

LINKAGE AND HAPLOTYPE ANALYSIS OF FAMILIAL EARLY-ONSET ALZHEIMER DISEASE IN JAPANESE POPULATION

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Summary Linkage and haplotype analysis of eleven early-onset Alzheimer disease (AD) families was performed in relation to D21S210 and microsatellite DNA polymorphisms localized on chromosome 14q24.3. Linkage analysis of eight informative families out of eleven early-onset AD families disclosed the highest LOD score of 3.45 ($\theta=0.00$) at D14S77, while the locus of β /A4 amyloid protein precursor gene was formally excluded within 10 cM from D21S210, given the evidence of recombinations in five families. Transmission disequilibrium study between the patients and controls without dementia indicated significant differences at D14S43 ($p=0.0001$) and D14S71 ($p=0.02$). Association study be-

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tween genotypes linked or related to onset of AD and those of control also revealed a significant difference at D14S43 ($p < 0.05$), suggesting the existence of linkage disequilibrium. Moreover, the haplotypes at D14S43 linked with the onset of AD indicated a significant relationship with the mean age at onset. These results support that the major locus of early-onset familial AD is located on 14q24.3, and its close linkage to D14S43 and the existence of allelic heterogeneity were suggested.

Key Words Alzheimer disease, genetics, β /A4 amyloid protein precursor, chromosome 14

INTRODUCTION

The familial form of Alzheimer disease (AD) is an autosomal dominant disorder of the brain characterized by progressive dementia that occurs in middle or late life. The pathologic characteristics are the presence of neuritic plaques and neurofibrillary tangles, which are predominantly found in the cerebral cortex and the hippocampus. Research on familial Alzheimer disease (FAD) has been focused on elucidating the genetic factors for the onset of AD, and linkage analyses have been performed to identify the loci for early-onset FAD, defined by the age at onset of not more than 65 years old. Several early-onset AD families were shown to be linked with mutations of the β /A4 amyloid protein precursor (APP) gene (Goate *et al.*, 1991; Murrell *et al.*, 1991; Chartier-Harlin *et al.*, 1991; Mullan *et al.*, 1992a). The mutation producing the $^{717}\text{Val} \rightarrow \text{Ile}$ substitution at codon 717 of the APP gene had also been identified in Japanese early-onset AD families (Naruse *et al.*, 1991; Yoshioka *et al.*, 1991; Yoshizawa *et al.*, 1993). Large scale mutational analyses and linkage analyses, however, indicated that the APP gene is not a major causative gene of early-onset FAD (Kamino *et al.*, 1992; Tanzi *et al.*, 1992; Yoshizawa *et al.*, 1993; Fujigasaki *et al.*, 1994). Microsatellite polymorphisms mapped to 14q24.3 were shown to be linked in several early-onset AD families (Schellenberg *et al.*, 1992), and subsequent reports strongly suggested that a major locus for early-onset FAD is located on 14q24.3 (St George-Hyslop *et al.*, 1992; Van Broeckhoven *et al.*, 1992; Mullan *et al.*, 1992b). But the absence of linkage on 14q24.3 in Volga-German families (Schellenberg *et al.*, 1992) and Swedish families (Lannfelt *et al.*, 1993) indicated that early-onset FAD with relatively later age at onset could be caused by other genes at unknown loci.

Linkage analysis is a relevant strategy to define a disease locus, and relies upon recombination events in families with the disease. In the case of an adult-onset disease, linkage analysis is based on an assumption of age-dependent penetrance of the disease phenotype (Pericak-Vance *et al.*, 1979). Linkage disequilibrium, on the other hand, allows fine localization of gene loci, and linkage disequilibrium mapping has also been utilized for positional cloning (Harley *et al.*, 1991; Haestbacka *et al.*, 1994). Transmission disequilibrium study can be used to support mapping studies (Takiyama *et al.*, 1993). Linkage disequilibrium is based on the

restricted number of origins of disease mutations, called the founder effect. However, linkage disequilibrium studies are confounded by population admixture. In this respect, a single homogeneous population can be expected to yield more reliable information. Here, we investigated the genotypes of eleven Japanese early-onset FAD kindred using microsatellite polymorphisms on 14q24.3 and performed linkage and haplotype analysis. Our findings supported that a major locus of early-onset FAD is located on 14q24.3. Our results also suggested that the locus is localized close to D14S43, and that allelic heterogeneity exists in early-onset FAD.

