European Journal of Human Genetics (1999) 7, 652–658

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Linkage disequilibrium in the 13q12 region in Finnish late onset Alzheimer's disease patients

Mikko Hiltunen[1,2], Arto Mannermaa[2], Anne Maria Koivisto[1], Maarit Lehtovirta[1], Seppo Helisalmi[1,2], Markku Ryynänen[3], Paavo Riekkinen Sr[1,4] and Hilkka Soininen[1]

[1]*Department of Neurology, University Hospital and University of Kuopio*
[2]*Chromosome and DNA Laboratory of the Division of Diagnostic Services, University Hospital of Kuopio*
[3]*Unit of Clinical Genetics of the Department of Gynaecology and Obstetrics, University Hospital of Kuopio*
[4]*AI Virtanen Institute, Department of Neuroscience, University of Kuopio, Finland*

Alzheimer's disease (AD) is a complex neurodegenerative disorder, for which several disease-associated loci have been located on different chromosomes. We have used a population-based linkage disequilibrium mapping approach in order to find potential AD-associated loci on chromosome 13. To avoid population stratification, late onset AD patients and age-matched controls were carefully chosen from the same geographical area in Eastern Finland, where the population is mainly descended from a small group of original founders. During the initial screening with chromosome 13-specific microsatellite markers, tetranucleotide marker D13S787 was found to be in linkage disequilibrium in the 13q12 region. Screening this region with additional microsatellite markers revealed that marker D13S292 was also significantly associated with AD. Stratification of the AD patients and controls into groups according to apolipoprotein E, sex, and familial/sporadic status indicated that the 13q12 locus was associated with female familial AD patients regardless of ApoE genotype. Based on the physical data from the region 13q12, markers D13S292 and D13S787 were estimated to reside in a 810 kb long YAC clone 754h7 together with two infant brain-derived ESTs and the H,K-ATPase α-subunit protein gene (*ATP1AL1*). The localisation of these sequences at the linkage disequilibrium region suggests that they may be candidate genes involved in a sex-specific effect during development of AD.

Keywords: late onset Alzheimer's disease; linkage disequilibrium; chromosome 13

Introduction

Alzheimer's disease (AD) is the most common form of progressive dementia and affects 5–10% of the population over 65 years of age. It is a disorder characterised by global cognitive decline, including impairment of memory, orientation and intellectual functioning eventually leading to death. The familial form of AD (FAD), as well as sporadic AD, can be categorised as early or late onset cases by using 60 years of age as the cut-off point. Highly penetrant, autosomal dominant mutations in amyloid precursor protein (APP), presenilin 1 (*PS1*) and 2 (*PS2*) genes are responsible for the early onset FAD,[1–4] whilst the ε4 allele of apolipoprotein E (ApoE4) is associated with an increased risk of early and late onset FAD and sporadic AD.[5–8] Together, these four loci account for about half the total genetic etiology of AD. In addition to the well established

Correspondence: Mikko Hiltunen, MSc, Chromosome and DNA Laboratory, Kuopio University Hospital, PO Box 1777, FIN-70211 Kuopio, Finland. Tel: 358 17 172729; Fax: 358 17 172726; E-mail: mhiltune@messi.uku.fi
Received 11 September 1998; revised 2 March 1999; accepted 8 March 1999

effect of ApoE4, genetic analysis of late onset AD has indicated disease-associated genes, such as α1-antichymotrypsin, butyrylcholinesterase, and low-density lipoprotein receptor-related protein.[9–11] Screenings for additional late onset AD genes have also indicated several potential regions in various chromosomes including chromosome 13.[12,13]

Linkage disequilibrium is a powerful tool for high-resolution genetic mapping in an isolated founder population[14,15] and has been used successfully for fine mapping and cloning of several disease-causing genes.[16,17] Moreover, it has been useful for mapping recessive single gene disorders as well as dominant and complex disease loci.[18–20] Finns are among the ideal populations for large scale linkage disequilibrium mapping studies since they are derived from a small group of founders, and are more genetically homogeneous than other populations.

We have used a population-based linkage disequilibrium mapping approach in which Finnish late onset AD patients were used as subjects. Here we present positive association results from chromosome 13, in which linkage disequilibrium was found between AD patients and microsatellite markers D13S292 and D13S787 in the 13q12 region.

