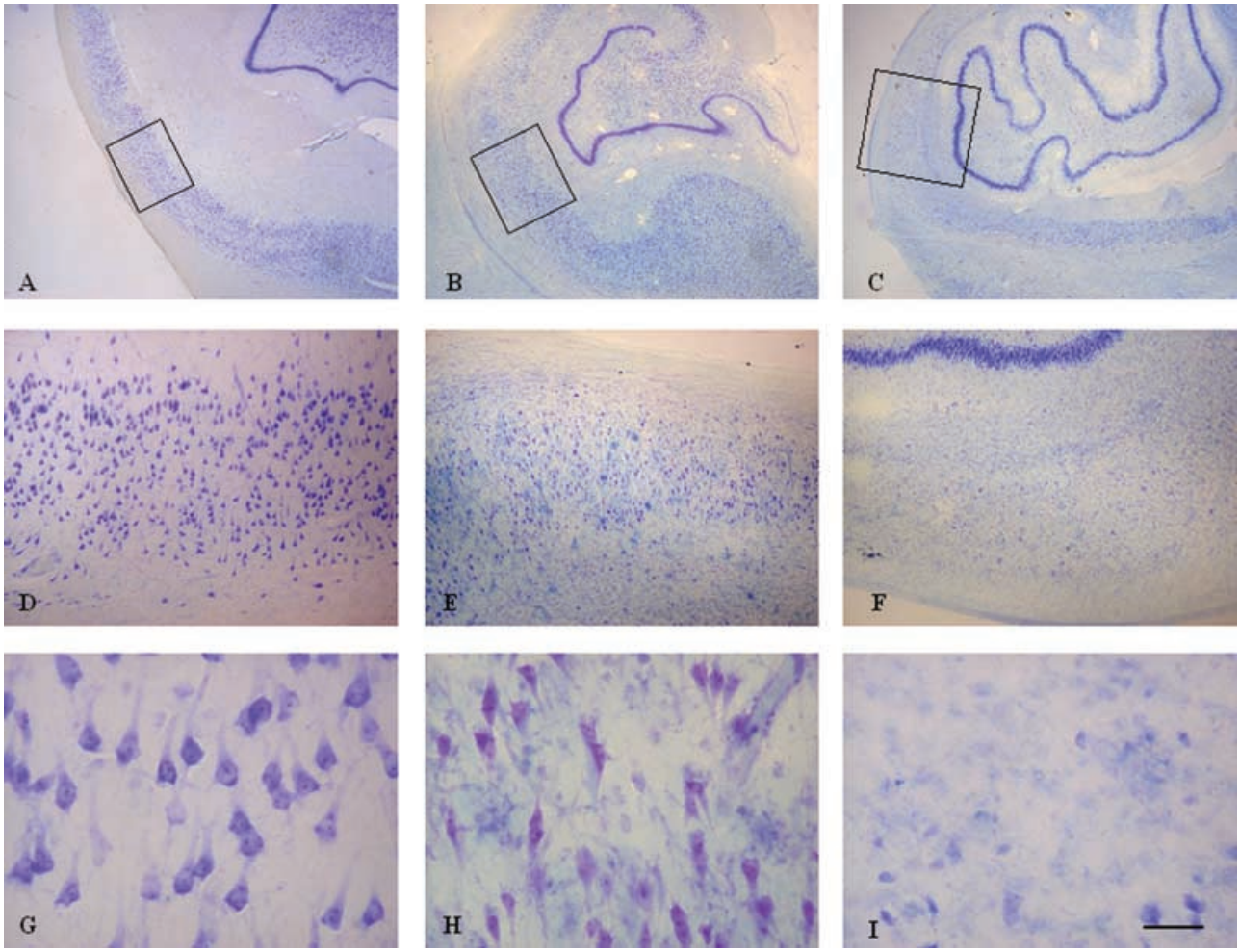

FIG. 1. Photomicrographs of the hippocampal CA1 field (*squared box*) stained with thionin from **(A)** a normal case (43); **(B)** familial Alzheimer's disease (FAD) (PS1[E280A]) patient without seizures (case 3156), and **(C)** FAD (PS1[E280A]) patient with seizures (case 40). Note the severe neuronal loss in the patient with FAD plus epilepsy **(C, F, I)** compared with either FAD **(B, E, H)** or control **(A, D, G)**. Scale bar, 800 μm in **A, B, C**; 250 μm in **B, D, F**; and 50 μm in **G, H, I**.