MATERIALS AND METHODS

Families and controls. Patients from eleven families with early-onset FAD were diagnosed according to the criteria of NINCDS-ADRDA (McKhann *et al.*, 1984), and blood samples were obtained with informed consent from 63 individuals. Autopsy findings confirmed the diagnosis in four families, TK-1 (IV-5, -10, -11, and -15), ME (II-4), KF (II-2 and -3), and AM (II-1) (Schellenberg *et al.*, 1991). Autopsy materials were obtained from IV-5, IV-10, and IV-15 of TK-1, and II-2 and II-3 of KF, and DNAs of these cases were extracted from paraffin blocks. In KT, AC-2, and HG-5, a blood sample was obtained from one patient in each family. The mean age at onset in all families was 43.3 ± 4.0 years old, and the pedigrees are shown in Fig. 1 (Nagano *et al.*, 1995). DNAs of 50 independent controls without dementia, including 10 normal spouses in TK-1, ME, and OS-3, were utilized to determine allele frequencies in the Japanese population.

Analysis with microsatellite markers. D21S210 (Warren *et al.*, 1993) was used to assess linkage with the APP locus. D14S53 (Wang and Wever, 1992), D14S43 (Sharma *et al.*, 1991), D14S71, D14S76, and D14S77 (Weissenbach *et al.*, 1992) were genotyped for the region on 14q24.3. To obtain better resolution for genotyping D14S77, the primer of 5'-CACTGTGCCAGCCTGAGAATT-3' was used as the CA strand primer. Linkage analyses of AM family using D21S210, D14S43, and D14S53 have been reported previously (Kamino *et al.*, 1992; Schellenberg *et al.*, 1992), but we determined the genotypes again to measure their allele sizes. First, 100 pmol of 5'-primer was endlabeled with 5.0 μ l of [γ - 32 P]-ATP (5,000 Ci/mmol) using 10 U of T4 polynucleotide kinase in a total volume of 20 μ l and the mixture was diluted to 1 pmol 5'-primer/ μ l with H₂O. The PCR reaction was carried out in a total volume of 10 μ l and was composed of 100 ng DNA, 200 μ M of each dNTP, 0.4 μ M 5'-primer, 0.1 μ M 32 P-endlabeled 5'-primer, 0.5 μ M 3'-primer, 1.5 mM MgCl₂ (2.0 mM for D14S53 and D14S76, 1.0 mM for D14S77), 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 0.001% gelatin and 0.25 U/10 μ l of *Taq* DNA polymerase. The PCR thermocycling was as follows: 94°C for 45 sec, 55°C for 30 sec, 72°C for 45 sec with an additional 1 sec in each cycle (35 cycles) for D14S71 and D14S76; 94°C for 45 sec, 60°C for 30 sec, 72°C for 45 sec with an additional 1 sec in each cycle (35 cycles) for D14S77; 96°C for 1 min, 51°C for 30 sec, 72°C