Materials and Methods

Subjects

Forty-seven late onset AD patients, who fulfilled the NINCDS–ADRDA criteria for possible or probable AD[21] and 51 unrelated voluntary controls who had no signs of dementia were examined at Kuopio University Hospital. The AD patients and controls were derived from a restricted area of eight communities in the province of Northern Savo in Eastern Finland. The area was settled in the late 16th and early 17th century.[22,23] To confirm the homogeneity of the study groups, the birthplaces of the patients, controls and their parents were recorded. The group of AD patients included 29 (61.7%) women and 18 (38.3%) men with a mean onset age of 75.1 ± 5.6 years and mean Mini Mental Status Examination of 18.6 ± 4.6 (mean ± SD).[24] The control group included 34 (66.7%) women and 7 (33.3%) men with a mean age of 70.1 ± 4.6 years. AD patients and controls did not differ significantly in age or gender distribution. There were 20 (44.4%) patients with positive familial history of AD, but insufficient evidence for clear autosomal dominant transmission and 25 patients (56.8%) with sporadic AD. Patients were considered to be familial cases if at least two first-degree relatives with dementia in two different generations were documented.[25] Familial patients were unrelated and for two patients there was no reliable family history available.

DNA and RNA Analysis

Genomic DNA was isolated from the peripheral blood lymphocytes of patients and controls. Chromosome 13-specific microsatellite markers (fluorescent-labelled) from the sixth version of the Weber lab (Table 1) were PCR amplified using standard procedures.[26] PCR products were pooled and their sizes were determined by a ABI PRISM 310 genetic analyser (Perkin Elmer, Foster City, CA, USA). Eight additional markers (Table 2) from the region 13q11–q12 were amplified using multiplex PCR. The order of the markers was obtained from http://cedar.genetics.soton.ac.uk/, http://WWW.gdb.org/, and from Guilford *et al*.[27] Female specific genetic map was used for estimating the distances between individual markers. ApoE alleles were analysed as previously described.[28] Expression status of the H,K–ATPase α-subunit protein (ATP1AL1) (EMBL/Genbank accession number U02076) in the human postmortem temporal cortex was determined using reverse transcriptase-PCR (RT-PCR) with the ATP1AL1 specific primers[29] and cycle sequencing.

Statistical Analysis

The linkage disequilibrium analysis at the initial screening was performed using the CONTING program, version 2.51.[30] Testing the significance of an individual marker allele was carried out by pooling other alleles into one group and calculating the results using the contingency-table χ^2 test with the Yates correction. Linkage disequilibrium of the markers at 13q11–q12 were calculated using two-tailed Fisher's exact test (Monte Carlo estimate with 99% confidence level) with SPSS software, version 6.1.3 and the likelihood-ratio statistic from the EH program, version 1.12.[30] A gene frequency of 0.01, phenocopy frequency of 0.001, and a dominant disease transmission with 70% penetrance were assumed as specified parameters in the case-control sampling option of the EH program, although the familial cases were not necessarily compatible with monogenic inheritance of AD. For stratified data with markers D13S292 and D13S787, the Bonferroni correction was applied by multiplying the *P* values with the

Table 1 Linkage disequilibrium results from initial screening with chromosome 13-specific microsatellite markers

Locus	*Marker*[a]	*cM*[b]	χ^2	*df*	*P*
D13S787	GATA23C03	0	10.5	4	0.03
	GGAA29H03	19	8.4	6	0.21
D13S894	GATA86H01	9	3.6	5	0.61
D13S325	GATA6B07	7	5.9	9	0.76
D13S788	GATA29A03	6	8.3	6	0.22
D13S800	GATA64F09	10	12.8	9	0.17
D13S317	GATA7G10	10	8.9	7	0.26
D13S793	GATA43H03	15	8.4	5	0.13
D13S779	ATA26D07	10	3.3	4	0.50
D13S796	GATA51B02	11	6.1	6	0.41
D13S173	26lyg5	3	7.6	7	0.37
D13S285	309VA9	20	14.7	10	0.14

[a]Markers are aligned in order from the pericentromeric region to the q telomer.
[b]Approximated distances in centiMorgans (cM) between adjacent markers.