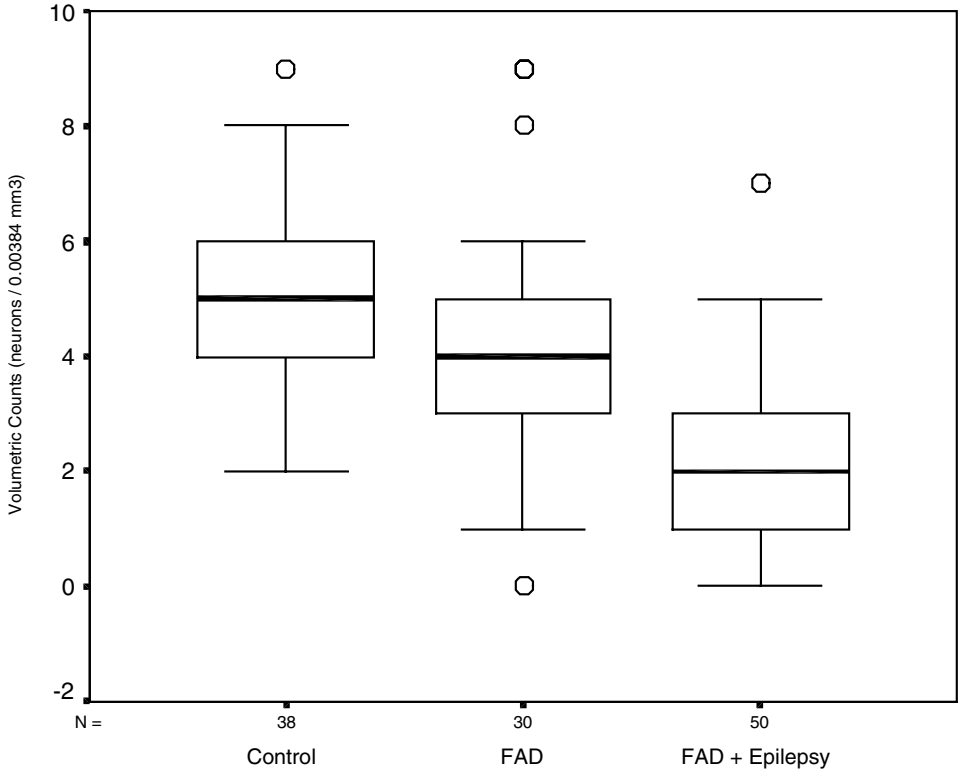

FIG. 2. Bar graph showing neuronal loss in CA1 field in controls and familial Alzheimer's disease patients with and without seizures. Statistical significance was set at $p < 0.05$. Data are presented by using box and whiskers plot.