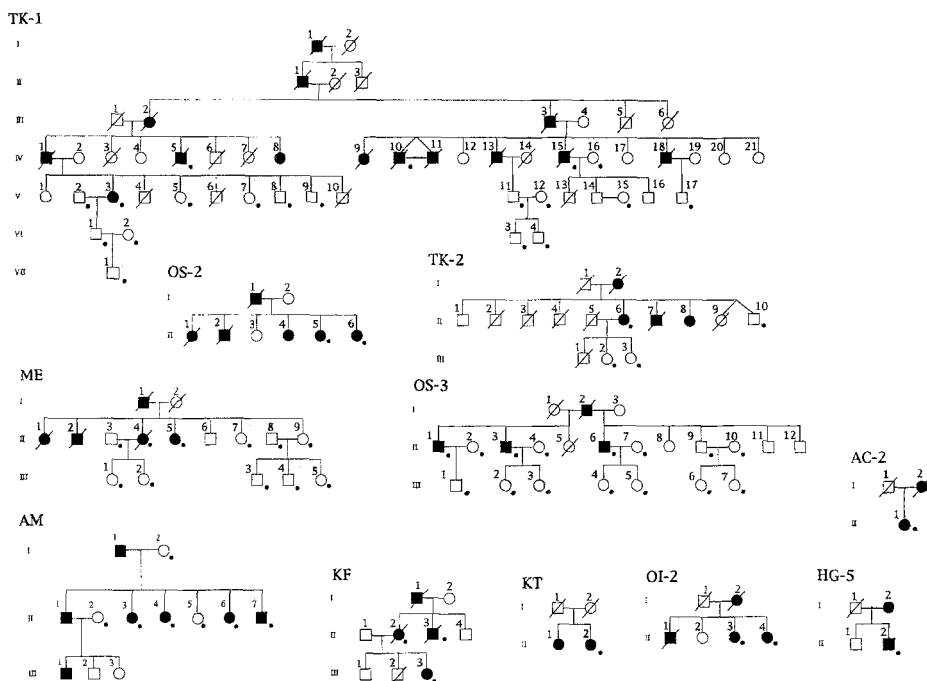


Fig. 1. Pedigrees of 11 Japanese early-onset FAD families. Small black circles represent persons whose DNA was analyzed. □, males; ○, females; ■, ●, affected; □, ○, unaffected.

for 90 sec (30 cycles) for D21S210; 96°C for 1 min, 55°C for 30 sec, 72°C for 1 min with an additional 1 sec in each cycle (35 cycles) for D14D53 and D14S43.

The PCR products were mixed with an equal volume of formamide loading buffer (95% formamide, 20 mM EDTA pH 8.0, 0.05% bromphenol blue and 0.05% xylene cyanol), denatured at 95°C for 3 min and applied to a 6% denaturing polyacrylamide DNA sequencing gel containing 46% urea, 30% formamide and 2 × TBE (1 × TBE: 0.1 M boric acid, 2 mM EDTA and 0.09 M Tris-HCl pH 8.3). Gels were fixed, dried and exposed to Fuji Rx film at -80°C for several days. Allele sizes for D14S76, D14S71, and D14S77 were determined by comparison with those of the CEPH reference sample 134,702, and those of D21S210, D14S53, and 14S-43 by comparison with M13 DNA sequencing ladder. DNA typing was performed without prior knowledge of the pedigree structure. In each case, the samples for the reference allele were run concurrently as a size control. Mendelian segregation of alleles was observed in all cases. Genotyping using DNA obtained from paraffin blocks was confirmed by identical results of at least two independent samples.

Statistical analysis. Linkage analyses were performed for eight Japanese early-onset AD families. Two-point lod scores were calculated using MLINK and LODSCORE of the LINKAGE package, version 5.1. Early-onset FAD

was considered as an autosomal dominant disease with a frequency of 0.001, and equal female to male recombination rates were applied. Thirteen penetrance classes were used based on the cumulative risk curve, which was obtained from the mean age at onset of the patients in each family (Ott, 1991).

Transmission disequilibrium between the early-onset FAD locus and marker loci was assessed by χ^2 statistics according to the method previously defined for multiallelic loci (Weir and Cockerham, 1978). Association due to linkage disequilibrium was evaluated by comparison of allele frequencies of the linked alleles in the patients and the controls basically as described (Yu *et al.*, 1994). Namely, when one common haplotype or allele was observed among the patients, only a single allele was counted. In the case of recombination, both alleles were counted. When the linked allele could not be determined, two alleles of a patient were included. The allele that was overrepresented among early-onset FAD chromosomes was defined as one allele, and all of the other alleles were combined to form a second allele. The usual χ^2 test for 2×2 tables was then applied to determine significance. A p-value of less than 0.05 was considered to indicate significance.

