Journal of Alzheimer's Disease 87 (2022) 863–871
DOI 10.3233/JAD-215627
IOS Press

Association Study and Meta-Analysis of Polymorphisms and Blood mRNA Expression of the *ALDH2* Gene in Patients with Alzheimer's Disease

Mariko Ueno, Yuta Yoshino, Hiroaki Mori, Yu Funahashi, Hiroshi Kumon, Shinichiro Ochi, Tomoki Ozaki, Ayumi Tachibana, Taku Yoshida, Hideaki Shimizu, Takaaki Mori, Jun-ichi Iga* and Shu-ichi Ueno
Department of Neuropsychiatry, Molecules and Function, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime, Japan

Accepted 9 March 2022
Pre-press 5 April 2022

Abstract.
Background: Late-onset Alzheimer's disease (LOAD) is a complex disease in which neuroinflammation plays an important pathophysiological role, and exposure to neurotoxic substrates such as aldehydes may contribute. Blood mRNA expression levels of neuroinflammation-related genes appear to be potential biological markers of LOAD. A relationship between *ALDH2* and LOAD has been suggested.
Objective: Our objective was to examine blood *ALDH2* expression in Japanese LOAD patients, conduct a genetic association study, and add new studies to an extended meta-analysis of the Asian population.
Methods: A blood expression study (45 AD subjects, 54 controls) in which total RNA was isolated from whole peripheral blood samples and *ALDH2* expression measured was conducted. In addition, a genetic association study (271 AD subjects, 492 controls) using genomic DNA from whole peripheral blood samples was conducted. Finally, a meta-analysis examined the relationship between ALDH2*2 frequency and the risk of LOAD.
Results: *ALDH2* mRNA expression was significantly higher in LOAD than in controls, and also higher in men with LOAD than in women with LOAD ($p = 0.043$). The genotypes in the two classified groups and the allele frequency were significantly different between AD and control subjects. The meta-analysis showed a significant difference in the ALDH2*2 allele, with an increased AD risk (OR = 1.38; 95% CI = 1.02–1.85; $p = 0.0348$, $I^2 = 81.1\%$).
Conclusion: There was a significant increase in blood *ALDH2* expression, and a genetic association with ALDH2*2 in LOAD. ALDH2 may have significant roles in the pathogenesis of LOAD in the Asian population.

Keywords: ALDH2, Alzheimer's disease, gene expression, rs671, meta-analysis

INTRODUCTION

Late-onset Alzheimer's disease (LOAD) is a complex disease affected by multiple genetic factors including *APOE* [1, 2], environmental factors such as lifestyle, and comorbidities [3]. Neuroinflammation is one of the important factors in the pathophysiology of LOAD [4], and exposure to neurotoxic substrates

*Correspondence to: Jun-ichi Iga, Department of Neuropsychiatry, Molecules and Function, Ehime University Graduate School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan. Tel.: +81 89 960 5315; Fax: +81 89 960 5317; E-mail: iga.junichi.it@ehime-u.ac.jp.; ORCID iD: https://orcid.org/0000-0003-4409-3096

such as aldehydes may contribute to Alzheimer's disease (AD)-associated pathologies [5]. Several studies have reported that blood mRNA expression levels of neuroinflammation-related genes were potential biological markers of LOAD [6–9].

Currently, extensive research has reported that mitochondrial dysfunction may be associated with various AD pathologies [10]. The aldehyde dehydrogenase (ALDH) superfamily plays a crucial role in several biological processes including development and detoxification pathways in the organism [11]. *ALDH2*, a gene that codes a protein localized in mitochondria, is crucial in the oxidative metabolism of toxic aldehydes in the brain, such as catecholaminergic metabolites 3,4-Dihydroxyphenylacetaldehyde (DOPAL) and 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL) [12]. *ALDH2* also protects against oxidative stress, such as that caused by the principal product of the lipid peroxidation process, 4-hydroxy-2-nonenal (4-HNE) [12]. Animal studies also suggested that *ALDH2* was associated with age-dependent memory impairment and AD-like pathological changes [13, 14].

The rs671(A) allele of the *ALDH2* gene causes the substitution of glutamate (Glu) to lysine (Lys) at codon 487 and inactivates ALDH2 enzyme [15]. The G allele and A allele are shown as ALDH2*1 and ALDH2*2, respectively. Thus, the genotype was shown as having three types, 1/1, 1/2, and 2/2. The A allele is very rare in Caucasians and widely prevalent in Asian populations [16]. ALHD2 is also a mitochondrial enzyme involved in the metabolism of acetaldehyde, a metabolite of ethanol. Deficiency of the ALDH2 enzyme due to ALDH2*2 has been reported to elevate ROS upon ethanol intake, increasing the risk of cytotoxicity and AD-related pathologies [5]. Kamino et al. showed that ALDH2*2 was associated with the development of LOAD in the Japanese population [17]. The recent meta-analysis including five studies (1,057 LOAD and 1,136 healthy controls from Asian populations) showed no significant association between ALDH2*2 and LOAD overall, but men with ALDH2*2 had a higher risk of LOAD [18]. Unfortunately, this meta-analysis is now outdated, and several large-scale genetic association studies of ALDH2*2 in the Asian population have been conducted since then.

