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A multigenerational pedigree of late-onset Alzheimer's disease implies new genetic causes

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We describe the clinical phenotype and pathology of a new autosomal dominant late-onset familial form of Alzheimer's disease in four extensive kindred originated in a genetically isolated population. Twelve affected and 16 unaffected members of these kindred were examined clinically, and a brain post-mortem study was carried out in one case. The preliminary genetic assessment included complex segregation analysis, evaluation of the power to detect linkage, and exclusion of candidate genes. Dementia has been recorded for six generations in ancestors of examined cases. Review of death certificates allowed linking of all subjects in four extensive pedigrees. Affected individuals examined had progressive memory loss with onset between 57 and 74 years of age, along with seizures, myoclonus and parkinsonism in advanced stages. The brain of the case examined post-mortem showed widespread neocortical neuritic plaques and neurofibrillary tangles (stage VI of Braak), amyloid angiopathy, and Lewy bodies restricted to limbic areas. Sequencing exons 16 and 17 of amyloid precursor protein, and exons 4–12 of presenilin 1 and presenilin 2 genes did not disclose any mutations. Genotyping with markers D21S265, D14S71, D14S77, D1S2850 and D1S479 located 1–3 cM from the previously reported genes further excluded linkage to these genes. Seven out of 12 cases were apolipoprotein E (APOE) ε3/3, although the presence of an APOE ε4 allele was associated with an increased risk of dementia (odd ratio 6.17; 95% confidence interval: 1.15–33.15), but not to an earlier age of onset. Complex segregation analysis showed that the best model fitting the data was that of a major gene (dominant) with a gene frequency close to 3% in this population. Simulation analysis predicted an average logarithm of odds (LOD) of 2.2 at θ = 0.05. These four families, which seem to be part of a common extended pedigree originated by a founder arriving in this region in the 18th century, represent an autosomal dominant late-onset familial Alzheimer's disease not linked to previously known genetic loci. The simulation analysis suggests that it will be feasible to locate a novel responsible gene in these kindred.

Keywords: genetic isolates; familial Alzheimer's disease; complex segregation analysis; late onset dementia; genetics

Abbreviations: Aβ = amyloid β; *APOE* = apolipoprotein E; *APP* = amyloid precursor protein; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; CI = confidence interval; LOD = logarithm of odds; MIM = Mendelian Inheritance in Man database; *PSEN1* = presenilin 1; *PSEN2* = presenilin 2

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Introduction

Alzheimer's disease is considered genetically complex. Three genes have so far been identified in early-onset autosomal dominant Alzheimer's disease—presenilin 1 (*PSEN1*) [Mendelian Inheritance in Man (MIM) 104311)], presenilin 2 (*PSEN2*) (MIM 633044) and amyloid precursor protein (*APP*) (MIM 104760) (Levy *et al.*, 1990; Van Broeckhoven *et al.*, 1990; Sherrington *et al.*, 1995; Levy-Lahad *et al.*, 1996)—along with apolipoprotein E (*APOE*) (MIM 107741),

which has been consistently reported as a risk factor contributing to late-onset Alzheimer's disease (Corder *et al.*, 1993; Saunders *et al.*, 1993). Additional risk genes or loci have been reported in selected populations (Pericak-Vance *et al.*, 2000; Ertekin-Taner *et al.*, 2001; Rocchi *et al.*, 2003). However, these genes explain only a small proportion of familial Alzheimer's disease and leave an important gap in late-onset forms, which are those most closely resembling sporadic disease.

The two ways used to identify new genes in late-onset Alzheimer's disease have been the study of multiple small families and the examination of a single extensive pedigree. Both have important drawbacks. The studies of multiple families are expensive and hampered by heterogeneity, which decreases the signal to noise ratio and thus hinders finding the signal. Identification of a large kindred has potential to detect a mutation segregated with the disease with less intensive laboratory work. For different reasons (e.g. comorbidity, death or migration), however, it is extremely rare to find kindred with late-onset Alzheimer's disease and a number of cases large enough to allow detection of linkage. In a survey of the medical literature, we found only three reports of families with autosomal dominant late-onset Alzheimer's disease and at least three generations of affected individuals where *APP*, *PSEN1* and *PSEN2* genes were excluded (Pericak-Vance *et al.*, 1996; Cai *et al.*, 1997; Martin *et al.*, 1997). Even when this search is extended to dates before the discovery of these genes, very few kindred are reported (Bird *et al.*, 1989; St George-Hyslop *et al.*, 1989).

We present here the clinical, neuropathological and genetic data of a cluster of related families with late-onset hereditary Alzheimer's disease appearing in a genetic isolate. These families support the existence of a genetically identifiable subtype of late-onset autosomal dominant Alzheimer's disease in this population. Furthermore, our analysis suggests that they are suitable for a genome-wide screen to find a new locus linked to the appearance of late-onset Alzheimer's disease.