The early-onset FAD families were divided into three groups based on the D14S43 allele co-segregating with the disease, and the differences of age at onset were evaluated by analysis of variance (ANOVA). Then, differences of the mean age at onset between the groups were further evaluated by Fisher's PLSD test.

RESULTS

Linkage analysis

To evaluate the linkage between the onset of FAD and the APP gene, Japanese early-onset FAD families were genotyped at D21S210 (Table 1), which is localized within 1 cM from the APP gene (Warren *et al.*, 1993). Recombinations between the onset and D21S210 were found in five families, and overall linkage was formally excluded within 10 cM from D21S210 (Table 2). Moreover, direct sequencing of exon 17 of the APP gene did not show any mutations in TK-1, TK-2, ME, KT, and OS-2 (Yoshioka *et al.*, 1991). No mutations in exon 16 and 17 of the APP gene were identified in the eleven families by mutational analysis using mismatched PCR-RFLP (submitted data). These results indicate that the APP gene is not a causative gene.

Then we investigated the linkage of FAD to chromosome 14q24.3 (Table 1). Linkage analysis between D14S77 and early-onset FAD indicated positive linkage, and no recombinations were found ($Z_{\max}=3.45$ at $\theta=0.00$; 1.50 for AM family and 1.95 for the others). No recombinations between D14S43 and AD phenotype were also observed ($Z_{\max}=0.95$ at $\theta=0.00$). Recombination events were observed in D14S53 and D14S76 for TK-1, and in D14S71 for KF. All of these recombinations were detected in the cases in which DNAs were extracted from paraffin blocks. The overall two-point linkage analysis is summarized in Table 2.

Table 1. Two-point linkage analysis of eight Japanese early-onset FAD families.

DNA marker	family\θ	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D21S210	TK-1	-inf	-2.50	-1.19	-0.68	-0.28	-0.12	0.05
	TK-2	0.05	0.05	0.04	0.03	0.02	0.01	0.00
	OS-2	-0.14	-0.13	-0.11	-0.08	-0.05	-0.02	-0.00
	OS-3	-2.40	-1.36	-0.72	-0.44	-0.19	-0.08	-0.02
	ME	-0.59	-0.59	-0.55	-0.45	-0.25	-0.11	-0.03
	KF	-2.70	-1.29	-0.63	-0.37	-0.15	-0.05	-0.01
	O1-2	-2.70	-1.38	-0.72	-0.44	-0.19	-0.08	-0.02
	AM	-3.20	-2.18	-0.91	-0.42	-0.05	0.06	0.06
D14S53	TK-1	-3.11	-1.73	-0.90	-0.52	-0.20	-0.07	-0.01
	TK-2	0.12	0.11	0.10	0.08	0.05	0.02	0.01
	OS-2	0.10	0.09	0.08	0.06	0.04	0.02	0.00
	OS-3	0.01	0.01	0.01	0.01	0.01	0.00	0.00
	ME	0.68	0.66	0.60	0.52	0.35	0.18	0.05
	KF	-0.20	-0.19	-0.16	-0.12	-0.06	-0.03	-0.01
	O1-2	-0.20	-0.19	-0.16	-0.12	-0.06	-0.03	-0.01
	AM	1.80	1.77	1.65	1.48	1.12	0.72	0.29
D14S43	TK-1	-0.20	-0.18	-0.11	-0.06	-0.01	-0.00	-0.00
	TK-2	0.26	0.25	0.22	0.18	0.11	0.05	0.01
	OS-2	0.20	0.19	0.17	0.14	0.08	0.04	0.01
	OS-3	0.21	0.21	0.18	0.15	0.09	0.04	0.01
	ME	0.61	0.59	0.52	0.44	0.27	0.13	0.04
	KF	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	O1-2	-0.11	-0.10	-0.08	-0.07	-0.04	-0.02	-0.00
	AM	-0.03	-0.02	0.01	0.03	0.03	0.02	0.01
D14S77	TK-1	0.74	0.72	0.64	0.55	0.35	0.19	0.07
	TK-2	0.09	0.08	0.07	0.06	0.03	0.02	0.00
	OS-2	-0.24	-0.23	-0.18	-0.14	-0.07	-0.03	-0.01
	OS-3	0.59	0.58	0.52	0.46	0.31	0.17	0.05
	ME	0.63	0.62	0.56	0.48	0.31	0.16	0.05
	KF	-0.09	-0.09	-0.07	-0.05	-0.02	-0.01	-0.00
	O1-2	0.23	0.23	0.20	0.16	0.10	0.05	0.01
	AM	1.50	1.47	1.36	1.22	0.91	0.56	0.20