Table 2 Linkage disequilibrium results with microsatellite markers located in the region 13q11–q12

			Number of chromosomes						*Number of chromosomes*		
Marker and allele[a]	*AD*	*C*	*Single allele* χ^2*(P)*	*Fischer's exact test P*	*Likelihood ratio P*	*Marker and allele*[a]	*AD*	*C*	*Single allele* χ^2*(P)*	*Fischer's exact test P*	*Likelihood ratio P*
D13S308e (–4.5)						D13S115 (–3.0):					
A1	1	2	0.00 (1.00)			B1	4	4	0.00 (1.00)		
A2	1	0	0.00 (1.00)			B2	4	2	0.27 (0.61)		
A3	13	13	0.00 (0.99)	0.96	0.77	B3	32	35	0.00 (1.00)		
A4	34	35	0.00 (0.90)			B4	39	43	0.00 (1.00)	0.99	0.94
A5	10	14	0.19 (0.66)			B5	14	14	0.00 (0.98)		
A6	35	38	0.00 (1.00)			B6	0	1	0.00 (1.00)		
						B7	0	1	0.00 (1.00)		
						B8	1	1	0.00 (1.00)		
						B9	0	1	0.00 (1.00)		
D13S232 (–0.8):						D13S292 (–0.25):					
C1	15	22	0.67 (0.41)			D1	0	6	3.89 (0.05)		
C2	29	31	0.00 (1.00)			D2	13	4	4.88 (0.03)		
C3	21	25	0.04 (0.85)			D3	45	45	0.15 (0.70)	0.002	0.003
C4	1	2	0.00 (1.00)			D4	30	45	2.59 (0.11)		
C5	4	6	0.04 (0.85)	0.79	0.34	D5	6	2	1.44 (0.23)		
C6	4	3	0.01 (0.91)								
C7	2	0	0.59 (0.44)								
C8	17	12	1.09 (0.30)								
C9	1	1	0.00 (1.00)								
D13S787 (0):						D13S1243 (0.25):					
E1	13	6	2.68 (0.10)			F1	15	22	0.32 (0.57)		
E2	50	48	0.51 (0.47)			F2	33	28	1.00 (0.32)		
E3	22	35	2.32 (0.13)	0.032	0.019	F3	9	16	1.14 (0.29)		
E4	9	8	0.03 (0.86)			F4	22	20	0.22 (0.64)	0.25	0.39
E5	0	5	2.96 (0.09)			F5	14	10	0.75 (0.39)		
						F6	1	3	0.18 (0.67)		
						F7	0	3	1.20 (0.27)		
D13S252 (1.5):						D13S246 (6.0):					
G1	18	21	0.01 (0.94)			H1	1	0	0.00 (0.97)		
G2	4	6	0.04 (0.85)			H2	5	4	0.02 (0.90)		
G3	15	19	0.09 (0.76)			H3	5	9	0.45 (0.50)		
G4	25	30	0.08 (0.78)	0.90	0.51	H4	11	9	0.18 (0.67)		
G5	30	25	0.99 (0.32)			H5	8	3	1.91 (0.17)	0.52	0.49
G6	1	0	0.00 (0.97)			H6	10	13	0.06 (0.81)		
G7	1	1	0.00 (1.00)			H7	50	58	0.14 (0.71)		
						H8	3	6	0.31 (0.58)		
						H9	1	0	0.00 (0.97)		
D13S893 (20.0):											
I1	7	7	0.00 (1.00)								
I2	29	21	2.20 (0.14)								
I3	32	44	1.34 (0.25)	0.25	0.33						
I4	25	25	0.03 (0.86)								
I5	1	5	1.31 (0.25)								

[a]Relative location of the microsatellite markers from the D13S787 is indicated in parenthesis as cM after each marker.

number of alleles examined. The level of statistical significance was defined as $P < 0.05$. The distribution of genotypes for each marker in the AD and control groups was in Hardy–Weinberg equilibrium. Estimated haplotype frequencies were obtained from the EH program.

Results

The distribution of ε2/3/4 alleles of ApoE in the AD and control groups was 0.02/0.62/0.36 and 0.02/0.83/0.15, respectively (χ^2, $P = 0.0023$). The ApoE4 allele was strongly associated with AD giving an odds ratio of 3.9 (95% CI 1.7–9.1).

Initial screening with the chromosome 13-specific microsatellite markers indicated that markers D13S787 was in linkage disequilibrium at the 13q12 region in AD group (Table 1). Although the single allele tests with D13S787 did not reveal statistically significant association with any allele, the likelihood-ratio indicated that the disease and marker alleles were in linkage disequilibrium (Table 2) and that allele E1 displayed the strongest association with AD. Genotyping of eight additional markers located in the 13q11–q12 region indicated that marker D13S292, adjacent to D13S787, was also in linkage disequilibrium in AD group (Table 2). According to the single allele test, allele D2 displayed the strongest suggestive association with AD (Bonferroni corrected $P = 0.07$). As markers flanking D13S292 and D13S787 were in equilibrium, the size of the linkage disequilibrium region at 13q12 was estimated to be approximately 0.2–1.3 cM.