Therefore, we examined blood *ALDH2* expression in Japanese LOAD patients. Then, a genetic association study was conducted using our Japanese cohort, and new studies were added to the extended meta-analysis of the Asian population.

Table 1
Demographic data and clinical characteristics of control and LOAD subjects for the gene expression study

	Control ($n=54$)	LOAD ($n=45$)	p
Male, n (%)	18 (33.3)	15 (33.3)	
Female, n (%)	36 (66.7)	30 (66.7)	0.948
Age, years	77.1 ± 5.57	77.3 ± 4.39	0.848
Diabetes mellitus (n)	–	6	
MMSE score	–	19.0 ± 3.90	
NPI score	–	12.31 ± 18.3	
ADAS score	–	19.12 ± 8.66	
CDR score	–	1.42 ± 0.64	

Sex; χ^2 test, age; Student's t test. Data are given as mean $\pm$ SD unless indicated otherwise. There were no differences in the sex and age between control and LOAD subjects (sex 0.948 χ^2 test; age 0.848 Student's t-test).

METHODS

Re-analysis of our transcriptome data using an AD mouse model (3xTg-AD)

We used our transcriptome data to reanalyze the Aldh2 mRNA levels in the AD mouse model (3xTg-AD) and control mice. Detailed methods were shown in our published paper [19]. In brief, mRNA was extracted from the blood and hippocampus of 3xTg-AD and control mice at different ages (at 12 and 52 weeks of age) and used for microarray analysis. The animal experiments were approved by the Animal Experiment Committee of Ehime University (#28–25) and were performed in accordance with the Guidelines for Animal Experiments at Ehime University. The microarray data were deposited in the GEO database (accession number GSE144459). From this database, we re-analyzed the Aldh2 mRNA levels.

Subjects for the blood expression study

Demographic data for each group of participants are shown in Table 1. A total of 45 AD subjects who visited Ehime University Hospital [mean age $\pm$ standard deviation (SD) = 77.31 ± 4.39 years] were diagnosed and classified as having probable AD dementia according to the criteria of the National Institute on Aging and the Alzheimer's Association [20]. The control participants were 54 elderly individuals [mean age $\pm$ SD = 77.1 ± 5.57 years] without cognitive impairment, psychiatric signs, or a history of neuropsychiatric diseases as determined by at least two certified psychiatrists based on clinical interviews.

Table 2
Demographic data and clinical characteristics of control and LOAD subjects for genetic association study

	Control ($n = 492$)	LOAD ($n = 271$)	p
Male, n (%)	149 (30.3)	74 (27.3)	
Female, n (%)	343 (69.7)	197 (72.7)	0.387
Age, y	80.8 ± 5.84	81.6 ± 6.41	0.078

Sex; χ^2 test, age; Student's t test. Data are given as mean $\pm$ SD unless indicated otherwise. There were no differences in sex and age between control and LOAD subjects (sex 0.387 χ^2 test; age 0.078 Student's t-test).

Subjects for the genetic association study

Demographic data for each group of participants are shown in Table 2. A total of 271 AD subjects (74 males and 197 females, mean age $\pm$ SD = 81.6 ± 6.41 years) who visited Ehime University Hospital, Saiseikai Saijo Hospital, Kuroda Hospital, Maajiro Kujira Rehabilitation Hospital, Heisei Hospital, Japan Community Health Care Organization Uwajima Hospital, Matsukaze Hospital, Nomura Hospital, or Zaidan Niihama Hospital, Ehime, Japan, from December 2013 to May 2015 were enrolled. AD subjects were diagnosed and classified as having probable AD dementia according to the criteria of the National Institute on Aging and the Alzheimer's Association [20]. The control participants were 492 elderly individuals (149 males and 343 females, mean age $\pm$ SD = 80.8 ± 5.84 years) without cognitive impairment, psychiatric signs, or a history of neuropsychiatric diseases as determined by at least two certified psychiatrists based on clinical interviews.

All participants were unrelated, of Japanese origin, and provided written, informed consent using forms that were approved by the institutional ethics committees of each hospital and of Ehime University Hospital (31-K8, 1901009, and 2109001).

