

# A new locus on chromosome 19 linked with late-onset Alzheimer's disease

S. E. Poduslo and X. Yin

Division of Neurology, Texas Tech University Health Sciences Center, 3601 Fourth Street, Lubbock, TX 79430, USA

<sup>CA</sup>Corresponding Author

Received 8 August 2001; accepted 20 September 2001

Alzheimer's disease (AD) is a complex neurodegenerative disorder, characterized by cognitive decline and distinctive neuropathology. APOE 4 and APOC1 A on chromosome 19 are risk factors for late-onset disease. Using large extended families with multiple siblings affected, we have identified several microsatellite markers which are also linked with late-

onset Alzheimer's disease. These microsatellites are distal from the apolipoprotein cluster on chromosome 19. It is likely that multiple genes will be involved with late-onset disease, either as risk factors or as causative agents. *NeuroReport* 12:3759-3761 © 2001 Lippincott Williams & Wilkins.

**Key words:** Alzheimer's disease; Chromosome 19

## INTRODUCTION

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by a decline in cognition and distinctive neuropathology. The disease is genetically heterogeneous in that mutations in genes on chromosomes 21, 14, and 1 cause some cases of early-onset disease (clinical symptoms before 65 years of age) [1].

APOE 4 has been associated with an increased risk for late-onset AD [1,2]. Our studies show that the A allele of APOC1 is also a risk factor for the disease, as the frequency of the A allele in AD patients is similar to that found with the APOE 4 allele [3]. In this study we analyzed microsatellites surrounding the apolipoprotein gene cluster for linkage with the disease.

## MATERIALS AND METHODS

**Ascertainment:** A community-based DNA bank of families with AD and other neurodegenerative disorders in Texas was established in 1993. All family members who enrolled into the DNA bank were informed of the study and asked to sign consent forms. Procedures for recruitment, requests for medical records, and consent forms were approved by the medical school's institutional review board (IRB). Medical records were requested for the probands and affected siblings; the diagnosis of senile dementia of Alzheimer's type was made, according to established criteria [4]. The diagnosis included a detailed medical history documenting the progressive loss of memory and worsening of cognitive functions, and appropriate blood work to rule out other medical conditions. A CT scan or MRI of the brain was also done to determine whether the dementia was due to a tumor, vascular disease, or cortical atrophy. In addition to small nuclear families, we have large extended families with multiple affected members

who have enrolled into the DNA bank. In most of the extended families there are a minimum of six siblings, with two or three also affected with AD. Some of the extended families have over 10 siblings; in most cases, a parent and several of the parent's siblings also had dementia. Four autopsies have been performed on deceased members of the extended families and almost 100 autopsies on the remainder since the start of the study; the autopsy findings confirmed the diagnosis, using criteria described [5]. The patients are of non-Hispanic, non-Black, and non-Indian descent. The extended families were used for the linkage analysis of markers on chromosome 19 surrounding the apolipoprotein gene cluster.

**Molecular and linkage analysis:** Genomic DNA was extracted from blood or transformed lymphocytes. Markers on chromosome 19 were amplified by PCR, analyzed on an ABI 377 sequencer, and the alleles called using GeneScan. Linkage analysis was performed with the FASTLINK software package, using LinkMap [6-9]. Simulations were done using FASTSLINK with unlinked markers having heterozygosities of 0.25 and 0.2 [10,11]. Linklods was used to obtain Lod scores from LinkMap and Homog was used to determine homogeneity [12]. The liability classes were assigned as described [13] and the gene frequency was set at 0.05. Normal allele frequencies were obtained from genotyping 100 unaffected (Caucasian) spouses enrolled in the DNA bank. The order of the microsatellite markers analyzed were D19S220, D19S210, APOE, APOC1, D19S226, D19S420, D19S246, D19S581, D19S198, D19S178, D19S879, D19S867, D19S418, D19S902, as obtained from the sequences in the human genome project.

**Table 1.** Statistics on four extended families.

Families	Total number analyzed	Total number sibs	Number affected	Number DNA samples
1	50	9	4	20
2	54	13	4	29
3	46	12	3	26
4	91	19	4	37

## RESULTS

**Clinical findings:** The proband in family 1 had a gradual mental decline, with age of onset at 77 years. At age 83, the proband was oriented only to place, name, and year and did not know the season, the month, or the day. Questions were at times answered inappropriately, due to lack of understanding. The family had noted a progressive decline in mental acuity and increased confusion. Past medical history was positive for hypertension, arthritis, and cataracts. The complete blood work up was normal. A CT scan of the head revealed that the ventricular system was prominent symmetrically with mild prominence of cortical sulci. There were no lesions. At age 88, the patient was less mentally sharp, spoke less, and had severe confusion. At age 89, the patient no longer spoke, but nodded in response to questions. The patient died at age 94. The proband had eight siblings; three siblings were also affected. Some 20 blood samples (which included three siblings) were collected and analyzed. The average age of onset was  $72.5 \pm 6.36$  years.

In family 2, the proband presented with decreasing memory problems with an age of onset at 72 years. At age 75, the patient had poor short term memory, attention span, recall, calculation, and proverb interpretation. The patient was occasionally delusional and hallucinated. Past medical history revealed degenerative arthritis. The complete blood work up was normal. The CT scan showed slightly prominent cerebral sulci with normal ventricles and no lesions. At age 77, the patient required 24h supervision and had episodes of severe agitation, hallucinations and delusions. Responses to questions were confabulated. The patient did not know the month, the day, the president, the location, serial sevens, and could not spell world backward. The patient died at age 82. The proband

had 12 siblings with a total of four affected. Here 29 blood samples (which included four siblings) were collected and analyzed. The average age of onset of clinical signs in these families was  $72.8 \pm 5.4$  years.