Patients and methods

Families

In the context of a clinical genetic study on familial Alzheimer's disease in Spain (the GENODEM project), we identified a geographic aggregate of 10 families with autosomal dominant Alzheimer's disease. These families came from a genetically isolated subpopulation living in a small area in the province of Guadalajara (villages P and I, located 5 kilometres apart, with population of 275 and 40 people, respectively); they share family names.

The investigation of these families proceeded from information provided by relatives of the patients and by checking official death certificates kept in the Municipal Register at the town halls of these villages, encompassing ~120 years (1872 to 2003) and 1825 individuals. Information in death certificates included name of parents, spouse and children, age and cause of death. Diagnoses supporting Alzheimer's disease in old death certificates were 'progressive softening of the brain', 'necrobiosis cerebri', 'early aging' or 'senile hysteria', with age at death <80 years. In addition, a few cases gave the diagnosis of cerebral haemorrhage.

Informed consent for the collection of blood samples and medical history was obtained from 16 healthy siblings, four cases with mild cognitive impairment or Parkinson's disease and by surrogate in 12 affected cases. The study was approved by the Ethical Committee of the Hospital Ramon y Cajal.

Clinical and functional investigations

Phenotype characterization was based on medical history and neurocognitive examination, along with routine blood tests and neuroimaging in all affected individuals. In addition, we obtained consent from the closest relatives to perform a brain-only autopsy of one patient who died during the study. Age at onset was defined as the age at which an individual first demonstrated signs of memory loss or a mood disorder followed by cognition impairment, as estimated from the reports of family members or medical records.

Genetic studies

Complex segregation analysis was performed to determine whether the observed familial aggregation had a genetic basis, and to estimate the best model of transmission and the relative effects of genetic and environmental factors shared by the members of the families. This analysis was carried out according to the unified model of complex segregation analysis implemented in the software POINTER (Lalouel and Morton, 1981; Lalouel *et al.*, 1983). The model partitions the total variation of the underlying liability for Alzheimer's disease into three independent components: (i) a diallelic single major locus component; (ii) a polygenic background; and (iii) a random environmental component.

Detailed information on the segregation analysis is available as supplementary data.

We calculated the power to detect linkage in these pedigrees using a simulation program for linkage analysis (SIMLINK version 4.12) (Boehnke, 2003). This program estimates the probability or power to detect linkage given family history information on a pedigree. For the simulation, we assumed a dichotomous autosomal dominant trait with the disease allele frequency set at 0.03 according to the complex segregation analysis. The trait was examined using an age-associated penetrance model (cumulative normal penetrance function from 0 to maximum penetrance, set about the mean ± SD age of onset of 65.4 ± 6.6 years). Five hundred replicates of each pedigree segregating a marker for $\theta = 0.00$–0.5 from the disease locus were generated. Cases with mild cognitive impairment or Parkinson's disease were not included in the analysis.

For molecular genetics studies, blood samples were taken from the 32 living members of the kindred and DNA was extracted. Investigation of the three genes involved in familial Alzheimer's disease was performed by sequencing and exclusion of segregation with the microsatellites D21S265, D14S71, D14S77, D1S2850 and D1S479 sited at 1–3 cM of the *APP*, *PSEN1* and *PSEN2* genes, respectively. Exons 4, 5, 6, 7, 8, 9, 10, 11 and 12 of *PSEN1* and *PSEN2*, and exons 16 and 17 of the *APP* were amplified by polymerase chain reaction (PCR) following the protocol described by Perez-Tur *et al.* (1995) and Goate *et al.* (1991).

For the genotype analysis, reaction protocols were performed with the available sequence information and conditions from the genome database online at http://gdbwww.gdb.org. This database was consulted for allele frequencies of microsatellite markers. Logarithm of odds (LOD) scores for these markers were calculated using the program LINKAGE (Terwilliger and Ott, 1994). The *APOE* genotype was determined according to the method of Hixson and Vernier (1990).

Pathology study

A complete post-mortem neuropathological study was carried out on case I3-A263. After routine fixation in buffered 4% formaldehyde, the brain was cut following the protocol employed by the Tissue Bank for Neurological Research, Madrid. Tissue blocks were obtained from several cortical regions [including Consortium to Establish a Registry for Alzheimer's Disease (CERAD) areas and areas to demonstrate Lewy bodies according to Newcastle criteria], all significant subcortical regions, the cerebellum and all brainstem levels. After paraffin embedding, sections were stained with haematoxylin (H&E), *p*-amino-salicylic acid (PAS), Congo red, modified methenamine silver and Gallyas, and immunostained for tau (AT100, Pierce Endogen, Rockford, IL, USA), amyloid-β (Aβ) (6F/3D, Dako, HighWycombe, UK) and α-synuclein (KM51, Novocastra, Newcastle upon Tyne, UK). Antibody binding was visualized using the Envision kit (Vector, Burlingame, Ca, USA).