Blood sample preparation for the expression study

Total RNA was isolated from whole peripheral blood samples using PaxGene Blood RNA Systems tubes (BD, Tokyo, Japan) according to the standard protocol. The RNA concentration and purity were measured with a NanoDrop-1000 (Thermo Fisher Scientific, Yokohama, Japan), and the 260/280 ratio was between 1.8 and 2.0. RNA (1.0 μg) was used to synthesize cDNA with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time quantitative reverse transcription-PCR was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems). Specific TaqMan probes were Hs01007998_m1 (Applied Biosystems) for *ALDH2* and Hs.PT.39a.22214836 (IDT) for *GAPDH*. *GAPDH* was used as a housekeeping gene because previous studies, including ours, consistently identified *GAPDH* as a suitable housekeeping gene for blood gene expression analysis using the Paxgene blood RNA system [21–24]. The final volume of reactions was 10 μL with TaqMan Universal Master Mix (Applied Biosystems). The expression levels were examined in triplicate. The $\Delta\Delta$Ct method was used to determine relative expression levels using StepOne software (Applied Biosystems).

Blood sample preparation for the genetic association study

For genotyping, genomic DNA (gDNA) was obtained from whole peripheral blood samples collected in potassium EDTA tubes and extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Tokyo, Japan) according to the standard protocol. PCR was performed with 10.0 ng gDNA, 2.5 μL of the 2 × TaqMan genotyping Master Mix (Thermo Fisher Scientific), 0.125 μL of the 20 × TaqMan Assay mix, and 2.375 μL of nuclease-free water. A specific TaqMan probe for the SNP analysis was selected for *ALDH2* rs671, assay ID: C__11703892_10 (TaqMan Assays, Applied Biosystems). Cycling conditions were as follows: denaturation at 95°C for 10 min, followed by 50 cycles of 95°C for 15 s, and 60°C for 1 min.

Meta-analysis methods

Data were collected from PubMed, the Cochrane Library, ClinicalTrials.gov, and MEDLINE. The following keywords were used for the search: 'aldehyde dehydrogenase 2' and 'Alzheimer's disease'. Published studies to examine the association between ALDH2 and LOAD were carefully selected by two independent investigators (J.I. and M.U.). Inclusion criteria were as follows: 1) investigated the association of ALDH2*2 and LOAD risk; 2) case-control studies; 3) sufficient information for genotype counts for calculating the odds ratio (OR) and its corresponding 95% CI. Studies not meeting the above criteria were excluded.

Seventy potential studies were selected through searching and 67 studies were excluded due to not meeting the inclusion criteria. A total of seven potentially relevant case-control studies were identified,

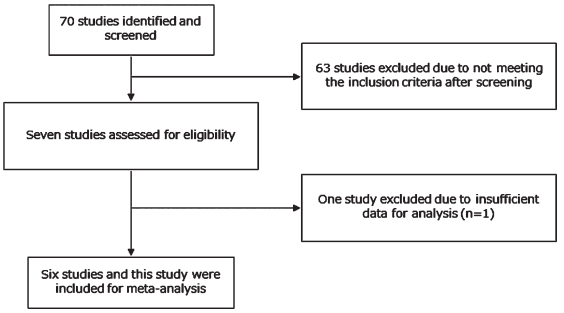

Fig. 1. The flow diagram of the literature search. Six studies and this study were included for meta-analysis.

but one paper was excluded because the paper did not show the number of the ALDH2*2 allele [25]. Among six studies, five were identical to the preceding meta-analysis [18] and one was newly added [26]. Thus, six studies and the present study were included for meta-analysis (Fig. 1). The meta-analysis examined the relationship between the frequency of ALDH2*2 and the risk of LOAD. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated for 1/1 versus the other carriers (1/2 and 2/2). To combine individual study results, the meta-analysis was conducted using EZR [27].

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 22.0 (IBM Corp., Tokyo, Japan). For analysis of the effects of *ALDH2* alleles on each parameter, these alleles were divided into two groups (1/1 versus 1/2 and 2/2) [17]. *ALDH2* mRNA expression levels and the participants' age were compared between AD and controls using Student's *t*-test or the Mann-Whitney U test after the Shapiro-Wilk test. Sex differences and distributions of *ALDH2* alleles were compared with the χ^2 test. Statistical significance was defined at the 95% level ($p = 0.05$). Meta-analysis and power calculations were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [27].

RESULTS

Gene expression study in AD mouse model (3xTg-AD)

Re-analysis of our transcriptome data using the AD mouse model (3xTg-AD) shows that *Aldh2* mRNA expression in blood is increased with age in wild-type mice (ANOVA $p < 0.01$, Dunnett's multiple comparison test: C12 versus C52 $p < 0.01$) and that in the hippocampus is significantly elevated in 3xTg-AD mice (ANOVA $p < 0.001$, Dunnett's multiple comparison test: C12 versus AD12 $p < 0.01$ and C12 versus AD12 $p < 0.0001$) (Fig. 2).