In family 3, the proband presented with short term memory loss with an age of onset at 66 years. At age 74 the patient was disoriented and could not tell the date, month, or time. Between 74 and 77, the patient experienced hallucinations at night, was agitated, and had mood swings. At age 78 the patient had difficulty communicating, did not speak much, and exhibited a generalized loss of higher cortical function. The patient could walk without difficulty and had normal muscle tone. A CT scan revealed a prominence of the cortical sulci, basilar cisterns, cerebral ventricles, and cerebellar folia. No other lesions were noted. The proband had 11 siblings with a total of three affected. Twenty-six blood samples (which included three siblings) were collected and analyzed. The average age of onset was  $66.7 \pm 9.0$  years.

In family 4 the proband presented with difficulty in expression, a flat affect, was less talkative and withdrawn at age 65. A year later, the dementia had worsened and the patient needed help dressing and bathing, was restless at night, and had occasional hallucinations. Two years later, the proband no longer communicated and developed moderate parkinsonism. The proband died at age 72. The proband was one of 19 sibs, four of whom were affected. Some 37 blood samples were collected from this family and analyzed. The average age of onset was  $68 \pm 3.6$  years.

**Analysis:** Fourteen microsatellites on chromosome 19 surrounding the apolipoprotein cluster, as well as the polymorphisms in APOE and APOCI, were analyzed against the disease in these families. Using LinkMap, Linklods, and Homog, four large extended families showed evidence of linkage with several markers. These four extended families had a minimum of nine sibs with three or four affected with the disease (Table 1).

Simulation studies on the four families gave an average expected Lod score of 1.568. Linkage analysis showed that placing the disease between D19S246 and D19S581 gave the highest Lod scores (total, 10.541) in these four extended families (Table 2). Placing the disease within the apolipoprotein cluster (between APOE and APOCI) gave negative

**Table 2.** Linkage analysis for four extended families on chromosome 19. Order of markers: D19S220-D19S210-APOE-APOCI-D19S226-D19S420-D19S246-D19S581-D19S198-D19S178-D19S879-D19S867. LOD scores at markers with theta of 0.35.

Families	D19S226-Disease-D19S420	D19S420-Disease-D19S246	D19S246-Disease-D19S581
1	0.940	2.611	2.877
2	1.874	0.126	0.298
3	-1.912	0.360	4.429
4	-0.725	2.782	2.936
Total	0.178	5.878	10.541
Families	D19S581-Disease-D19S198	D19S198-Disease-D19S178	D19S178-Disease-D19S879
1	0.533	1.399	1.109
2	1.250	4.766	2.161
3	2.031	2.768	0.106
4	1.132	-0.398	-0.990
Total	4.947	8.534	2.386

Lod scores ( $-2.7$  for family 1,  $-1.5$  for family 2,  $-1.6$  for family 3, and  $-3.5$  for family 4).

## DISCUSSION

In the initial report of linkage of AD with chromosome 19, results from two markers (D19S13 and D19S9) and polymorphic sites in three genes were presented [14]. After APOE 4 was identified as a risk factor for the disease, Lod scores of 2.4 [2] and of 2.77 [15] were obtained when the disease was analyzed with APOE. Our data suggest that this area of chromosome 19 may have another mutated gene linked with the disease that is distal from APOE.

There is no question that AD is quite heterogeneous; multiple genes will either be linked with late-onset disease or will serve as risk factors for the disease. We have found that a milder form of the disease is linked with markers on chromosome 3 [16]. Now we have found that others of our extended families are linked with markers on chromosome 19. Whether these links will ultimately identify risk factors or genes contributing to the disease is unknown at this time, but is under intense investigation.

Acknowledgements: This research was supported by funds from the State of Texas. We would like to thank the families for their active participation in the DNA bank.

## REFERENCES

1. Roses AD. *Am J Med Genet* **81**, 49–57 (1998).
2. Saunders AM, Strittmatter WJ, Schmechel D *et al.* *Neurology* **43**, 1467–1472 (1993).
3. Poduslo SE, Neal M, Herring K *et al.* *Neurochem Res* **23**, 361–367 (1998).
4. McKhann GM, Drachman D, Folstein M *et al.* *Neurology* **34**, 939–944 (1984).
5. Mirra SS, Hart MN and Terry RD. *Arch Pathol Lab Med* **117**, 132–144 (1993).
6. Cottingham RW, Idury RM and Schaeffer AA. *Am J Hum Genet* **53**, 252–263 (1993).
7. Schaeffer AA, Gupta SK, Shriram K *et al.* *Hum Hered* **44**, 225–237 (1994).
8. Lathrop GM, Lalouel J-M, Julier C *et al.* *Proc Natl Acad Sci USA* **81**, 3443–3446 (1994).
9. Terwilliger JD and Ott J. *Handbook of Human Genetic Linkage*. Baltimore, MD: Johns Hopkins University Press; 1994.
10. Ott J. *Proc Natl Acad Sci USA* **86**, 4175–4178 (1989).
11. Weeks DE, Ott J and Lathrop GM. *Am J Hum Genet* **47**, A204 (1990).
12. Ott J. *Genet Epidemiol Suppl.* **1**, 251–257 (1986).
13. Farrer LA and Cupples LA. *Am J Hum Genet* **54**, 374–383 (1994).
14. Pericak-Vance MA, Bebout JL, Gaskell PC *et al.* *Am J Hum Genet* **48**, 1034–1050 (1991).
15. Blacker D, Haines JL, Rodes L *et al.* *Neurology* **48**, 139–147 (1997).
16. Poduslo SE, Yin X, Hargis J *et al.* *Hum Genet* **105**, 32–37 (1999).