DISCUSSION

We report for the first time that dramatic cell loss in the CA1 field of the hippocampus in the PS1[E280A] FAD mutation is related with seizures with a duration of 2 to 10 min and a daily or weekly frequency. Recently Takao et al. (28) reported clinical, neuropathologic, and genetic data from an individual affected with familial AD PS1[S169L] mutation and epilepsy. The patient had an onset of dementia at age 29 years, followed by generalized seizures a year later, and died at age 40 years. At least eight mutations in PS1 (P117L, N135D, I143T, L166P, S169L, S169P, L235P, A434C) are associated with this clinical phenotype that includes seizures at the early stage of disease (28 and references therein). In contrast, we found that seizures occurs in FAD PS1[E280A] during the late stage of the disorder [6 ($n = 3$) and 1 ($n = 2$) years after the first symptom of AD; 1–4 years before death). These contrasting observations might be explained by differences in the penetrance of each mutation, which would lead to early onset of AD and severe Aβ and tau deposition. Accordingly, patients bearing the mutation PS1[E280A] had an onset of dementia at mean age 49 years ($n = 8$), start epileptic seizures at mean age 59 years, and exhibit neuropathologic alterations consisting in severe deposition of tau and Aβ in the form of neuritic plaques throughout the cortex. Conversely, the PS1[S169L] mutation induces earlier onset of dementia (at age 29 years) and seizures (at age 30 years), and has severe deposition of Aβ in the form of diffuse deposits both in the cerebral and cerebellar cortices and in the subcortical white matter. In addition, numerous ectopic neurons containing tau-immunopositive NFTs in the white matter of the frontal and temporal lobes were observed. Taken together, these data suggest that depending on the type of PS1 mutation analyzed, FAD patients could develop an early–early or an early–late onset of dementia and the seizures phenotype, respectively, with the accompanying differential patterns in Aβ deposition and selective neuronal damage. However, mutations per se appear not to be a causative factor of seizures, because three of eight PS1[E280A] FAD patients did not exhibit generalized tonic–clonic seizures.

These results prompted us to evaluate cell density at the CA1 field, because it has been extensively reported that loss of hippocampal neurons is related to clinical measures of AD (16), and cellular hippocampal alterations after status epilepticus are common (29). Initially we confirmed the severe deposition of NFT and Aβ plaques in the CA1 field, evidenced by thioflavine-S staining, as previously reported (26). We observed a high density of Aβ plaques and NFTs in all FAD cases, except in case 2970, which showed a low density of NFTs. These results suggest that Aβ may lead to NFT formation. This assumption is supported by Lewis et al. (30) and Gotz et al, (31) who

demonstrated that Aβ1-42 accelerates the formation of NFTs in transgenic mice. Moreover, Aβ1-42 and its derived peptide (Aβ25-35) have been found to be toxic in vitro (32 and references therein) and in vivo (33). Accordingly, we found a remarkable neuronal loss at CA1 field in both the FAD and FAD epilepsy groups when compared with controls (Figs. 1 and 2). Furthermore, we found a significant difference in cell loss between FAD and FAD epilepsy groups (Fig. 2). Although the pathological substrate of the seizures is not known, our data suggest that neuronal damage induced by Aβ1-42 might be associated with epileptic attacks. Therefore why do some of [E280A] FAD patients (63%) manifest seizures during the late stage of the disorder? What could be the mechanism mediating the link between PS1[E280A] mutation, Aβ1-42, cell death, and the subsequent development of epilepsy?

Two possible hypotheses exist. First, in PS1[E280A) FAD without and with epilepsy, the following scenario of sequential molecular events may take place. These events are initiated by the increase of Aβ1-42 induced by PS1[E280A]. This uncontrollable Aβ1-42 generation induces the formation of NFTs and neuronal death by either apoptosis (34–37) or necrosis (26,38–40). This neuronal death might be stronger in some patients, affecting particular γ-aminobutyric acid (GABA)ergic inhibitory circuits, and resulting in an imbalance between excitation and inhibition that might induce the appearance of seizures (4,27). The second hypothesis is that the different degree of neuron loss is generated by epileptic activity (41). It is known that hippocampal damage can be induced after repeated kindled seizures in the rat (31,42–44). Therefore a possibility exists that epileptic activity originating primarily in extrahippocampal cortical structures could induce neuronal loss in the hippocampus in these patients. Further studies using detailed electrophysiologic and neuropathologic methods should be performed, the better to understand the course of this brain disease and its association with epilepsy.

Acknowledgment: This research was supported by a grant awarded to P.C.G. from Colciencias (1115-05-110095), and by grants awarded to J.D.F. from the Spanish Ministry of Science and Technology (BFI2003-02745) and the "Comunidad de Madrid" (08.5/0027/2001).