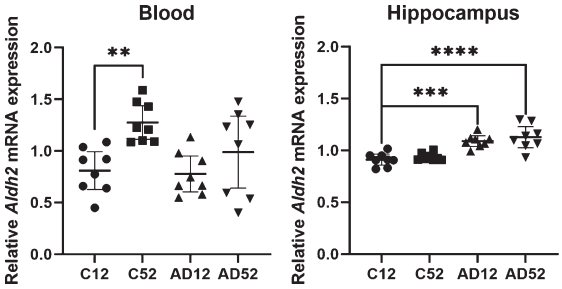

Fig. 2. Re-analysis of our transcriptome data using the AD mouse model (3xTg-AD). *Aldh2* mRNA expression in blood is increased with age in wild-type mice (ANOVA $p < 0.01$, Dunnett's multiple comparison test: C12 versus C52 $p < 0.01$) and that in the hippocampus is significantly elevated in 3xTg-AD mice (ANOVA $p < 0.001$, Dunnett's multiple comparison test: C12 versus AD12 $p < 0.01$ and C12 versus AD12 $p < 0.0001$). Lines are at means with 95% CI [19]. AD12, AD mouse at 12 weeks of age; AD52, AD mouse at 52 weeks of age; C12, control mouse at 12 weeks of age; C52, control mouse at 52 weeks of age.

Gene expression study

The *ALDH2* mRNA expression level was significantly higher in subjects with LOAD than in controls (Fig. 2). *ALDH2* mRNA expression levels were significantly higher in men with LOAD than in women with LOAD (men 1.51 ± 0.43 versus women 1.28 ± 0.31, Student's *t*-test: $p = 0.043$). *ALDH2* mRNA expression levels were not associated with the number of ALDH2*2 ($r = 0.158$ $p = 0.307$) or other clinical factors (presence of diabetes mellitus: $r = -0.004$ $p = 0.987$, Mini-Mental State Examination (MMSE): $r = -0.11$ $p = 0.942$, Neuropsychiatric inventory (NPI): $r = -0.106$ $p = 0.497$, Alzheimer's Disease Assessment Scale (ADAS): $r = 0.237$ $p = 0.184$, Clinical Dementia Rating (CDR): $r = -0.018$ $p = 0.911$). The number of ALDH2*2 was not correlated with clinical factors (presence of diabetes mellitus: $r = -0.224$ $p = 0.330$, MMSE: $r = -0.086$ $p = 0.578$, NPI: $r = -0.152$ $p = 0.337$, ADAS: $r = 0.076$ $p = 0.681$, CDR: $r = -0.030$ $p = 0.851$).

Genetic association study

LOAD and control subjects did not differ in sex or age. The distributions of *ALDH2* and rs671 are

Table 3
Genotype and allele frequencies of the *ALDH2* gene in patients with LOAD and control subjects

Genotypes N (%)	1/1	1/2	2/2	*p*	Allele frequencies N (%)	1	2	*p*
LOAD ($n=271$)	142 (52.4)	112 (41.3)	17 (6.3)	0.015	LOAD	396 (73.1)	146 (26.9)	0.044
Control ($n=492$)	299 (60.8)	175 (35.6)	18 (3.7)		Control	773 (78.6)	211 (21.4)	

Differences in the ratio for each genotype and allele frequency between LOAD and control were significant according to the χ^2 test.

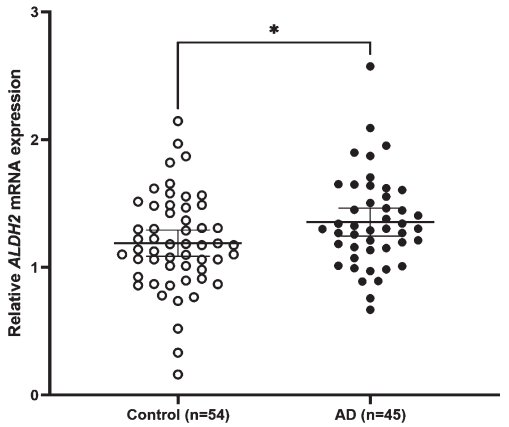

Fig. 3. Relative *ALDH2* mRNA expression levels in AD and control subjects. The mean expression level is significantly higher in AD subjects (1.35 ± 0.36) than in control subjects (1.19 ± 0.38) (Student's *t*-test, $p = 0.030$). Lines are at means with 95% CI.

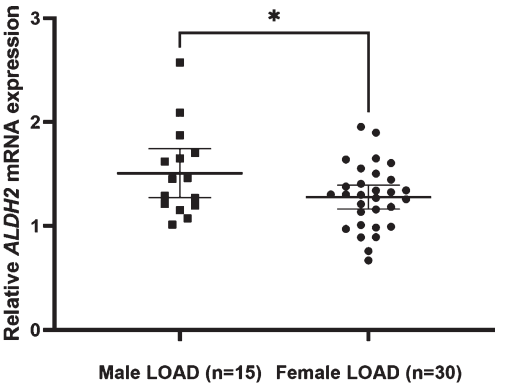

Fig. 4. Relative *ALDH2* mRNA expression levels in male and female LOAD. The mean expression level is significantly higher in male LOAD subjects (1.51 ± 0.43) than in female LOAD subjects (1.28 ± 0.31) (Student's *t*-test: $p = 0.043$). Lines are at means with 95% CI.

shown in Table 3. The genotypes in the two classified groups and the allele frequency in AD were significantly different from those of control subjects (genotypes, $p = 0.015$; allele frequencies, $p = 0.044$).