Results

Family overview

The earliest recorded year of birth is 1790. Twelve individuals (eight females and four males) were examined by at least one of the authors and classified as affected with Alzheimer's disease. Sixteen siblings (seven females and nine males men) were escapees (i.e. 'at risk' persons who were symptom-free >75 years) (Fig. 1). Male-to-male, male-to-female, female-to-male and female-to-female transmissions were observed. No instance of skipping a generation was noted in the six generations traced back and 37% of adults members developed Alzheimer's disease.

Genealogic investigation allowed us to cluster the 10 families in three extensive pedigrees [P-a, P-b, I; i.e. two pedigrees from village P and one from village I) and an additional family (I_3) from village I (Fig. 1) and indicated that the families originate from an isolated population because 99% of the recorded individuals were born and died in villages P or I over the last 200 years. Although we were not able to link all families into a common pedigree using genealogy, their origin in two small isolated villages only 5 km apart and their sharing of a few unusual family names—along with the similar clinical pattern and age at onset—supports the existence of a common founder. In addition, pedigree P-b had individuals with ancestors in both villages.

Clinical findings

The clinical features of the affected patients whose clinical records were available are presented in Table 1. The overall mean age at death was 74.3 (SD = 7.6) years; in the last generation the mean age at onset was 65.8 (range: 57–76) years and the age at death was 73, 82 and 91 years in the three cases deceased since the start of the study. When considered separately, there were slight differences in the age at onset in the three main kindred affected in the last generation: P-a, mean = 60.2 (range = 57–66) years; P-b, mean = 65.0 (range = 57–70) years; I, mean = 68.4 (57–74) years.

Affected subjects fulfilled CERAD and Alzheimer's Disease and Related Disorder Association/National Institute of Neurological Disorders and Stroke (ADRDA/NINDS) criteria for probable or definite Alzheimer's disease. Two patients developed seizures and three patients had frequent spontaneous focal myoclonus that increased with local stimulation. Two developed parkinsonism and another sibling of the P-a kindred has had levodopa (L-dopa) responsive parkinsonism for the last 6 years (Hoehn-Yahr stage 3) without cognitive impairment until time of examination at 74 years of age.

There were no noteworthy differences in clinical features or rate of progression between the three main kindred. All cases have cortical atrophy in brain CT without vascular lesions.

Neuropathology of case I3-A263 (Fig. 2)

The brain weighted 850 g. Gross examination revealed a diffuse symmetric cortical atrophy, particularly marked in the medial temporal lobes, and moderate dilatation of the lateral ventricles. Tissue sections of the isocortex stained with Gallyas and Congo red techniques, and immunostained for Aβ showed a high density of senile plaques, predominantly neuritic plaques, corresponding to CERAD's frequent level, and thus fulfilling criteria for the neuropathological diagnosis of definite Alzheimer's disease. Numerous neurofibrillary and neuropil threads in a bilaminar arrangement were also observed with a distribution and density corresponding to a Braak and Braak Stage VI. Amyloid-staining techniques revealed widespread vascular deposition of Aβ in leptomeningeal and intracortical vessels in both cerebral and cerebellar cortices. α-Synuclein immunostains showed abundant cortical Lewy bodies and neurites in deep cortical layers restricted to limbic areas. There were Lewy neurites and diffuse somatic α-synuclein labelling in hippocampal sector CA2 and the substantia nigra, but neither Lewy bodies nor evidence of neuronal loss were present in the latter. In addition, α-synuclein immunostains labelled senile plaques in a transcortical distribution in extralimbic regions. Neither microinfarcts nor haemosiderin deposits were present.

Genetic studies

Complex segregation analysis identified 21 nuclear components. There were 36 affected and 11 probands ($\pi = 0.28$). Ten models were compared using the likelihood ratio test (Table 2). The hypothesis of non-familial transmission of Alzheimer's disease in these families (cohort effect) was refuted, with strong significance, when it was compared with both multifactorial and major gene models [comparison between model 1 and 2: $\chi^2(1) = 20.86$, $P < 0.0001$; model 1 and 6: $\chi^2(5) = 29.29$, $P < 0.0001$]. The hypothesis of a multifactorial component compared with that of the existence of a major gene only (comparison between model 2 and model 6) was rejected [$\chi^2(2) = 8.43$, $P = 0.015$].

