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Deficiency in Mitochondrial Aldehyde Dehydrogenase Increases the Risk for Late-Onset Alzheimer's Disease in the Japanese Population

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Mitochondrial aldehyde dehydrogenase 2 (ALDH2) deficiency is caused by a mutant allele in the Mongoloids. To examine whether genetic constitutions affecting aldehyde metabolism influence the risk for late-onset Alzheimer's disease (LOAD), we performed a case–control study in the Japanese population on the deficiency in ALDH2 caused by the dominant-negative mutant allele of the *ALDH2* gene (*ALDH2*2*). In a comparison of 447 patients with sex, age, and region matched nondemented controls, the genotype frequency carrying the *ALDH2*2* allele was significantly higher in the patients than in the controls (48.1% vs 37.4%, $P = 0.001$). Logistic regression analysis indicates that carriage of the *ALDH2*2* allele is an independent risk for LOAD of the ϵ4 allele of the apolipoprotein E gene (*APOE-ϵ4*) ($P = 0.002$). Moreover, the odds ratio for LOAD in carriers of the *ALDH2*2* allele was almost twice that in noncarriers, irrespective of status with regard to the *APOE-ϵ4* allele. Among patients homozygous for the *APOE-ϵ4* allele, age at onset of LOAD was significantly lower in those with than without the *ALDH2*2* allele. In addition, dosage of the *ALDH2*2* allele significantly affected age at onset of patients homozygous for the *APOE-ϵ4* allele. These results indicate that the ALDH2 deficiency is a risk for LOAD, synergistically acting with the *APOE-ϵ4* allele. © 2000 Academic Press

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Late-onset Alzheimer's disease (LOAD) is a complex disease caused by multiple genetic and environmental factors upon aging. It was pointed out that alcohol intake could affect the development of LOAD, since ethanol and its metabolite, acetoaldehyde, are directly neurotoxic, and patients with alcohol abuse showed alterations in neurotransmitting molecules in the brain such as muscarinic cholinergic receptor and serotonin (1–3). This notion is supported by evidence that a history of alcohol abuse was related to the rate of cognitive decline and to reduce survival in patients with LOAD, and was a high relative risk for LOAD in a Swedish population (4–6). On the other hand, epidemiological studies concluded that alcohol intake was not a risk for AD in Caucasians and a Japanese population, but rather, protective for LOAD in a French population (7–10). It was also shown that an even higher intake of alcohol is not a risk for LOAD (8, 10). Although alcohol abuse is different from normal alcohol intake in respect to the amount consumed daily and to nutritional imbalance, these conflicting results could be explained by genetic constitutions that modify ethanol metabolism and potentially influence alcohol-drinking behavior.

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) is known to metabolize acetaldehyde produced from ethanol into acetate. In the Japanese population, the ALDH2 deficiency is caused by a mutant allele of the

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ALDH2 gene (*ALDH2*2*), and is related to ethanol-related sensitivity responses such as facial flushing (11, 12). The *ALDH2*2* allele encodes a Glu to Lys amino acid substitution at the 14th last codon, acting in a dominant negative fashion both *in vivo* and *in vitro* (13–16).

To examine whether the ALDH2 deficiency influences the development of LOAD, we performed a large-scale case-control study on 447 Japanese patients with LOAD and age, gender and region matched non-demented controls on the deficiency in mitochondrial ALDH2 caused by the *ALDH2*2* allele. Moreover, we examined synergistic interactions of the *ALDH2*2* allele with another genetic risk on LOAD, the $\epsilon 4$ allele of the apolipoprotein E gene (*APOE-$\epsilon 4$*) (17, 18).