REFERENCES

1. Allen SH. Alzheimer's disease: past, present, and future themes. In: Dawbarn D, Allen SJ, eds. *Neurobiology of Alzheimer's disease*. Oxford: Oxford University Press, 2001:1–32.
2. Reynolds EH. Introduction: epilepsy in the world. *Epilepsia* 2002;43(suppl 6):1–3.
3. Esiri MM. The neuropathology of Alzheimer's disease. In: Dawbarn D, Allen SJ, eds. *Neurobiology of Alzheimer's disease*. Oxford: Oxford University Press, 2001:33–53.
4. DeFelipe J. Chandelier cells and epilepsy. *Brain* 1999;122:1807–22.
5. Hardy J. Genetic dissection of neurodegenerative disease. *Clin Neurosci Res* 2001;1:1134–41.
6. Clark RF, Hutton M, Fuldner RA, et al. The structure of presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. *Nat Genet* 1995;11:219–22.
7. Lendon CL, Martinez A, Behrens IM, et al. E280A PS-1 mutation causes Alzheimer's disease but age of onset is not modified by ApoE allelles. *Hum Mutat* 1997;10:186–95.
8. Lopera F, Arcos M, Madrigal L, et al. Demencia tipo Alzheimer con agregación familiar en Antioquia, Colombia. *Acta Neurol Colombiana* 1994;10:173–87.
9. Lopera F, Ardilla A, Martinez A, et al. Clinical features of early-onset Alzheimer disease in a large kindred with an E280A presenilin-1 mutation. *JAMA* 1997;277:793–9.
10. Lemere C, Lopera F, Kosik KS, et al. The E280A presenilin1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. *Nat Med* 1996;2:1146–50.
11. Sisodia SS, Annaert W, Kim S-H, et al. γ-Secretase: never more enigmatic. *Trends Neurosci* 2001;24:S2–6.
12. Sisodia S, St. George-Hyslop PH. γ-Secretase, notch, aβ and Alzheimer's disease: where do the presenilins fit in? *Nat Rev Neurosci* 2002;3:281–90.
13. Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid β-protein. *J Alzheimer's Dis* 2001;3:75–81.
14. DeLeon MJ, Convit A, Tarshish C, et al. MRI studies of the hippocampal formation: contributions to the early diagnosis of Alzheimer's disease. In: DeLeon MJ, ed. *An atlas of Alzheimer's disease*. New York: The Parthenon Publishing Group, 1999:31–55.
15. Bobinski M, Wegiel J, Tarnawski M, et al. Relationships between regional neuronal loss and neurofibrillary changes in the hippocampal formation and duration and severity of Alzheimer disease. *J Neuropathol Exp Neurol* 1997;56:414–20.
16. Bobinski M, de Leon MJ, Tarnawski M, et al. Neuronal and volume loss in CA1 of the hippocampal formation uniquely predicts duration and severity of Alzheimer disease. *Brain Res* 1998;805:267–9.
17. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 1989;30:389–99.
18. Avoli M, Rogawski MA, Avanzini G. Generalized epileptic disorders: an update. *Epilepsia* 2001;42:445–57.
19. Berkovic SF, Scheffer IE. Genetics of the epilepsies. *Epilepsia* 2001;42(suppl 5):16–13.
20. Armon C, Peterson GW, Liwnicz BH. Alzheimer's disease underlies some cases of complex partial status epilepticus. *J Clin Neurophysiol* 2000;17:511–8.
21. Babb TL, Pretorius JK. Pathologic substrates of epilepsy. In: Wyllie E, ed. *The treatment of epilepsy: principles and practice*. Philadelphia: Lea & Febiger, 1993:55–70.
22. Honavar M, Meldrum BS. Epilepsy. In: Graham DI, Lantos PI, eds. *Greenfield's neuropathology*. London: Edward Arnold, 1997:931–71.
23. Meldrum BS, Bruton CJ. *Epilepsy*. 5th ed. London: Edward Arnold, 1992.
24. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD), part II: standardization of the neurophatologic assessment of Alzheimer's disease. *Neurology* 1991;41:479–86.
25. West MJ, Gundersen HJ. Unbiased stereological estimation of the number of neurons in the human hippocampus. *J Comp Neurol* 1990;296:1–22.
26. Velez-Pardo C, Lopera F, Jimenez Del Rio M. DNA damage does not correlate with beta-plaques and neurofibrillary tangles in familial Alzheimer's disease presenilin1 [E280A] mutation. *J Alzheimer's Dis* 2000;2:47–57.
27. Arellano JI, Muñoz A, Ballesteros-Yáñez I, et al. Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus. *Brain* 2004;127:45–64.
28. Takao M, Ghetti B, Murrell JR, et al. Ectopic white matter neurons, a developmental abnormality that may be caused by the PSEN1 S169L mutation in a case of familial AD with myoclonus and seizures. *J Neuropathol Exp Neurol* 2001;60:1137–52.
29. MacDonald RL, Kapur J. Acute cellular alterations in the hippocampus after status epilepticus. *Epilepsia* 1999;40(suppl 1):S9–20.
30. Lewis J, Dickson DW, Lin WL, et al. Enhanced neurofibrillary