Among the models postulating a major locus, co-dominant and recessive models were rejected when compared with

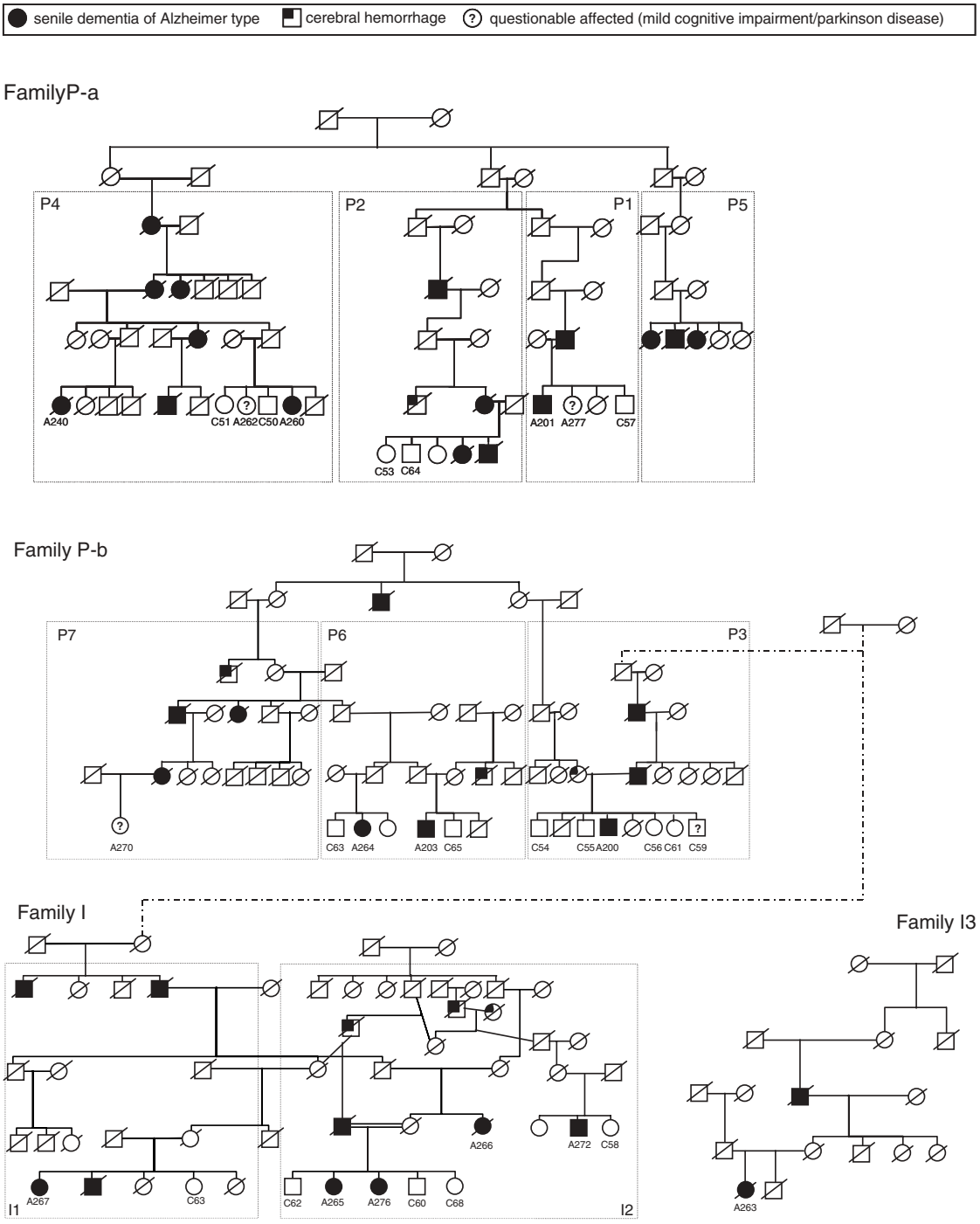

Fig. 1 Pedigrees of the four families examined. A = affected cases with available DNA sample; C = not affected cases with DNA available.

that of a dominant major gene model [model 4 versus 3: $\chi^2(1) = 4.48$, $P = 0.034$; model 5 versus 3: $\chi^2(1) = 4.89$, $P = 0.027$]. Comparison of the major gene dominant model with its mixed counterpart rejected the presence of a polygenic effect [model 3 versus 7: $\chi^2(1) = 8.43$, $P < 0.01$]. By first inspection, it is obvious that mixed counterparts for co-dominant and recessive models are rejected. Finally, the model of no major gene effect (t1 = t2 = t3) (comparison of models 10 and 6) was rejected [$\chi^2(1) = 10.08$, $P < 0.0001$]. Parameters may be selected from the major gene dominant model or the major gene model with unrestricted d; these are the most parsimonious models.

Thus far, segregation analyses were consistent with a major dominant gene with an allele frequency of 0.03 and a penetrance for genotypes carrying the susceptibility allele in liability classes reaching age of affection close to a probability of 0.55.