MATERIALS AND METHODS

Patients and controls. Patients with LOAD in whom cognitive declines were noticed at the age of 65 years and older, and control subjects with no cognitive decline were collected from the Kansai (Osaka and Hyogo) and Kanto areas of Japan. In the Osaka area, 167 patients with dementia were registered at the Department of Geriatric Medicine, Osaka University Medical School. Patients were evaluated by routine clinical and neurological examinations, and 34 patients were excluded because of history of alcohol abuse and syncope attack, and/or cerebrovascular regions on brain imaging studies. One hundred thirty-three patients fit the diagnosis of probable Alzheimer's disease (AD) according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (19). Control subjects ($n = 285$) were collected from among patients in its affiliated hospitals. In the Hyogo area located to the west of Osaka, patients with LOAD ($n = 265$) and control subjects ($n = 226$) were outpatients at the Department of Psychiatry, Hyogo Prefectural Amagasaki Hospital. In the Kanto area, patients with LOAD ($n = 65$) and control subjects ($n = 119$) were outpatients at the Department of Internal Medicine II, Nippon Medical School (Tokyo) and its affiliated hospitals. Twenty-three patients and control subjects ($n = 30$) were outpatients at Omiya Medical Center of Jichi Medical School, and these subjects were combined in the Kanto area. Three patients in the Osaka, 25 in the Hyogo, and 11 in the Kanto area were excluded because of lack of information about age at onset or gender. Gender, age and region-matched controls were selected among 660 control subjects, where the patients and control subjects in the Osaka and Hyogo areas were combined, because these two areas are closely located and several of the hospitals affiliated with Osaka University Medical School are located in the Hyogo area. In 447 patients with LOAD, mean [SD] age at blood drawing was 76.7 [6.9] with a range of 65–98 years, and mean [SD] age at onset was 74.3 [6.3] with a range of 65-94 years. Mean [SD] age in 447 control subjects was 77.1 [7.2] with a range of 65-101 years. Informed consent was obtained from the subject or a relative to participate in the study of geriatric disorders.

Genotyping. The genotype of the *ALDH2* gene was determined by the mismatched PCR-RFLP method reported previously (20) with minor modifications. In brief, 5 ng of DNA was amplified in 15 μl of PCR mixture with the following primers: sense primer: 5′-TTACAGGGTCAACTGCTATG-3′, and antisense primer: 5′-CCACACTCACAGTTTTCTCTT-3′, for the amplification of a 131 bp DNA fragment including exon 12 of the *ALDH2* gene. The PCR product was digested with 1.5 unit of *Ear*I in 100 μg/ml bovine serum albumin and the reaction buffer provided by the manufacturer (New England Biolab, Beverly, MA). Digested DNA was separated by 2.5% agarose electrophoresis, where the common *ALDH2*1* allele shows 108 and 23 bp, and the mutant *ALDH2*2* allele 131 bp. Samples showing undigested patterns were repeatedly genotyped by the digestion using 2 units of *Ear*I to verify the genotypes. The genotype frequency of the *APOE* gene of the Osaka and Hyogo areas has been reported previously (21, 22), but the genotypes of all samples were again determined by the PCR-RFLP method according to the reported procedure (23).

TABLE 1

Genotype Frequencies of the *ALDH2* Gene in Patients with LOAD and Controls

	Number of genotype [frequency]			
Subjects	1/1	1/2	2/2	1/2 and 2/2
Patients ($n = 447$)	232 [0.519]	183 [0.409]	32 [0.072]	215 [0.481]*
Controls ($n = 447$)	280 [0.626]	138 [0.309]	29 [0.065]	167 [0.374]

Note. The frequency of allele *ALDH2*1* and allele *ALDH2*2* was 0.724 and 0.276 in the patients, and 0.781 and 0.219 in the controls, respectively ($P = 0.005$). *$P = 0.001$, OR = 1.6 (95% CI = 1.19–2.03).

Statistics. Genotype frequencies were compared by χ^2 test, where P values less than 0.05 were considered significant. If the number of subjects was insufficient for χ^2 test, Fisher's exact test was done. Logistic regression analysis using linear regression was performed using SPSS statistics version 6.1.1, where the dominant effect of the *APOE-$\epsilon 4$* allele on the risk for LOAD and the dominant negative effect of the *ALDH2*2* allele on ALDH2 activity were considered (14, 15, 24). Age at onset of LOAD was compared by Student's t test. Trend of dosage of the *ALDH2*2* allele on age at onset of total patients with Alzheimer's disease (AD) was evaluated by regression analysis.