Haplotype analysis

To evaluate linkage disequilibrium, transmission disequilibrium between the early-onset FAD locus and marker loci on 14q24.3 was investigated by comparison

Table 2. Summary of two-point linkage analysis of eight Japanese early-onset.

Marker \ θ	0.00	0.01	0.05	0.10	0.20	0.30	0.40	$Z_{max}(\theta)$
D21S210	—inf	—9.38	—4.79	—2.85	—1.14	—0.39	—0.07	
D14S53	—0.80	0.53	1.22	1.39	1.25	0.81	0.32	1.39(0.11)
D14S76	—inf	1.66	2.07	1.98	1.50	0.91	0.33	2.07(0.05)
D14S43	0.95	0.94	0.91	0.81	0.53	0.26	0.08	0.95(0.00)
D14S71	—1.09	0.32	0.90	0.98	0.81	0.48	0.19	0.98(0.10)
D14S77	3.45	3.38	3.10	2.74	1.92	1.11	0.37	3.45(0.00)

Table 3. Transmission disequilibrium study of Japanese early-onset FAD patients with microsatellite polymorphisms on 14q24.3.

Marker	D14S53			D14S76			D14S43			D14S71			D14S77		
	(bp) Control AD			(bp) Control AD			(bp) Control AD			(bp) Control AD			(bp) Control AD		
1	144	11	4	163	1	0	161	18	4	193	5	3	201	2	0
2	146	1	0	165	1	0	167	0	4	195	8	7	203	0	2
3	147	1	0	167	14	10	175	8	0	197	21	2	205	1	1
4	148	4	0	169	0	1	181	3	0	199	3	0	207	15	10
5	149	3	0	171	21	14	183	1	3	201	8	3	209	1	0
6	150	1	0	173	1	1	185	17	2	203	12	2	211	1	0
7	151	60	34	177	9	2	186	0	1	205	2	5	213	10	2
8	153	5	2	179	47	20	187	28	25	207	37	18	215	37	13
9	155	9	9	181	3	2	189	9	6	209	3	0	217	5	4
10	157	1	1	183	3	0	191	14	1	211	1	2	219	6	1
11	159	4	0				193	1	0				221	5	1
12							195	1	0				223	0	2
13													225	3	2
14													227	1	0
15													229	3	0
16													231	5	0
17													233	2	3
18													235	2	0
19													237	1	1
Total No. of chromosomes	100	50		100	50		100	46		100	42		100	42	
df	10			9			11			9			18		
χ^2 value	10.2			7.80			36.9			19.4			23.9		
p	0.42			0.55			0.0001			0.022			0.16		

Allele frequencies of the patients and of Japanese controls, and allele sizes are shown. Transmission disequilibrium was statistically evaluated by means of χ^2 statistics for each marker. D14S43 and D14S71 showed significant differences.

of allele frequencies of the patients and those controls (Table 3). D14S43 and D14S71 out of five marker loci revealed significant differences ($p=0.0001$, $p=0.02$, respectively). To evaluate allelic association, the frequencies of the linked alleles

from each family and those of controls were evaluated (Table 4). Statistical evidence of association was found between early-onset FAD and D14S43 polymorphism ($p < 0.05$). These results suggest that the early-onset FAD locus is located close to D14S43.