Stratification of the AD patients and controls according to the ApoE4 status indicated that the allelic association of D13S292 and D13S787 was not further modified by the ApoE genotypes ($ApoE4^+$ group; $P = 0.02$ and $P = 0.48$ and $ApoE4^-$ group; $P = 0.11$ and $P = 0.15$ for D13S292 and D13S787, respectively). Stratification according to sex and familial/sporadic data attributed stronger allelic association for D13S292 and D13S787 in the female and familial groups than in the unstratified group (Table 3). Combined female and familial data indicated overlapping association effect for the 13q12 locus, which was accompanied with statistically significant single allele association of the D2 and E1 alleles in the AD group. Stratification according to the female/familial data did not influence the overall equilibrium of the flanking markers D13S232 and D13S1243 (data not shown).

As marker phases of individual patients and controls were not known, haplotype frequencies of unstratified and stratified cases were estimated using the EH program (Table 4). Using the female/familial stratification data, haplotype D5–E1 occurred in 13% of AD chromosomes, but only in 1.5% of control chromosomes, indicating a possible founder haplotype for the disease. Haplotypes D3–E1, D2–E3, D2–E2, D4–D4 and D2–E4 were also more frequent in AD chromosomes. When taking into account the possible disease allele (DA), significant evidence for an association (likelihood-ratio statistic, $P = 0.0002$) was found between the disease and marker alleles, and the haplotypes DA–D5–E1 and DA–D2–E2 displayed the strongest association with AD.

Discussion

Our findings indicate linkage disequilibrium between the Finnish late onset AD patients and markers D13S292 and D13S787 in the chromosomal region 13q12. Stratification of the AD and control groups according to the ApoE4, sex and familial/sporadic status suggests that the allelic association of 13q12 locus is attributed by female patients with positive familial history of AD (FAD). Although ApoE4 allele is also significantly associated with the AD population studied, it appears that 13q12 locus is associated with female FAD patients regardless of ApoE genotype. However, we cannot rule out the possibility that the independence of ApoE genotypes may be due to the small sample sizes in $ApoE4^+$ and $ApoE4^-$ subgroups. This is also true of certain other subgroups. Nevertheless, the 13q12 region may harbour a susceptibility gene, which may independently contribute sex-specific effects to the development of AD. Other genetic modelling studies have also suggested factors, which could account for sex differences independently or in concert of ApoE genotypes in AD.[31,32]

Based on the genetic map information, the size of the linkage disequilibrium region is approximately 0.2–1.3 cM, and the possible disease-associated haplotypes are observed with two adjacent markers. This is a relatively small area considering that the possible ancestral AD mutation(s) was introduced into the population 15–25 generations ago, in which case the extended haplotypes should be found approximately 1–12 cM around the disease gene.[33,34] According to the haplotype estimation analysis with markers D13S292 and D13S787, there may exist more than one disease-associated haplotype. Therefore, only the chromosomal regions displaying the strongest association can be

Table 3 Linkage disequilibrium results with the microsatellite markers D13S292 and D13S787 after stratification of the AD and control (C) groups according to female, familial and female/familial data

	Female[a]					*Familial*[b]					*Female/Familial*[c]				
	Number of chromosomes					*Number of chromosomes*					*Number of chromosomes*				
Marker and allele	*AD*	*C*	*Single allele* $\chi^2(P)$[d]	*Corrected* P[e]	*Fisher's exact test P*	*AD*	*C*	*Single allele* $\chi^2(P)$[d]	*Corrected* P[e]	*Fisher's exact test P*	*AD*	*C*	*Single allele* $\chi^2(P)$[d]	*Corrected* P[e]	*Fisher's exact test P*
D13S292:															
D1	0	5	2.72 (0.10)	0.18		0	6	1.22 (0.27)	0.59		0	5	1.05 (0.30)	0.64	
D2	10	2	5.86 (0.02)	0.03		7	4	5.63 (0.02)	0.03		6	2	5.96 (0.01)	0.02	
D3	24	30	0.02 (0.90)	1.00	0.005	16	45	0.07 (0.80)	1.00	0.002	12	30	0.03 (0.87)	1.00	0.002
D4	20	30	0.84 (0.36)	1.00		12	45	1.83 (0.18)	0.61		8	30	1.99 (0.16)	0.51	
D5	4	1	1.20 (0.27)	0.60		5	2	4.75 (0.03)	0.05		4	1	3.85 (0.05)	0.07	
D13S787:															
E1	10	3	4.27 (0.04)	0.09		8	6	4.95 (0.03)	0.06		7	3	3.70 (0.05)	0.02	
E2	31	31	0.49 (0.48)	1.00		20	48	0.02 (0.90)	1.00		16	31	0.24 (0.63)	1.00	
E3	13	25	2.42 (0.12)	0.40	0.024	5	35	5.72 (0.02)	0.05	0.002	4	25	4.42 (0.04)	0.10	0.008
E4	4	5	0.00 (1.00)	1.00		7	8	1.91 (0.17)	0.46		3	5	0.00 (1.00)	1.00	
E5	0	4	1.87 (0.17)	0.30		0	5	0.85 (0.36)	0.77		0	4	0.64 (0.42)	0.87	
Total	58	68				40	102				30	68			