RESULTS

Patients with LOAD and controls in three areas were combined to examine the effect of the *ALDH2*2* allele on the risk for LOAD, because the frequency of the *ALDH2*2* allele is not significantly different between the Osaka, Hyogo and Kanto areas (20, 25). Genotype frequencies of the *ALDH2* gene both in the patients and controls were in Hardy–Weinberg equilibrium. The frequency of the *ALDH2*2* allele was higher in the patients than in the controls ($P = 0.005$). Since the ALDH2 deficiency is compatible to the carriage of either one or two *ALDH2*2* alleles, carriers with the allele were combined when evaluating the risk for LOAD. The frequency of carriers with the *ALDH2*2* allele (*1/2* and *2/2* in Table 1) was significantly higher in the patients than in the controls (odds ratio (OR) = 1.6 [95% confidence interval (CI) = 1.19–2.03], $P = 0.001$). This trend was evident in both males (OR = 1.9 [95% CI = 1.14–3.17], $P = 0.01$) and females (OR = 1.4 [95% CI = 1.06–1.97], $P = 0.02$) (Table 2).

To examine the interaction between the *APOE-$\epsilon 4$* and *ALDH2*2* alleles for the risk of LOAD, the *APOE* genotype was also examined (Table 3). In our samples,

TABLE 2

Genotype Frequency of the *ALDH2* Gene Separated by Sex

	Patients		Controls	
	Male	Female	Male	Female
1/1	62 [0.504]	170 [0.525]	81 [0.659]	199 [0.614]
1/2	53 [0.431]	130 [0.401]	35 [0.285]	103 [0.318]
2/2	8 [0.065]	24 [0.074]	7 [0.057]	22 [0.068]
1/2 and 2/2	61 [0.496]*	154 [0.475]**	42 [0.341]	125 [0.386]
Total	123	324	123	324

* $P = 0.01$, OR = 1.9 (95% CI = 1.14–3.17), ** $P = 0.02$, OR = 1.4 (95% CI = 1.06–1.97).

when compared to carriers with no *APOE-ϵ4* allele, the odds ratios of carriers with one and two *APOE-ϵ4* alleles for the patients were 3.7 (95% CI = 2.72–5.11, $P < 10 \times 10^{-10}$) and 14.6 (95% CI = 5.14–41.5, $P = 10 \times 10^{-10}$), respectively. Logistic regression analysis indicates a significant effect of the carriage of the *ALDH2*2* allele for the patients ($P = 0.002$), supporting that carriage of the *ALDH2*2* allele is an independent risk for LOAD of the *APOE-ϵ4* allele. The odds ratio in those with the *ALDH2*2* allele was almost twice that in those without the allele irrespective of status with regard to the *APOE-ϵ4* allele (Table 4). Thus, the carriage of the *ALDH2*2* allele is a synergistic risk for LOAD in those with the *APOE-ϵ4* allele.

Next, the effect of these alleles on age at onset was examined (Fig. 1). In all patients with LOAD, the difference in age at onset was not significant between patients with zero, one and two *APOE-ϵ4* alleles (mean ± SE: 74.6 ± 0.42, 74.2 ± 0.51, and 73.3 ± 1.02 years, respectively). In contrast, among patients with the *ALDH2*2* allele, those with two *APOE-ϵ4* alleles (71.1 ± 1.41 years) had significantly earlier ages at onset than those with one *APOE-ϵ4* allele (74.3 ± 0.64 years, $P < 0.04$) and those with no *APOE-ϵ4* allele (74.8 ± 0.64 years, $P = 0.03$). A significant effect on age at onset in those with the *ALDH2*2* allele was detected only among patients with two *APOE-ϵ4* alleles ($P < 0.05$). Finally, we compared age at onset among patients homozygous for the *APOE-ϵ4* allele including early onset AD patients. As shown in Fig. 2, trend of dosage of the *ALDH2*2* allele on age at onset of AD was significantly evident by regression analysis ($P = 0.023$). Therefore, we conclude that the *ALDH2*2* and *APOE-ϵ4* alleles synergistically affect not only the frequency of LOAD, but also the age at onset of total AD.