Meta-analysis

Seven case-control studies using Asian populations were included in the meta-analysis, which consisted of 1,824 cases and 4,300 controls. The association between the number of ALDH2*2 and AD was tested by estimating the ORs for 1/1 versus 1/2 + 2/2 carriers. There was a significant difference in the ALDH2*2 allele, with an increased AD risk under both random and fixed models (OR = 1.38; 95% CI = 1.02–1.85; $p = 0.0348$, $I^2 = 81.1\%$) (Fig. 3). Although statistical analysis for publication bias could not be done due to the small sample size, the funnel plots seemed not to have a significant bias (Supplementary Figure 1).

DISCUSSION

There were three major findings in this study. First, this was the first study to show elevated blood *ALDH2* mRNA expression in LOAD. This result was consistent with the re-analysis of our transcriptome study using an AD mouse model (3xTg-AD) showing that *ALDH2* mRNA expression in blood was increased with age in WT mice, and that in the hippocampus, it was significantly elevated in both young and old 3xTg-AD mice (Fig. 2) [19]. ALDH2*2 genotypes did not alter the ALDH2 mRNA expression in our study, which is consistent with the previous studies showing that this SNP did not change relative ALDH mRNA levels in genetically engineered knock-in mice that express the human ALDH2*1 (wild-type allele) or ALDH2*2 gene (mutant allele) but reduced the ALDH enzyme activity [28]. *ALDH2* is a nuclear gene, but it is transported and functions in the mitochondrial matrix [29]. Mitochondria are well known to be impaired from the early stage of LOAD [30–34]. ALDH2 is involved in metabolizing aldehydes [35, 36] such as acetaldehyde, 4-HNE, 3,4-dihydroxyphenylacetaldehyde (DOPAL, MAO product of dopamine), and 3,4-dihydroxyphenylglycoaldehyde (PDPEGAL, MAO product of norepinephrine), which accumulates in AD patients' brains from the early stage [37–39]. Because ALDH2 activity is known to protect neurons against neurotoxicity induced by toxic aldehydes during oxidative

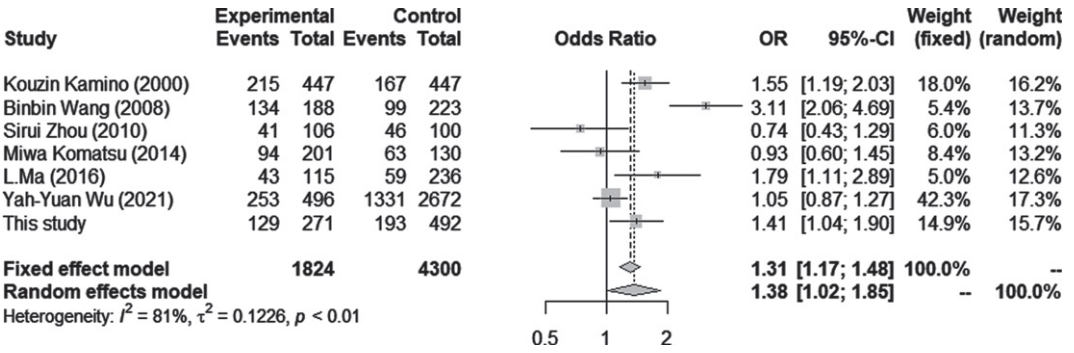

Fig. 5. Forest plots for the meta-analysis in the Asian population. Pooled ORs and 95% CIs were examined for 1/1 versus 1/2 + 2/2. Both fixed effect and random effects models showed significantly higher odds ratios for ALDH2*2 in LOAD.

stress and plays a role in neurodegenerative conditions such as AD [40, 41], elevated blood ALDH2 expression may be associated with a protective response to toxic aldehydes in mitochondria. We found that *ALDH2* mRNA expression levels were significantly higher in men with LOAD than in women with LOAD. This may be related to the sex differences in alcohol consumption because it is reported that drinking behavior in Japanese elderly men was significantly higher than that in women and ALDH2*2 was a major factor associated with drinking behavior [42]. Moreover, regardless of ALDH2*2 genotype, ALDH2 mRNA in peripheral blood of healthy young Japanese volunteers increased following ethanol ingestion [43]. These studies indicate that alcohol consumption should be included as a covariate in a future study on ALDH2 gene expression and association.

Second, there was a significant association between ALDH2*2 and the development of LOAD in the present Japanese cohort. The frequencies of ALDH2*2 were significantly increased in subjects with LOAD compared to those of control subjects. The odds ratio was 1.41, which is consistent with the preceding study using another Japanese cohort [17]. The variant from G to A in exon12 of *ALDH2* (ALDH2*2) causes very low levels of mitochondrial aldehyde dehydrogenase activity [44]. *ALDH2*-KO mice showed decreased cognitive function [13], and Alda-1, which activates the ALDH2 enzyme, improved the pathological and clinical outcome in the AD mouse model [45].