[a]Fisher's exact test P values for male data were 0.51 and 0.93 for D13S292 and D13S787, respectively.
[b]Fisher's exact test P values for sporadic data were 0.10 and 0.47 for D13S292 and D13S787, respectively.
[c]Fisher's exact test P values for male/familial data were 0.75 and 0.18 for D13S292 and D13S787, respectively.
[d]χ^2 values were derived using Yates correction.
[e]Bonferroni corrected P values are derived from χ^2 values without using Yates correction.

Table 4 Estimated haplotype frequencies of the AD and control (C) chromosomes with microsatellite markers D13S292 and D13S787

	Unstratified		*Female/Familial*	
Haplotype[a]	*AD* (n=*94*)	*C* (n=*102*)	*AD* (n=*30*)	*C* (n=*68*)
D3–E2	0.287	0.247	0.267	0.230
D4–E2	0.174	0.210	0.200	0.204
D5–E1	0.064	0.020	0.133	0.015
D3–E1	0.046	0.024	0.100	0.015
D2–E3	0.000	0.015	0.100	0.000
D2–E2	0.071	0.000	0.067	0.000
D4–E4	0.041	0.000	0.067	0.000
D2–E4	0.038	0.000	0.033	0.000
D3–E3	0.130	0.075	0.033	0.122
D4–E3	0.104	0.208	0.000	0.193
D2–E1	0.029	0.015	0.000	0.015
D3–E4	0.016	0.078	0.000	0.074
D1–E3	0.000	0.045	0.000	0.052
D4–E5	0.000	0.023	0.000	0.044
D3–E5	0.000	0.016	0.000	0.000
D1–E2	0.000	0.014	0.000	0.021
D2–E5	0.000	0.010	0.000	0.015

[a]Haplotype frequencies indicated are EH program output under the assumption of linkage disequilibrium between markers D13S292 and D13S787.

observed, while the flanking regions of disease-associated haplotypes disappear due to the population-based sampling strategy used. In addition, locus heterogeneity as well as nongenetic etiological factors may further reduce the size of the linkage disequilibrium region around the mutated gene.

Although D13S292 and D13S787 displayed a statistically significant overall association ($P < 0.01$) with female FAD patients, the magnitude of single allele association was moderate when compared with studies performed with single gene disorders. As multiple tests were performed with the alleles of locus 13q12 markers, a rather conservative Bonferroni correction was applied, in which case the corrected P values for presumed AD-associated alleles D2 ($P = 0.02$) and E1 ($P = 0.02$) still remained moderately significant in the stratified female FAD group. On the other hand, a weak-to-moderate association, in which the occurrence of a certain allele is 1.5 to 3 times more frequent in patients than in control subjects appears to be a common trait in the association studies performed so far with complex diseases.[35] Thus, the moderate association presented here may be due to the locus and/or allele heterogeneity known to exist in AD.

The physical data available from the chromosome 13 specific contig map[27] indicates that the markers D13S292 and D13S787 reside in a 810 kb YAC clone 754h7 together with two infant brain-derived ESTs and the *ATP1AL1* gene. RT-PCR showed that ATP1AL1 is expressed in the human temporal cortex (data not shown), which is affected during the pathogenesis of AD. As the *ATP1AL1* and ESTs are located in the linkage disequilibrium region, we suggest that one of these genes may contribute to late onset AD. On the basis of current information, however, it is difficult to determine the importance of association for AD. Therefore, additional means such as reconstruction of actual haplotypes by sampling the spouses and children, and sequence analysis of the genes in the vicinity of the markers D13S292 and D13S787 of female FAD patients are necessary.

Acknowledgements

The authors thank Claes Wadelius and the Nordic marker resource at Clinical Genetics in the University Hospital of Uppsala, Sweden, for supplying the microsatellite markers used in the study. The authors also thank Mrs Pirjo Halonen and Ms Marjo Heikkinen for their excellent help. The study was supported by the Health Research Council of the Academy of Finland and an EVO grant (5032) from Kuopio University Hospital.

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