

## Chromosome 12 and late-onset Alzheimer's disease

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### Abstract

Alzheimer's disease is a complex neurodegenerative disorder, characterized by cognitive decline and distinctive neuropathology. Using large extended families with multiple affected, we found that three markers on chromosome 12 were linked with late-onset Alzheimer's disease. These markers were downstream from the gene for alpha-2 macroglobulin. It is likely that multiple genes will be identified either as risk factors or as causative agents for late-onset Alzheimer's disease. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Alzheimer's disease is a complex neurodegenerative disorder characterized by cognitive decline and distinctive neuropathology. Mutations in three genes (encoding the amyloid precursor protein, presenilins 1 and 2) have been identified as linked with early-onset Alzheimer's disease [5,7,16]. Risk factors, including APOE 4, APOC1 A, and polymorphisms in the alpha-2 macroglobulin, have also been found [2,8,13,15]. We found no association of the two polymorphisms in the gene for alpha-2 macroglobulin (A2M) in our patients and families [14]. However, we did find strong evidence of linkage with markers downstream from the A2M gene in late-onset disease.

A community-based DNA Bank for families having probands with memory problems or other neurodegenerative disorders was started in 1993 in Texas. To enroll, family members completed detailed histories on the patients; those with power of attorney signed release forms for the medical records of those affected. The criteria for the clinical diagnosis of Alzheimer's disease was according to the guidelines by NINCDS-ADRDA [9] which included a progressive decline in memory and cognitive function and appropriate blood work to rule out other medical conditions. A CT scan or MRI of the brain which showed cortical atrophy was also included. In addition to small nuclear families, there were large extended families with 6–11 siblings and multiple affected with late-onset disease (clinical symptoms after the age of 65) that enrolled. Four autopsies have been

performed on deceased members of the extended families which confirmed the diagnosis, using criteria described [10]. The patients are of non-Hispanic, non-Black, and non-Indian descent. Procedures for recruitment, requests for medical records, and consent forms were approved by the medical school's institutional review board (IRB). All participants and those having power of attorney for the patients were advised of the study and gave informed consent, in accordance with the IRB guidelines.

Genomic DNA was extracted from blood or transformed lymphocytes. Markers on chromosome 12 were amplified by PCR. The reaction mix consisted of 1.00  $\mu$ l 10 $\times$  PCR buffer, 1.00  $\mu$ l 2.5 mM dNTP, 1.00  $\mu$ l 2.5 mM MgCl<sub>2</sub>, 0.25  $\mu$ l each primer 1 and 2, 4.47  $\mu$ l ddH<sub>2</sub>O, 0.08  $\mu$ l Amplitaq Gold, and 2.00  $\mu$ l 20–50 ng DNA. The amplification consisted of 95°C for 5 min; 95°C for 15 s; 55°C for 15 s; 72°C for 30 s; steps 2–4 for 10 cycles followed by 89°C for 15 s; 55°C for 15 s; 72°C for 30 s; steps 6–9 for 20 cycles followed by 72°C for 10 min. The samples were analyzed on an ABI 377 sequencer and the alleles called using GeneScan. (Reagents were from Perkin/Elmer/Applied Biosystems, Foster City, CA).

Linkage analysis was performed with the FASTLINK software package, using Mlink, Ilink, and LinkMap [3]. Linklods and Homog were also used [11]. The liability classes were assigned as described [4] and the gene frequency was set at 0.038. Normal allele frequencies were obtained from genotyping 100 unaffected (Caucasian) spouses enrolled in the DNA Bank. The order for the markers used was D12S351, D12S367, D12S346, D12S79, D12S86, A2M, D12S352, D12S310, D12S326,

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Table 1  
Statistics on three extended families

Families	Total number analyzed	Total number sibs	Number affected	Number DNA samples
1	71	10	4	30
2	42	6	4	21
3	118	8	4	24

D12S83, D12S345, D12S364, D12S78, D12S85, D12S324, D12S99, D12S368, as obtained from the sequences in the human genome project.

Sixteen markers on chromosome 12 and the two A2M polymorphisms were analyzed against the disease in the extended families. Using LinkMap, Linklods, and the Homog software, three large extended families with late-onset disease showed evidence of linkage with several markers. These three families had 6 to 10 siblings, 4 of whom were affected (Table 1). Linkage analysis showed that placing the disease between D12S364 and D12S78 gave the highest Lod scores in these three families (Table 2). Placing the disease between the two A2M polymorphisms gave negative Lod scores. Numerous studies have failed to show an association with the A2M polymorphisms (for references, see [17]).

There have been several other studies that have reported linkage of Alzheimer's disease with markers on chromosome 12. Upon completion of a genome screen on late-onset Alzheimer's disease, there was a report of linkage with markers on chromosome 12, especially D12S1042 [12]. The study on A2M also reported linkage with markers D12S98 and D12S358 [2]. The results of a third study and a second genome screen showed linkage with the marker D12S98 [6,18].

The reports mentioned above used families collected from the National Institute of Mental Health AD Genetics Consortium (NIMH) [1]. These reported markers are centromeric from the markers linked with the disease in our study. Their reported marker D12S358 gave a negative Lod score with our families. Marker D12S1042 is 4.4 cM from

D12S345 which also gave a negative Lod score in our families.

Alzheimer's disease is a complex disorder. It is likely that multiple genes will be involved with late-onset disease. Subdividing families according to initial clinical symptoms, neuropathology, and genetic links, as well as age of onset of clinical symptoms, will ultimately make therapeutic intervention more meaningful.

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Table 2  
Linkage analysis for extended families on chromosome 12<sup>a</sup>

Families	D12S345-disease-D12S364	D12S364-disease-D12S78
1	0.053	1.126
2	2.160	1.647
3	-0.156	3.601
Total	2.057	6.374

  

Families	D12S78-disease-D12S85	D12S85-disease-D12S324
1	1.649	2.563
2	2.070	3.136
3	0.901	-0.109
Total	4.620	5.591

<sup>a</sup> LOD scores of markers at theta of 0.35.

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