Third, the present comprehensive meta-analysis combining seven genetic studies also showed a significant association between ALDH2*2 and the development of LOAD in the Asian population. This is the first meta-analysis to establish a significant association between ALDH2*2 and LOAD in the Asian population. Although none of the recent genome wide association studies using Caucasian samples [46] or Asian samples [47, 48] detected genome-wide significance of the *ALDH2* gene, this analysis suggested that ALDH2*2 has some role in the pathogenesis of LOAD in the Asian population. Interestingly, a recent genome-wide associated study in a Han Chinese population showed that ALDH2*2 was associated with serum uric acid by affecting alcohol consumption [49]. Similarly, the role of ALDH2*2 in the pathogenesis of LOAD may be affected by alcohol consumption and sex effects, which may have made it difficult for past genome-wide association studies to confirm the significance of the *ALDH2* gene.

There are some limitations in this study. The power analysis using EZR revealed the minimum number of AD and controls required for our gene expression analysis were 77 and 92, respectively. Thus, our results for gene expression analysis may not have adequate power. Due to the low power, the negative findings in this study cannot be interpreted as showing no associations. Elevated blood *ALDH2* mRNA expression in LOAD subjects may arise from other factors such as glucose [50, 51]. Although the presence of diabetes mellitus did not affect *ALDH2* mRNA expression in the present study, the effects of other comorbidities need to be examined. A receiver operating characteristic curve using ALDH2 mRNA levels for diagnosis of LOAD revealed that the area under the curve was 0.623. This was relatively small, so this biomarker alone was insufficient for proper diagnosis. Combination with other biomarkers may increase the sensitivity and specificity [19, 22]. In addition, the effect of alcohol consumption, which may affect the gene expression of *ALDH2*

and the development of LOAD, was not examined. This study included only studies from East Asia, because ALDH2*2 is very rare in other populations. Therefore, in the future, larger studies from various regions in the world are needed. Furthermore, other approaches, such as epigenetic analysis and functional analysis of mitochondria, may be useful for demonstration of the involvement of ALDH2 in the pathophysiology of LOAD. ALDH2 protein levels and enzyme activity should be measured in a future study because increased mRNA levels are not necessarily indicative of increased protein levels [52].

Conclusion

There was a significant increase in blood *ALDH2* expression and a genetic association with ALDH2*2 in LOAD. ALDH2 may have significant roles in the pathogenesis of LOAD in the Asian population.

ACKNOWLEDGMENTS

The authors would like to thank Ms. Chiemi Onishi for technical assistance. This study was supported by the Japan Agency for Medical Research and Development (JP20dk0207025 and JP20dk0307076) and JSPS KAKENHI Grant Numbers 18H02752, 18K07564, 20K07971, and 20K16628.

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/21-5627r2).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: https://dx.doi.org/10.3233/JAD-215627.

REFERENCES

[1] Liu H, Lutz M, Luo S, Alzheimer's Disease Neuroimaging Initiative (2021) Association between polygenic risk score and the progression from mild cognitive impairment to Alzheimer's disease. *J Alzheimers Dis* **84**, 1323-1335.

[2] Wightman DP, Jansen IE, Savage JE, Shadrin AA, Bahrami S, Holland D, Rongve A, Børte S, Winsvold BS, Drange OK, Martinsen AE, Skogholt AH, Willer C, Bråthen G, Bosnes I, Nielsen JB, Fritsche LG, Thomas LF, Pedersen LM, Gabrielsen ME, Johnsen MB, Meisingset TW, Zhou W, Proitsi P, Hodges A, Dobson R, Velayudhan L, Heilbron K, Auton A; 23andMe Research Team, Sealock JM, Davis LK, Pedersen NL, Reynolds CA, Karlsson IK, Magnusson S, Stefansson H, Thordardottir S, Jonsson PV, Snaedal J, Zettergren A, Skoog I, Kern S, Waern M, Zetterberg H, Blennow K, Stordal E, Hveem K, Zwart JA, Athanasiu L, Selnes P, Saltvedt I, Sando SB, Ulstein I, Djurovic S, Fladby T, Aarsland D, Selbæk G, Ripke S, Stefansson K, Andreassen OA, Posthuma D (2021) A genome-wide association study with 1,126,563 individuals identifies new risk loci for Alzheimer's disease. *Nat Genet* **53**, 1276-1282.

[3] Yu JT, Xu W, Tan CC, Andrieu S, Suckling J, Evangelou E, Pan A, Zhang C, Jia J, Feng L, Kua EH, Wang YJ, Wang HF, Tan MS, Li JQ, Hou XH, Wan Y, Tan L, Mok V, Tan L, Dong Q, Touchon J, Gauthier S, Aisen PS, Vellas B (2020) Evidence-based prevention of Alzheimer's disease: Systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. *J Neurol Neurosurg Psychiatry* **91**, 1201-1209.