Relationships between haplotype and age at onset

Three alleles at D14S43 were determined by haplotype analysis. Then, we classified early-onset FAD families into three groups according to D14S43 alleles;

Table 4. Linkage disequilibrium on 14q24.3 in Japanese early-onset FAD families.

Family \ Marker	D14S53	D14S76	D14S43	D14S71	D14S77
TK-1	144/151*	179/167*	187	201	217
TK-2	151/151	179/179	161/187	195/201	207/215
OS-2	151	171	187	207	215
OS-3	151	171	161	205	233
ME	155	179	187	195	217
OI-2	151	179	187	207	215/223
AM	155	171	187	207	207
KF	151	179/179	167	205/207	215
KT	151/157	179/179	167/189	203/207	205/215
AC-2	151/151	167/179	187/191	205/207	213/215
HG-5	151/151	167/179	183/187	193/207	213/219
No. of alleles in FAD	144-1 151-12 155-2 157-1	167-3 171-3 179-11	161-2 167-2 183-1 187-8 189-1 191-1	193-1 195-2 201-2 203-1 205-3 207-7	205-1 207-2 213-2 215-6 217-2 219-1 223-1 233-1
Total No. of alleles	16	17	15	16	16
Frequency of representative allele in FAD (Control)	151-0.75 (151-0.60)	179-0.65 (179-0.47)	187-0.53 (187-0.28)	207-0.44 (207-0.37)	215-0.38 (215-0.37)
χ^2	1.3	1.8	3.9	0.27	0.0015
p value	0.25	0.18	0.049	0.61	0.97

The alleles linked to the onset in each family are shown as the allele size. When recombinations were found (indicated as asterisks), both alleles were counted as being linked to the onset. Frequencies of representative allele in early-onset FAD chromosome are also summarized. A significant association ($p < 0.05$) was found between the onset and D14S43.

Table 5. Relation between haplotype of D14S43 and mean age at onset of early-onset FAD.

Group (D14S43)	(D14S71)	Family	Mean±SD (range) age at onset (years old)	Overall mean±SD age at onset (years old)
A (187)	195	ME	34.8±2.05 (33-38)	42.8±6.74
		TK-1	47.5±3.29 (45-56)	
	TK-2	47.7±4.04 (43-50)		
	207	OS-2	45.0±4.24 (42-48)	
		OI-2	40.5±2.12 (39-42)	
		AM	41.7±3.64 (36-46)	
		AC-2	33.0±0.00 (33-33)	
HG-5		41.0±19.8 (27-55)		
B (161)	205	OS-3	52.5±5.07 (49-60)	52.5±5.07
C (167)	207	KT	64.0±0 (64-64)	40.0±13.5
		KF	34.0±1.63 (32-36)	

The haplotype linked to the onset in each family, the mean age and range at the onset are shown. Significant differences of the mean age at the onset were found between group A and B, and between group B and C ($p < 0.05$).

Group A was classified as families in which the onset of AD was linked with the 187 bp allele, and consisted of ME, TK-1, TK-2, OS-2, OI-2, AM, AC-2, and HG-5; Group B was families with the 167 bp allele, and consisted of OS-3; Group C was families with the 161 bp allele, and consisted of KT and KF. In TK-2, it was not determined by haplotype analysis whether the 161 bp allele or the 187 bp allele was linked with the disease. But TK-2 had the same geographic origin as TK-1 and the 187 bp allele was shared between the families. Therefore, TK-2 was included in group A. The mean±SD age at onset in group A, B, and C was 42.8 ± 6.74 , 52.5 ± 5.07 , and 40.0 ± 13.5 years, respectively (Table 5). According to ANOVA, the age at onset was significantly different among the groups ($p < 0.05$), and the significant differences of the mean age at onset were detected between group A and B, and between group B and C ($p < 0.05$). This result suggests the existence of allelic heterogeneity in early-onset FAD.