The computer simulations using a model that assumed a major autosomal dominant locus revealed that this pedigree

Table 1 Clinical features of affected individuals at time of the study

Family Patient	P-a A201	P-a A240	P-a A260	Pb A201	P-b A203	P-b A264	I A267	I A265	I A266	I A276	I A272	I3 A263	Normal siblings (n = 16)
Demographic data													
Gender (M/F)	M	F	F	M	M	F	F	F	F	F	M	F	9/7
Age at onset (years)	66	57	60	58	71	70	68	64	75	57	71	72	
Age at death (years)		73							91			82	
Disease duration (years)	6	16	7	12	3	2	4	4	15	14	3	10	
Initial symptoms													
Depression		+	+				+			+			
Memory loss	+			+	+	+		+	+	+	+	+	
Evolution symptoms													
Depression		+	+		+		+			+			
Memory loss	+	+	+	+	+	+	+	+	+	+	+	+	
Seizures		+		+									
Myoclonus		+		+					+				
Parkinsonism		+	+		+								
Stroke-like episodes			+	+									
Aphasia										+			
Neuropsychometry													
MMSE	12	<10	16	<10	22	21	22	16	<10	<10	23	<10	
CDR	2	3	2	3	1	2	1	2	3	2	2	3	
APOE genotype	3/4	3/3	3/3	3/3	3/3	3/3	3/4	4/4	3/4	4/4	3/3	3/3	3/3 (13) 3/4 (2) 3/2 (1) 4.4 (0)

has enough power to perform a total genome search with markers spaced 10 cM apart. This analysis suggested that the pedigree could generate a maximum LOD score of 6.7, but predicted an average maximum LOD score of 3.4 if a linked marker shows no recombination with the disease. The predicted maximum and average maximum LOD scores at $\theta = 0.05$ and $\theta = 0.1$ are shown in Table 3. The estimated mean maximum LOD score for an unlinked marker (mean exclusion value) is −4.9 ($\theta = 0.01$) with a range from −10.6 (minimum) to 0.29 (maximum). This means that the sum of these kindred has enough power to detect linkage in a 10 cM wide genome search followed by a fine mapping with polymorphic markers of selected regions.

Three patients bore the *APOE* ε3/4 genotype and two the ε4/4; the seven remaining were 3/3, while two out of 16 not-affected siblings were 3/4 [odds ratio for Alzheimer disease in carriers of an *APOE* ε4 allele is 6.17; 95% confidence interval (CI): 1.15–33.15]. *APOE* ε4 carriers did not show an earlier onset or more severe clinical manifestations. No mutations were found in the coding exons of *PSEN1*, *PSEN2* and exons 16 and 17 of *APP*. All microsatellite tests for *APP*, *PSEN1* and *PSEN2* genes were informative. No common haplotype was found for any of these markers in the affected or the unaffected that may suggest segregation of a causal mutation in the genes *APP*, *PSEN1* and *PSEN2*. Calculated LOD scores were negative for these markers (Fig. 3).

Discussion

These kindred represent a multigenerational and extended pedigree with autosomal dominant late-onset definite Alzheimer's disease appearing in a genetically isolated population, which reduces the genetic heterogeneity of the disease. The importance of this pedigree relies in its genetic profile because, having ruled out its association with known Alzheimer's disease causal genes, it supports the implication of a novel gene. Sequencing of *APP*, *PSEN1* and *PSEN2* coding regions, along with the examination of the markers of these loci, rules out involvement of these genes in these kindred. Furthermore, most affected cases lack the APOE ε4 allele and, therefore, Alzheimer's disease is not influenced in these cases by this strong susceptibility factor. Thus, this kindred may be suitable to describe new genetic causes of the disease.

There are few reports of multigenerational autosomal dominant Alzheimer's disease kindred with a late age at onset and exclusion of candidate genes. These include the kindred reported by Martin *et al.* (1997) and by Cai *et al.* (1997), where *APOE* ε4 has a strong effect in the onset of the disease, and an Amish kindred with all the affected cases being *APOE* 3/3 (Pericak-Vance *et al.*, 1996). These reports did not include pathological confirmation of the disease. Other reports on late onset kindred were before the discovery that *APP*, *PSEN1* and *PSEN2* genes are involved in familial Alzheimer and, therefore, lack molecular analysis of the genes (Bird *et al.*, 1989; St George-Hyslop *et al.*, 1989).

Complex segregation analysis of this kindred points to the segregation of a major gene with an autosomal dominant pattern for Alzheimer's disease. The results presented in Table 2 show that, compared with the environmental or the recessive effect models, the major dominant gene effect model is the most parsimonious to explain the mode of inheritance. In contrast to the usual notion in late onset familial

