SHORT REPORT

New Alzheimer's disease locus on chromosome 8

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Background: Family history is one of the most consistent risk factors for dementia. Therefore, analysis of families with a distinct inheritance pattern of disease can be a powerful approach for the identification of previously unknown disease genes.

Objective: To map susceptibility regions for Alzheimer's disease.

Methods: A complete genome scan with 369 microsatellite markers was carried out in 12 extended families collected in Sweden. Age at disease onset ranged from 53 to 78 years, but in 10 of the families there was at least one member with age at onset of ≤ 65 years. Mutations in known early-onset Alzheimer's disease susceptibility genes have been excluded. All people were genotyped for APOE, but no clear linkage with the $\epsilon 4$ allele was observed.

Results: Although no common disease locus could be found in all families, in two families an extended haplotype was identified on chromosome 8q shared by all affected members. In one of the families, a non-parametric multi-marker logarithm of the odds (LOD) score of 4.2 ($p=0.004$) was obtained and analysis based on a dominant model showed a parametric LOD score of 2.4 for this region. All six affected members of this family shared a haplotype of 10 markers spanning about 40 cM. Three affected members in another family also shared a haplotype in the same region.

Conclusion: On the basis of our data, we propose the existence of a dominantly acting Alzheimer's disease susceptibility locus on chromosome 8.

Family history is the most consistent risk factor for Alzheimer's disease after advanced age. In a recent study of Swedish twins, genetic factors were estimated to account for up to 79% of disease susceptibility.¹ Mutations in the genes of amyloid precursor protein (APP), presenilin 1 (PS1) and 2 (PS2), cause an early-onset, dominantly acting form of familial Alzheimer's disease.² However, mutations in these genes are rare and cannot explain all genetic susceptibility to dementia. Only one gene, apolipoprotein E (APOE), has been identified as a susceptibility factor for late-onset Alzheimer's disease.³ Yet, not everyone carrying the high-susceptibility APOE $\epsilon 4$ allele will develop illness, and many who lack the $\epsilon 4$ allele will develop Alzheimer's disease. It is estimated that APOE contributes to about 50% of the total genetic variance in Alzheimer's disease.⁴ Thus, a large proportion of the genetic variance in Alzheimer's disease must be determined by unidentified genes.

Several disease susceptibility regions have been identified by genome scans, the most consistent of which are on chromosomes 6, 9, 10 and 12.⁵ Recently, several candidate genes in these regions have been examined, although none has shown a stable association with late-onset Alzheimer's disease in all studies. Genes located in the chromosome-10 region have been most extensively investigated—for example,

the gene encoding insulin degrading enzyme (IDE) has been examined by many research groups. However, the association to Alzheimer's disease was observed only in some studies.^{6–8} Recently, Grupe *et al*⁹ published results from an extensive single-nucleotide polymorphism scan of the chromosome-10 region. They identified a gene with an as yet unknown function, which was associated with Alzheimer's disease in several case–control series.

Some families with Alzheimer's disease have an inheritance pattern consistent with an autosomal dominant model. Analysis of such families can be a powerful approach for the identification of new disease genes. Several attempts have been made to locate susceptibility genes in extended families with Alzheimer's disease and several disease-linked chromosomal regions have been identified.^{10–11}

In this study, we have used 12 Swedish families, with an autosomal dominant inheritance pattern of Alzheimer's disease, in an attempt to identify disease-causing genes. We have hypothesised that these families might share a common disease locus.

MATERIALS AND METHODS

Pedigrees

All 12 families were collected in Sweden and were of Swedish origin. Clinical follow-up of affected people was carried out by neurological examination of incident patients, review of medical records and interviews of first-degree relatives. In five members from four families, diagnosis of Alzheimer's disease was confirmed by neuropathological examination. For deceased patients, diagnosis was obtained through family informants. A diagnosis of definite or probable Alzheimer's disease was made according to NINCDS-ADRDA diagnostic criteria.¹² Age at onset ranged from 53 to 78 years. Patients with Alzheimer's disease, and recorded cardiovascular disease or stroke, were categorised as Alzheimer's disease with vascular symptoms. The selection of families was based on at least one family member being diagnosed with Alzheimer's disease at or before ≤ 65 years of age. However, this restraint was lifted in two cases, families 8 and 9, as these families included a substantial number of affected family members. Furthermore, families with numerous affected members were selected, even though DNA samples could not be obtained for all members. From all 12 families, DNA samples from 44 affected members and 47 healthy family members were available for analysis (table 1).

This study was approved by research ethics committees at the Uppsala University Hospital.