TABLE 3

Genotype Frequency of the *ALDH2* Gene Separated by the *APOE-ϵ4* Status

	Number of patients				Number of controls			
APOE	*ϵ4/ϵ4*	*ϵ4/−*	*−/−*	Total	*ϵ4/ϵ4*	*ϵ4/−*	*−/−*	Total
ALDH2								
1/1	20	90	122	232	3	48	229	280
1/2	15	71	97	183	1	20	117	138
2/2	2	16	14	32	0	7	22	29
1/2 and 2/2	17	87	111	215	1	27	139	167
Total	37	177	233	447	4	75	368	447

TABLE 4

Relative Risks of LOAD by the *APOE* and *ALDH2* Gene

Genotype	Odds ratio (95% CI)	*P*
APOE-ϵ4 (2+)/*ALDH2*2* (+)	31.9 (4.20–242)	3.9×10^{-7} #
APOE-ϵ4 (2+)/*ALDH2*2* (−)	12.5 (3.65–43.0)	8.6×10^{-7} #
APOE-ϵ4 (1+)/*ALDH2*2* (+)	6.1 (3.73–9.82)	$<1 \times 10^{-10}$
APOE-ϵ4 (1+)/*ALDH2*2* (−)	3.5 (2.32–5.32)	9.0×10^{-9}
APOE-ϵ4 (−)/*ALDH2*2* (+)	1.5 (1.08–2.09)	0.017
APOE-ϵ4 (−)/*ALDH2*2* (−)	1	Reference

Fisher's exact test.

DISCUSSION

This study indicates that carriage of the *ALDH2*2* allele is a risk for LOAD in the Japanese population by the case-control study, and a synergistic risk in those with the *APOE-ϵ4* allele.

In previous case–control studies on AD, specific alleles of very low density lipoprotein (VLDL) receptor and α2-macroglobulin gene have been reported to be significantly associated with AD (26, 27). In addition, it

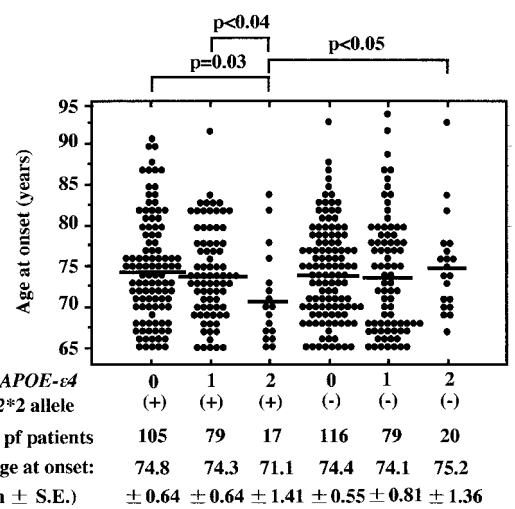

FIG. 1. Gene-dose effect of the *APOE-ϵ4* allele on age of onset of late-onset of Alzheimer's disease. Patients were separated by the gene dose of the *APOE-ϵ4* allele and each closed circle presents age at onset of each patient. Horizontal lines indicate mean ages at onset of each group. Student's *t* test shows significance between indicated groups in difference of onset age as shown by *P* values.

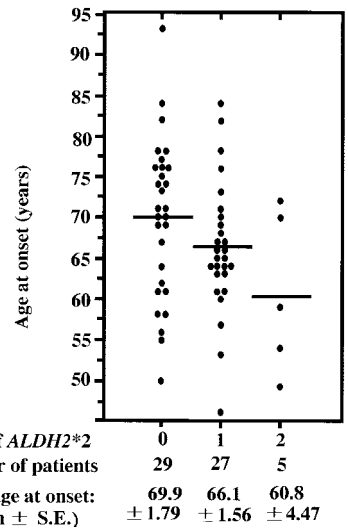

FIG. 2. Gene-dose effect of the *ALDH2*2* allele on age at onset of total patients, including early-onset patients, who are homozygous for the *APOE-ϵ4* allele. Patients homozygous for the *APOE-ϵ4* allele were separated by the dose of the *ALDH2*2* allele. Each closed circle indicates onset age of the corresponding patient. Horizontal lines indicate mean ages at onset of each group. Trend of each group depending upon the dosage of the *ALDH2*2* allele is significant by regression analysis ($P = 0.0023$).