[4] Maccioni RB, Navarrete LP, Gonzalez A, Gonzalez-Canacer A, Guzman-Martinez L, Cortes N (2020) Inflammation: A major target for compounds to control Alzheimer's disease. *J Alzheimers Dis* **76**, 1199-1213.

[5] Joshi AU, Van Wassenhove LD, Logas KR, Minhas PS, Andreasson KI, Weinberg KI, Chen CH, Mochly-Rosen D (2019) Aldehyde dehydrogenase 2 activity and aldehydic load contribute to neuroinflammation and Alzheimer's disease related pathology. *Acta Neuropathol Commun* **7**, 190.

[6] Sao T, Yoshino Y, Yamazaki K, Ozaki Y, Mori Y, Ochi S, Yoshida T, Mori T, Iga JI, Ueno SI (2018) MEF2C mRNA expression and cognitive function in Japanese patients with Alzheimer's disease. *Psychiatry Clin Neurosci* **72**, 160-167.

[7] Sao T, Yoshino Y, Yamazaki K, Ozaki Y, Mori Y, Ochi S, Yoshida T, Mori T, Iga JI, Ueno SI (2018) TREM1 mRNA expression in leukocytes and cognitive function in Japanese patients with Alzheimer's disease. *J Alzheimers Dis* **64**, 1275-1284.

[8] Lee T, Lee H (2020) Prediction of Alzheimer's disease using blood gene expression data. *Sci Rep* **10**, 3485.

[9] Kumon H, Yoshino Y, Funahashi Y, Mori H, Ueno M, Ozaki Y, Yamazaki K, Ochi S, Mori T, Iga JI, Nagai M, Nomoto M, Ueno SI (2021) PICALM mRNA expression in the blood of patients with neurodegenerative diseases and geriatric depression. *J Alzheimers Dis* **79**, 1055-1062.

[10] Swerdlow RH (2018) Mitochondria and mitochondrial cascades in Alzheimer's disease. *J Alzheimers Dis* **62**, 1403-1416.

[11] Vasiliou V, Pappa A, Petersen DR (2000) Role of aldehyde dehydrogenases in endogenous and xenobiotic metabolism. *Chem Biol Interact* **129**, 1-19.

[12] Deza-Ponzio R, Herrera ML, Bellini MJ, Virgolini MB, Herenu CB (2018) Aldehyde dehydrogenase 2 in the spotlight: The link between mitochondria and neurodegeneration. *Neurotoxicology* **68**, 19-24.

[13] D'Souza Y, Elharram A, Soon-Shiong R, Andrew RD, Bennett BM (2015) Characterization of Aldh2 (-/-) mice as an age-related model of cognitive impairment and Alzheimer's disease. *Mol Brain* **8**, 27.

[14] Ohsawa I, Nishimaki K, Murakami Y, Suzuki Y, Ishikawa M, Ohta S (2008) Age-dependent neurodegeneration accompanying memory loss in transgenic mice defective in mitochondrial aldehyde dehydrogenase 2 activity. *J Neurosci* **28**, 6239-6249.

[15] Yoshida A, Huang IY, Ikawa M (1984) Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proc Natl Acad Sci U S A* **81**, 258-261.

[16] Goedde HW, Agarwal DP, Harada S, Rothhammer F, Whittaker JO, Lisker R (1986) Aldehyde dehydrogenase polymorphism in North American, South American, and Mexican Indian populations. *Am J Hum Genet* **38**, 395-399.

[17] Kamino K, Nagasaka K, Imagawa M, Yamamoto H, Yoneda H, Ueki A, Kitamura S, Namekata K, Miki T, Ohta S (2000) Deficiency in mitochondrial aldehyde dehydrogenase increases the risk for late-onset Alzheimer's disease in the Japanese population. *Biochem Biophys Res Commun* **273**, 192-196.

[18] Liu H, Ge W, Chen W, Kong X, Jian W, Wang A (2020) Association between ALDH2 gene polymorphism and late-onset Alzheimer disease: An up-to-date meta-analysis. *Curr Alzheimer Res* **17**, 105-111.

[19] Ochi S, Iga JI, Funahashi Y, Yoshino Y, Yamazaki K, Kumon H, Mori H, Ozaki Y, Mori T, Ueno SI (2020) Identifying blood transcriptome biomarkers of Alzheimer's disease using transgenic mice. *Mol Neurobiol* **57**, 4941-4951.

[20] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[21] Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408.

[22] Watanabe SY, Iga J, Ishii K, Numata S, Shimodera S, Fujita H, Ohmori T (2015) Biological tests for major depressive disorder that involve leukocyte gene expression assays. *J Psychiatr Res* **66-67**, 1-6.

[23] Watanabe S, Iga J, Nishi A, Numata S, Kinoshita M, Kikuchi K, Nakataki M, Ohmori T (2014) Microarray analysis of global gene expression in leukocytes following lithium treatment. *Hum Psychopharmacol* **29**, 190-198.