Genotype and sequence analysis

A genome-wide screen was carried out using 369 microsatellite markers from the Weber V.6 marker set. The average distance between markers used in the analysis was 10.6 cM.

Abbreviations: APOE, apolipoprotein E; APP, amyloid precursor protein; IDE, insulin degrading enzyme; LOD, logarithm of the odds; PS1, presenilin 1; PS2, presenilin 2; SSCP, single-strand conformation polymorphism

Table 1 Families with an autosomal dominant inheritance pattern of Alzheimer's disease included in the genome scan

Family	Number of affected members	Genotyped members affected or unaffected or unknown	Age at onset	Clinical symptoms
1	7	3/3	60–78	AD, vascular symptoms in two cognitively healthy members
2*	10	6/7	57–70	Pathologically confirmed AD, vascular symptoms in three cases of AD
3	6	3/10	59–75	Pathologically confirmed AD, vascular symptoms in one patient with AD
4*	5	3/3	54–75	Pathologically confirmed AD
5	4	3/3	60–69	AD
6	9	4/7	65–76	AD
7	8	5/2	61–78	AD
8	8	5/1	67–75	AD
9	10	2/2	72–78	Pathologically confirmed AD
10	8	4/2	58–70	AD
11	12	1/5	55–70	AD
12	6	5/2	53–72	AD, vascular symptoms in one case of AD

AD, Alzheimer's disease.

*Families in which linkage to chromosome 8 was observed.

People with available DNA samples have been genotyped. In some cases, unaffected people have been described as unknowns as they had not yet reached the age at disease onset of affected people in their respective families.

At a second stage, seven additional markers were analysed in regions showing evidence of linkage on chromosomes 4 and 8. Marker positions were determined using the Marshfield marker map.

Mutations in the A β region of the APP gene have been ruled out in these families by the direct sequencing of exons 16 and 17. Involvement of the PS1 and PS2 genes has been excluded by direct sequencing of all translated exons, by single-strand conformation polymorphism (SSCP) or by linkage analysis. All people were genotyped for APOE.

Statistical analysis

Parametric and non-parametric statistical analysis was carried out using the GeneHunter V.2.1 software.¹³ Some of the least informative, unaffected people were removed from the analysis to meet computational requirements. Unaffected people have been described as unknowns, if they had not yet reached the age at disease onset of affected members in their respective families. For the haplotyping, SIMWALK V.2.8 was used.¹⁴ Allele frequencies were estimated using the Mendel software¹⁵ from all genotyped people and taking into account family relationships.

RESULTS

We conducted a genomewide screen in 12 Swedish families with an autosomal dominant inheritance pattern of Alzheimer's disease. No significant logarithm of the odds (LOD) scores were observed for the whole data set, indicating that there is no common single disease-causing gene in all these families. Therefore, every family was examined individually. Linkage results were considered significant only if confirmed by haplotype analysis.

In the centromeric region of chromosome 8 in family 2 (table 2), a non-parametric multimarker LOD score of 3.7 ($p = 0.004$) was obtained. Analysis based on a dominant model showed a parametric LOD score of 1.8 for this region (table 2). All five affected members of this family shared a haplotype of seven markers spanning around 40 cM. Affected members of another family, family 4, shared a six-marker haplotype in this region. At the second stage we analysed four additional markers in the region in families 2 and 4. We also genotyped additional members in family 2. This analysis

confirmed a shared haplotype identified in the first stage; however, this did not reduce the size of the region. Analysis that included additional markers and members of family 2 showed increased evidence of linkage with a non-parametric LOD score of 4.3 ($p = 0.016$) and a parametric dominant model-based multipoint LOD score of 2.4 (fig 1).

In family 2, a four-marker haplotype shared only by affected members was also identified on chromosome 4p16–15 (table 2). This region gave a non-parametric LOD score of 2.1. At the second stage, three markers were added to this region. However, the second analysis did not confirm the results of the initial scan. In this family, a two-marker shared haplotype was also identified on chromosome 18p11 (table 2).

In family 5, shared haplotypes were identified on chromosomes 3q26–28 and 4q34–35, both with non-parametric LOD scores of 2.4 (table 2). In family 7, a two-marker shared haplotype was identified on chromosome 8q24, shared by all five affected members. Two unaffected members of this family do not carry this haplotype, although in the analysis they were defined as having unknown affection status, since they were <50 years of age (table 2).

Several other regions were suggested by linkage analysis of individual pedigrees. However, affected people usually shared only single-marker genotypes and no shared haplotypes were identified. It will be necessary to validate these regions by additional markers.