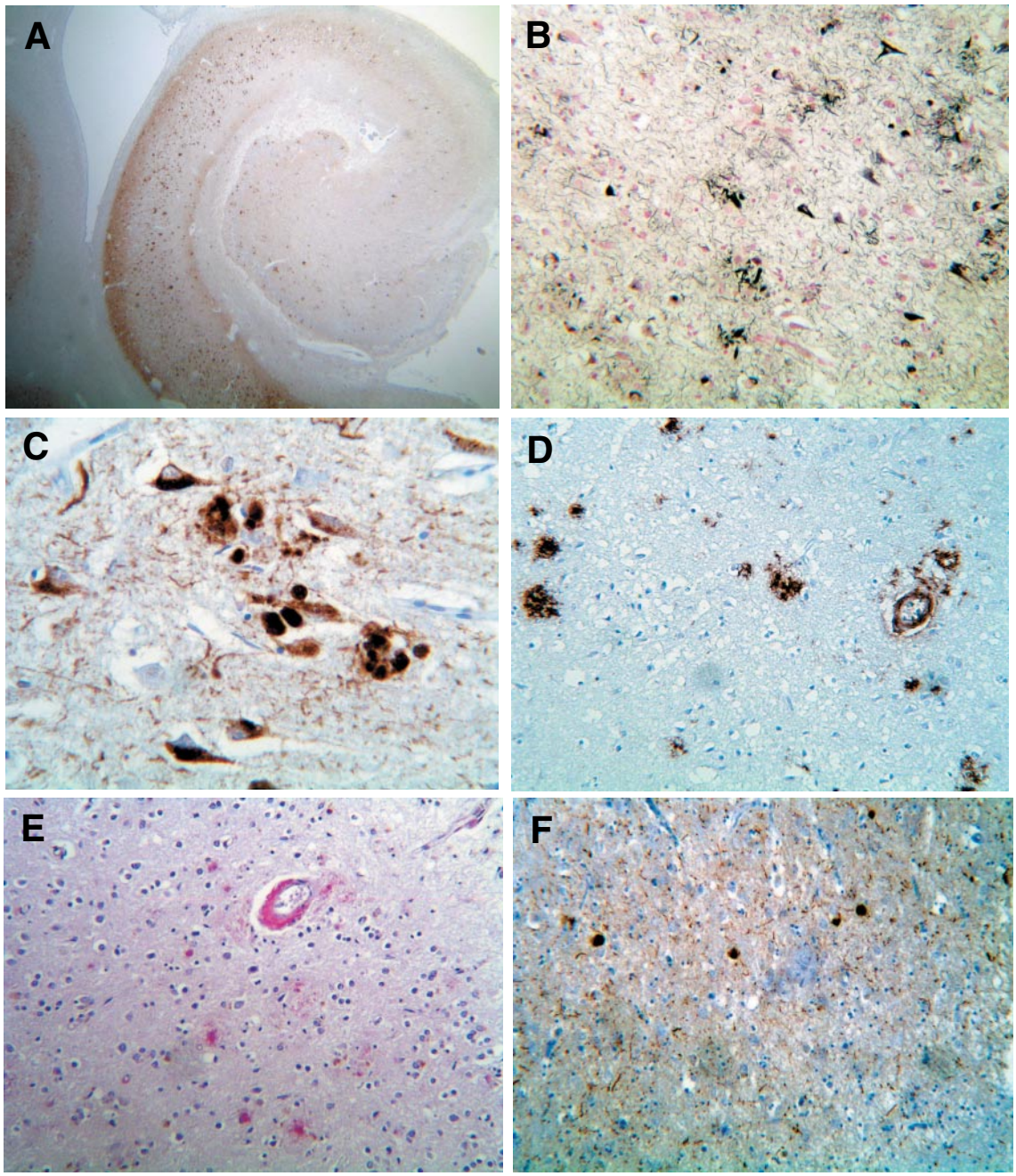

Fig. 2 Neuropathological findings; case I3-A263. (**A**) Low power view of hippocampus immunostained for tau with AT100 antibody demonstrating extensive tau deposition. (**B**) Numerous neuritic plaques and neurofibrillary tangles demostrated by Gallyas silver staining (temporal neocortex) and (**C**) tau immunohistochemistry (AT100 antibody) (hippocampus). Neocortical Aβ deposits in the neuropil and walls of arteriole, demonstrated by immunohistochemistry with Aβ antibody 6F/3D (**D**) or by Congo red (**E**). (**F**) Lingual gyrus layer 6, α-synuclein inmunohistochemistry, demonstrating cortical Lewy bodies.

Alzheimer's disease, where the disease is likely to be the result of several additive genes each of relatively small effect (polygenic hypothesis) (Daw *et al.*, 2000), here the disease is caused by the strong effect of a single gene.

The polygenic model is included in the multifactorial and mixed hypotheses of the complex segregation analysis. These models result from the assumption of the presence of a polygenic effect (represented by an infinite number of genes with identical effect), plus an environmental effect and a major gene effect in the mixed model. As demonstrated in the complex segregation analysis, these models are rejected when compared with models assuming only the presence of a major gene. The genetic isolation is surely the reason for this monogenic pattern.