degeneration in transgenic mice expressing mutant tau and APP. *Science* 2001;293:1487–91.
31. Gotz J, Chen F, Dorpe JV, et al. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Aβ42 fibrils. *Science* 2001;293:1491–5.
32. Velez-Pardo C, Garcia-Ospina G, Jimenez Del Rio M. Aβ[25-35] peptide and iron promote apoptosis in lymphocytes by a common oxidative mechanism: involvement of hydrogen peroxide (H_2O_2), caspase-3, NF-kappa B, p53 and c-Jun. *Neurotoxicology* 2002;23:351–65.
33. Geula C, Wu C-K, Saroff D, et al. Aging renders the brain vulnerable to amyloid beta protein β-neurotoxicity. *Nat Med* 1998;4:827–31.
34. Anderson AJ, Stoltzner S, Lai F, et al. Morphological and biochemical assessment of DNA damage and apoptosis in Down syndrome and Alzheimer's disease, and effect of postmortem tissue archival on TUNEL. *Neurobiol Aging* 2000;21:511–24.
35. Dragunow M, Faull RL, Lawlor P, et al. In situ evidence for DNA in Huntington's disease and Alzheimer's disease temporal lobes. *Neuroreport* 1995;6:1053–7.
36. Smale G, Nichols NR, Brady DR, et al. Evidence for apoptotic cell death in Alzheimer's disease. *Exp Neurol* 1995;133:225–30.
37. Su JH, Anderson AJ, Cummings BJ, et al. Immunohistochemical evidence for apoptosis in Alzheimer's disease. *Neuroreport* 1994;5:2529–33.
38. Lucassen PJ, Chung WCJ, Kamphorst W, et al. DNA damage distribution in the human brain as shown by in situ end labeling: area-specific differences in aging and Alzheimer's disease in the absence of apoptotic morphology. *J Neuropathol Exp Neurol* 1997;56:887–900.
39. Stadelman C, Bruck W, Bancher C, et al. Alzheimer disease: DNA fragmentation indicates increased neuronal vulnerability, but not apoptosis. *J Neuropathol Exp Neurol* 1998;57:456–64.
40. Velez-Pardo C, Tobon S, Lopera F, et al. Ultrastructure evidence of necrotic neural cell death in familial Alzheimer's disease presenilin-1 [E280A] mutation. *J Alzheimer's Dis* 2001;3:409–15.
41. Fujikawa DG, Shinmei SS, Cai B. Seizure-induced neuronal necrosis: implications for programmed cell death mechanism. *Epilepsia* 2000;41(suppl 6):S9–13.
42. Cavazos JE, Das I, Sutula TP. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. *J Neurosci* 1994;14:3106–21.
43. Kondratyev A, Selby D, Gale K. Status epilepticus leads to the degradation of the endogenous inhibitor of caspase-activated DNase in rats. *Neurosci Lett* 2002;319:145–8.
44. Sloviter RS. "Epileptic" brain damage in rats induced by sustained electrical stimulation of the perforant path, I: acute electrophysiological and light microscopic studies. *Brain Res Bull* 1983;10:675–97.