The ApoE ϵ 4 frequency in patients with Alzheimer's disease from these families was 52% and that in unaffected individuals 35%. The total non-parametric LOD score for the APOE region was 1.3.

DISCUSSION

We have studied 12 families with an autosomal dominant inheritance pattern of Alzheimer's disease. All families have been selected not to carry any of the previously identified mutations in the APP, PS1 or PS2 genes, and 10 of the selected families include at least one member with a disease onset at ≤ 65 years. As all the families are of Swedish origin, we might expect a somewhat limited genetic variance and the hypothesis was that these families might share a common disease locus. The results from a complete genome scan did not show any common chromosomal region that could be

A

B

Figure 1 Results of chromosome 8 linkage and haplotype analysis in family 2. (A) Haplotyping: a shared haplotype is indicated by a red line; individuals marked with "S" have been genotyped. (B) Parametric and non-parametric linkage analysis: graphs show parametric logarithm of the odds (LOD) and non-parametric LOD (NPL) score curves for the haplotype region.

Table 2 Shared chromosomal regions identified in families with Alzheimer's disease in the initial screen

Family	Chromosome	Marker with highest score	LOD score	NPL score	Haplotype sharing	
					Affected	Healthy
5	3q26–28	D3S2427	1.2	2.4	3/3	1/2*
2	4p16–15	D4S2408	1.2	2.1	5/5	0/2
5	4q34–35	D4S408	0.6	2.4	3/3	1/3
2	8p12–q22	D8S1119	1.8	3.7	5/5	0/3
4	8p12–q22	GATA12B06	1.2	0.9	3/3	0/3
7	8q24	D8S1128	0.7	1.8	5/5	0/2
2	18p11	D18S59	1.5	2.0	5/5	0/2

*Affection status of one individual who carries the disease haplotype is unclear.

The highest multimarker parametric LOD and non-parametric LOD (NPL) scores for the linked regions are given. Parametric LOD score was calculated assuming a dominant model with nearly complete penetrance.

linked to disease in all families. However, by analysis of individual families, several potential loci were identified. The most interesting locus is in the centromeric region of chromosome 8. This haplotype is around 40 cM long and is covered by 10 microsatellite markers in our analysis. The haplotype is shared by all nine affected members in two families and none of the unaffected family members share it. Interaction with the APOE locus cannot be excluded in these two families, as all patients with Alzheimer's disease carry the ApoE ϵ 4 allele; however, in each family one unaffected member also carries this allele. In family 2, which gave the highest LOD score for the chromosome 8 locus, several affected members have not only Alzheimer's disease symptoms but also vascular problems including stroke. One of two pathologically examined cases in this family also showed substantial atherosclerosis in addition to extensive neuronal loss, neuritic plaques and neurofibrillary tangles. In the pathologically examined brain from a patient in family 4, moderate atherosclerosis was observed, even though in this family no cases of Alzheimer's disease with vascular symptoms were recorded by clinical examination. Therefore, susceptibility genes involved in the vascular system positioned within this region of chromosome 8 could be of interest. Such candidate genes include tissue plasminogen activator, which has previously been studied for association with both Alzheimer's disease and vascular disease.^{16,17} Another candidate could be neuregulin 1, a putative susceptibility gene for schizophrenia.¹⁸ This gene was also associated with Alzheimer's disease, with symptoms of psychosis.¹⁹

In family 2 (table 2), two more shared regions were identified. Interestingly, association with the chromosome 18 region was also observed in the Finnish linkage disequilibrium scan.²⁰ We also found evidence of linkage to chromosome 4q34–35 in one family. The marker D4S1629 in this region was also linked to Alzheimer's disease in a recently published genome scan in families with Alzheimer's disease collected by the NIMH Genetics Initiative.²¹ On other chromosomes, several shared regions were identified in individual families. All these regions are rather wide and require further delineation. In some families, several chromosomal regions shared by affected members were found. This indicates generally high sharing between family members, and is a common problem for validation and fine mapping of disease linked regions in small families.

The frequency of the ApoE ϵ 4 allele was higher in affected members than in those unaffected, showing the importance of this gene for disease susceptibility also in these families. The significance of APOE was shown in other genetic analyses of Swedish families with Alzheimer's disease.^{22,23} Some of the samples from these studies may overlap with the families included in the present scan, as the samples originate from the same DNA collection.

We acknowledge the limited power of detecting disease loci in families of restricted size and are currently collecting additional members from the two families with linkage to chromosome 8. This will hopefully confirm and increase the evidence of linkage to this locus and perhaps also limit the size of the region. Selection of candidate genes in this region on chromosome 8 and mutation screening will subsequently follow.

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