The age of onset in these kindred ranges from the sixth to the seventh decades, as evidenced in the last generation of

Table 2 Hypotheses tested by complex segregation analysis

Hypothesis	Parameters								
	d	t	q	H	Z	t1	t2	t3	–2ln(L)+C
No transmission									
1 Sporadic (q = H = 0)	(0)	(0)	(0)	(0)	(1)	...	...	...	80.17
Multifactorial									
2 No cohort effect	(0)	(0)	(0)	0.64	(1)	...	...	...	59.31
Major locus									
3 Dominant	(1)	3.2	0.03	(0)	(1)	(1)	(0.5)	(0)	50.88
4 Co-dominant	(0.5)	4.3	0.023	(0)	(1)	(1)	(0.5)	(0)	55.36
5 Recessive	(0)	3.3	0.23	(0)	(1)	(1)	(0.5)	(0)	55.77
6 d not restricted	1.0	3.2	0.03	(0)	(1)	(1)	(0.5)	(0)	50.88
Mixed model									
7 Dominant	(1)	0.0	0.02	0.64	(1)	(1)	(0.5)	(0)	59.31
8 Co-dominant	(0.5)	0.0	0.17	0.64	(1)	(1)	(0.5)	(0)	59.31
9 Recessive	(0)	6.4	0.04	0.86	(1)	(1)	(0.5)	(0)	55.03
No major gene effect									
10 t1 = t2 = t3	1.0	3.2	0.03	0.30	(1)	0.97	0.97	0.97	73.13

Parameters of the model: q = frequency of high risk allele A; t = the displacement at the major simple locus between the two homozygotes; d = dominance (d = 0 corresponds to a recessive gene; d = 1 corresponds to a dominant gene; 0 < d < 1 corresponds to some grade of dominance; if d = 0.5, the gene is co-dominant); H = polygenic heritability in the offspring; Z = ratio for the inter-generational heritability (adult to child); t1, t2, t3 = probabilities that the genotypes AA, Aa, aa transmit the allele A, respectively. For example, if the simple major locus has a Mendelian heritability, then t1 = 1, t2 = 0.5, t3 = 0. If the t's are all the same, there is no major gene effect in the transmission. L = likelihood of the model. Models are compared using the likelihood ratio criterion, wherein [–2ln(L)+C]i – [–2ln(L)+C]j is distributed as a χ^2 with degrees of freedom equal to the difference in the number of estimated parameters (for i, j the number of the compared models) (Ray *et al.*, 1993).

Table 3 Estimated LOD score for a linked and unlinked marker of the pedigree

		Recombinant frequency (θ)		
		0.00	0.05	0.10
Linked marker	Maximum LOD	6.682	6.265	4.875
P = 0.8	Mean LOD	3.417	2.253	1.451
		Recombinant frequency (θ)		
		0.01	0.05	0.10
Unlinked marker	Minimum LOD	−10.597	−5.681	−3.553
P = 0.8	Maximum LOD	0.289	1.111	1.46
	Mean LOD	−4.928	−2.163	−1.405

affected subjects who have been clinically examined and as assumed in affected ancestors whose mean age at death was around 75 years. That is, these families have an age of onset similar to sporadic Alzheimer's disease; this is unlike most autosomal dominant familial Alzheimer's disease, in which an earlier age at onset is the rule. The age at onset did not decrease in subsequent generations arguing against the existence of anticipation.

The clinical presentation in all the cases studied is quite typical of Alzheimer's disease with a progressive cognitive deterioration of cortical type and functional impairment. In addition, seizures and myoclonus were documented in several cases in advanced stages. The long duration of the disease in the absence of atypical features did not differ from late onset sporadic Alzheimer's disease. This kindred presents also three clinical features deserving further consideration. First, the high prevalence of depression in early stages of the disease, a symptom that is increasingly taken into account in early stages of Alzheimer's disease (Powlishta *et al.*, 2004; Hwang *et al.*, 2004). Secondly, the presence of several cases with mild parkinsonism in the evolution of the disease, a fact that has been reported profusely in familial Alzheimer's disease caused by presenilin or *APP* mutations (Houlden *et al.*, 2001). Also, one case in these kindred has Parkinson's disease without cognitive deterioration so far. Whether she presents a phenotypic variant or the coincidence of another neurodegenerative disorder may be clarified in the future by genetic linkage or neuropathology. Finally, some subjects had stroke-like episodes and several ancestors died of a cerebral haemorrhage suggesting the presence of amyloid angiopathy, as found in the brain examined in the necropsy study.

The necropsy study of case I3-A263 allowed confirmation of the diagnosis of Alzheimer's disease. This case demonstrated a very high density of cortical neurofibrillary tangles and senile neuritic plaques fulfilling a Braak stage VI. In addition, cerebral amyloid angiopathy and α-synuclein positive limbic Lewy bodies and neurites were found. These two pathological features are common in both familial Alzheimer's disease caused by *APP* and presenilin mutations and sporadic Alzheimer's disease (Revesz *et al.*, 1997; Lippa