DISCUSSION

Our results indicate that the major locus for early-onset FAD is localized on 14q24.3, and is closely linked to D14S43. The order of markers on 14q24.3 has been determined by linkage and physical mapping as telomere-D14S53-D14S76-D14S43-D14S71-D14S77-centromere. In our study, linkage analysis revealed significant lod scores for only D14S77, and no recombination events were found for D14S77 and D14S43. The recombinations found in TK-1 and KF were dem-

onstrated using DNAs extracted from paraffin blocks. Contamination with other tissues might be possible during the process of paraffin embedding. Therefore, these recombinations seem not to be reliable. The highest lod scores of early-onset FAD were reported for D14S43 and D14S53 (Schellenberg *et al.*, 1992; St George-Hyslop *et al.*, 1992; Van Broeckhoven *et al.*, 1992; Mullan *et al.*, 1992b). The interval between these markers is estimated to be 2.1 cM (Weissenbach *et al.*, 1992). The reason why D14S77 showed the highest lod score in this study can be explained by its having a larger number of alleles than D14S53 and D14S43.

Comparison of allele frequencies between all early-onset FAD patients and controls indicated transmission disequilibrium between the early-onset FAD locus and D14S43 as well as D14S71, supporting the positive linkage. This phenomenon could be strongly influenced by the geographic origins of patients. Then, we selected the alleles linked with the onset of AD in each family, and an association due to linkage disequilibrium was detected only at D14S43. These results suggest that the major early-onset FAD locus is located close to D14S43. The families used in this study were distributed in Western Japan, and the most frequent alleles in the patients were the same as those of controls for all markers. But the 167 bp allele of D14S43 was not found in controls. Moreover, when early-onset FAD patients were divided into three groups by D14S43 allele, the age at onset among the groups was significantly different. These results suggest the existence of allelic heterogeneity for early-onset FAD. OS-3 was the only family linked with the 161 bp allele of D14S43. Suppression of the calcium response at S phase in cell cycle was reported in the fibroblasts of the patient with APP717:Val→Ile mutation and of the patient in OS-2, but not of the patient in OS-3 (Tatebayashi *et al.*, 1995). This observation supported the existence of allelic heterogeneity. This possibility will remain unconfirmed until a causative gene is isolated.

Linkage disequilibrium is based on a founder effect, but the ability to detect this phenomenon is strongly affected by the allele frequency of polymorphic marker analyzed. We used 50 controls distributed in the vicinity of Osaka and Hyogo prefectures, both of which are located in Western Japan. The allele frequency of D14S43 in the Japanese population has been reported (Takiyama *et al.*, 1993), but their result was different from that of our study, suggesting that population variation exists between Western and Eastern Japan. Our controls were composed of individuals without dementia; therefore, the characteristics of the control subjects could be another cause of the difference. Allele sizes at D14S43 in our study were 4 bp longer than those reported previously (Schellenberg *et al.*, 1992). But even when adjusting the allele sizes, allele frequency in the Japanese population was still significantly different from that reported in Caucasians (Wang and Weber, 1992). This difference might be due to difficulty in determining the allele size at D14S43.

Linkage disequilibrium has recently been applied to obtain fine mapping of disease genes. Recent studies indicated that linkage disequilibrium can be detected typically in regions of 1–2 cM in size or smaller (Yu *et al.*, 1994). AD is a hetero-

geneous disorder, and linkage study of early-onset FAD indicated non-allelic heterogeneity (Van Duijn *et al.*, 1994). But it should be noted that allelic association can be detected in the case that a major allele linked with the disease exists. Our results indicated that one of the closest markers is D14S43, which is located within the 3.0 cM region between D14S71 and D14S76 (Weissenbach *et al.*, 1992). It has been reported that the activity of α -ketoglutarate dehydrogenase in the brain and fibroblasts was reduced in AD patients (Gibson *et al.*, 1988; Sheu *et al.*, 1994). The α -ketoglutarate dehydrogenase complex is composed of three protein subunits, one of which is dihydrolipoamide succinyltransferase (DLST). The gene of DLST is localized to 14q24.3 (Ali *et al.*, 1993), and its sequence and D14S43 were found in the same clone of a yeast artificial chromosome library (unpublished data), suggesting that this gene is a candidate gene of early-onset FAD.

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