has been reported that a specific allele of the α1-antichymotrypsin gene modifies the risk of the *APOE-ϵ4* allele (28). Contradictory data have, however, been reported against these case–control studies (29–31). In this study, we carefully adopted controls matched with age, gender, and region. The frequencies of carriers with the *ALDH2*2* allele in two different regions have been reported in younger Japanese populations (mean age 33–42 years old), but regional difference in the frequency was not likely (43.1% in Osaka area and 41.9% in Kanto area) (20, 25). The frequency of subjects with the *ALDH2*2* allele in those aged 65 and older in this study (37.4%) is lower than that in the younger subjects (20, 25). To clarify the discrepancy, we performed a large-scale analysis of the *ALDH2*2* allele frequency in an area of Japan and found that the *ALDH2*2* allele frequency decreases depending on age (will be published elsewhere). This finding indicates that the lower *ALDH2*2* allele frequency of the aged control subjects described in this study should be due to the effect on susceptibility to other diseases and/or aging. Therefore, we conclude that the frequency of the *ALDH2*2* carriage in LOAD is significantly higher than that in the age-matched controls.

Moreover, we showed that the carriage of the *ALDH2*2* allele is a synergistic risk for LOAD in those with the *APOE-ϵ4* allele as shown in Table 4. The synergistic increase of the odds ratios cannot be due to contingent effects, supporting that the *ALDH2*2* allele is indeed associated with LOAD. When compared to carriers of the *APOE-ϵ3/ϵ3* genotype, the risks for LOAD in Japanese subjects with the *APOE-ϵ4* allele (OR [$\epsilon4/\epsilon4$] = 33.1; *OR* [$\epsilon3/\epsilon4$] = 5.6) are twice those in Caucasian subjects (OR [$\epsilon4/\epsilon4$] = 14.9; OR [$\epsilon3/\epsilon4$] = 3.2) (32). Our results suggest that the increased risks for LOAD in Japanese can partly be explained by the effect of the *ALDH2*2* allele, since this allele is very rare in populations except the Mongoloids (33). However, it is possible that the other polymorphism affecting the ALDH2 activity exists in Caucasians, because a sib-pair analysis of LOAD indicated a weak positive score around the region at chromosome 12q24.2 where the *ALDH2* gene is localized (34, 35).

Furthermore, we compared ages at onset among the patients and found significant dose effects of the *APOE-ϵ4* and *ALDH2*2* alleles on the ages at onset (Figs. 1 and 2). The *ALDH2*2* affects not only the frequency of LOAD but also the ages at onset in the patients with the *APOE-ϵ4* allele. Since compared among only the patients in this case, no influence by the non-dementia controls would be taken into consideration.

Twelve genes have been identified in the human *ALDH* gene family (36). The *ALDH2* gene has a similar primary structure to the *ALDH1* and *ALDH6* gene, but only the *ALDH2* gene is highly transcribed in the brain (37). Acetaldehyde is a neurotoxic product in the metabolic pathway of valine and threonine. The other aldehydes or aldehyde derivatives could be also neurotoxic. Thus, accumulation of toxic acetaldehyde or aldehyde derivatives could affect the development of the pathogenesis of AD.

Mitochondrial ALDH2 metabolizes acetaldehyde produced from ethanol into acetate. The *ALDH2* gene is the strongest genetic factor influencing alcohol drinking behavior and relates to the risk of alcoholism, because carriers of the *ALDH2*2* allele suffer from low tolerance of alcohol (38–40). On the other hand, a protective effect of alcohol drinking for LOAD has been suggested (8, 10). Therefore, it is unknown whether accumulation of acetaldehyde produced from ethanol affects the pathogenesis of AD. More careful study will be required for investigating the role of the ALDH2 deficiency.

In conclusion, we found a significant association between LOAD and the carriage of the *ALDH2*2* allele which is causative of the ALDH2 deficiency in mitochondria. Thus, aldehyde metabolism could be a therapeutic target for AD and LOAD.

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