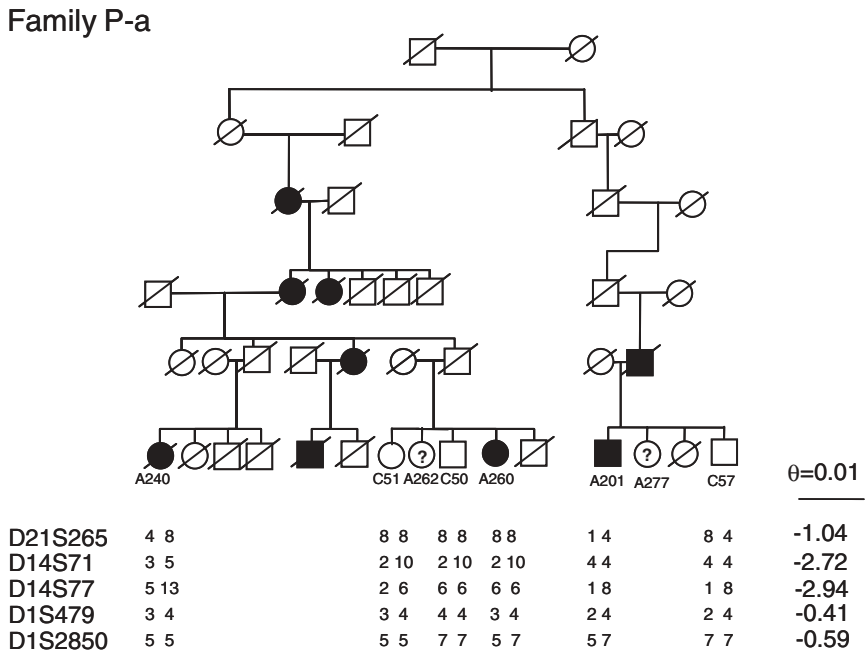

Fig. 3 Genotype results in family P-a.

et al., 1998; Singleton *et al.*, 2000) and are likely to correlate with the stroke-events and parkinsonian symptoms, respectively, present in these kindred. Frontotemporal dementia or other familial dementias such as Lewy body dementia or prion diseases can be ruled out because none of the affected cases had clinical, neuroimaging or EEG manifestations of these diseases. In addition, amyloid angiopathy and the clinical counterpart of this pathology, such as cerebral haemorrhage (very common in this kindred) is not present in these diseases.

This pedigree is, therefore, suitable for a genetic search study that can provide clues for new genetic markers associated with Alzheimer's disease. The number of affected individuals is large enough to perform a significant genome-wide screening according to the results of the computer simulation studies. Moreover, their genetic isolation increases the chances of finding a causative gene (Arcos-Burgos and Muenke, 2002; Heutink and Oostra, 2002) because this implies a lower heterogeneity and a monogenic or oligogenic disorder decreasing the genetic complexity of the disease. In addition, a region in linkage disequilibrium, containing the responsible gene, may be expected. According to the genealogical study, this genetic isolate can be considered a recent isolate (<20 generations), so a long disequilibrium region (>1 cM) should be likely.

Although we have not been able to link the four families into a single pedigree due to an absence of some old municipal registers, multiple data support the concept that these families have inherited the disease from a common founder who arrived in the region in the 18th century. Previous studies have confirmed that a genome-wide screening performed in highly selected populations can associate Alzheimer's disease with new loci, even with a low number of DNA samples examined. This was the case with Farrer *et al.* (2003), who studied five cases and five controls from a consanguineous tribal Arab–Israeli community or with Hiltunen *et al.* (2001), who examined 47 late-onset cases and 51 controls from a genetically isolated Finnish group.

The genetic study of these kindred will add information to the scant data on late-onset Alzheimer's disease in Europeans. To date, only four late-onset Alzheimer's disease genome-wide screenings have been conducted in extended families or sibling pairs (Pericak-Vance *et al.*, 1998; Kehoe *et al.*, 1999; Blacker *et al.*, 2003; Lee *et al.*, 2004). All of these have been performed in North American populations, with the exception of the study of Kehoe *et al.* (1999), which included 94 families from the UK. However, these families were examined along with other 357 North American families, thus increasing the genetic heterogeneity. No chromosomal region, except chromosome 19 around the APOE locus, showed evidence of linkage in all four studies. However, several regions on chromosomes 9, 10 and 12 showed evidence of linkage in at least two studies (Kamboh, 2004). These studies have been followed by examination of genes in these locations with particular interest in the region coding for the insulin-degrading enzyme and the urokinase-type plasminogen activator on chromosome 10 (Myers and Goate, 2001). In addition, the study of 86 kindred with late onset Alzheimer's disease on five chromosomes where linkage was previously reported has identified linkage to chromosome 19p13.2—a locus 40 cM away from the APOE gene (Wijsman *et al.*, 2004).

In summary, we describe the clinical and pathological features of a late-onset familial autosomal dominant Alzheimer's disease not caused by the previously reported genes, which

may help to locate new loci. As the result of the encouraging findings from computer simulation, we are pursuing linkage studies in an attempt to map the gene underlying Alzheimer's disease in these families.

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