[24] Hu N, Tan MS, Yu JT, Sun L, Tan L, Wang YL, Jiang T, Tan L (2014) Increased expression of TREM2 in peripheral blood of Alzheimer's disease patients. *J Alzheimers Dis* **38**, 497-501.

[25] Kim JM, Stewart R, Shin IS, Jung JS, Yoon JS (2004) Assessment of association between mitochondrial aldehyde dehydrogenase polymorphism and Alzheimer's disease in an older Korean population. *Neurobiol Aging* **25**, 295-301.

[26] Wu YY, Lee YS, Liu YL, Hsu WC, Ho WM, Huang YH, Tsai SJ, Kuo PH, Chen YC (2021) Association study of alcohol dehydrogenase and aldehyde dehydrogenase polymorphism with Alzheimer disease in the Taiwanese population. *Front Neurosci* **15**, 625885.

[27] Kanda Y (2013) Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* **48**, 452-458.

[28] Hirohashi K, Ohashi S, Amanuma Y, Nakai Y, Ida T, Baba K, Mitani Y, Mizumoto A, Yamamoto Y, Kikuchi O, Matsubara J, Yamada A, Miyamoto S, Seno H, Matsuda T, Muto M (2020) Protective effects of Alda-1, an ALDH2 activator, on alcohol-derived DNA damage in the esophagus of human ALDH2*2 (Glu504Lys) knock-in mice. *Carcinogenesis* **41**, 194-202.

[29] Chen CH, Ferreira JC, Gross ER, Mochly-Rosen D (2014) Targeting aldehyde dehydrogenase 2: New therapeutic opportunities. *Physiol Rev* **94**, 1-34.

[30] Mise A, Yoshino Y, Yamazaki K, Ozaki Y, Sao T, Yoshida T, Mori T, Mori Y, Ochi S, Iga JI, Ueno SI (2017) TOMM40 and APOE gene expression and cognitive decline in Japanese Alzheimer's disease subjects. *J Alzheimers Dis* **60**, 1107-1117.

[31] Spuch C, Ortolano S, Navarro C (2012) New insights in the amyloid-beta interaction with mitochondria. *J Aging Res* **2012**, 324968.

[32] Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA (2001) Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci* **21**, 3017-3023.

[33] Du H, Guo L, Fang F, Chen D, Sosunov AA, McKhann GM, Yan Y, Wang C, Zhang H, Molkentin JD, Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD (2008) Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat Med* **14**, 1097-1105.

[34] Gan X, Huang S, Wu L, Wang Y, Hu G, Li G, Zhang H, Yu H, Swerdlow RH, Chen JX, Yan SS (2014) Inhibition of ERK-DLP1 signaling and mitochondrial division alleviates mitochondrial dysfunction in Alzheimer's disease cybrid cell. *Biochim Biophys Acta* **1842**, 220-231.

[35] Guo JM, Liu AJ, Zang P, Dong WZ, Ying L, Wang W, Xu P, Song XR, Cai J, Zhang SQ, Duan JL, Mehta JL, Su DF (2013) ALDH2 protects against stroke by clearing 4-HNE. *Cell Res* **23**, 915-930.

[36] Kimura M, Yokoyama A, Higuchi S (2019) Aldehyde dehydrogenase-2 as a therapeutic target. *Expert Opin Ther Targets* **23**, 955-966.

[37] Fukuda M, Kanou F, Shimada N, Sawabe M, Saito Y, Murayama S, Hashimoto M, Maruyama N, Ishigami A (2009) Elevated levels of 4-hydroxynonenal-histidine Michael adduct in the hippocampi of patients with Alzheimer's disease. *Biomed Res* **30**, 227-233.

[38] Markesbery WR, Lovell MA (1998) Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging* **19**, 33-36.

[39] Lovell MA, Ehmann WD, Mattson MP, Markesbery WR (1997) Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol Aging* **18**, 457-461.

[40] Ohsawa I, Kamino K, Nagasaka K, Ando F, Niino N, Shimokata H, Ohta S (2003) Genetic deficiency of a mitochondrial aldehyde dehydrogenase increases serum lipid peroxides in community-dwelling females. *J Hum Genet* **48**, 404-409.

[41] Degl'Innocenti D, Ramazzotti M, Sarchielli E, Monti D, Chevanne M, Vannelli GB, Barletta E (2019) Oxadiazon affects the expression and activity of aldehyde dehydrogenase and acylphosphatase in human striatal precursor cells: A possible role in neurotoxicity. *Toxicology* **411**, 110-121.

[42] Sasaki T, Nishimoto Y, Hirata T, Abe Y, Takebayashi T, Arai Y (2021) ALDH2 p.E504K variation and sex are major factors associated with current and quitting alcohol drinking in Japanese oldest old. *Genes (Basel)* **12**, 799.

[43] Kimura Y, Nishimura FT, Abe S, Fukunaga T, Tanii H, Saijoh K (2009) A promoter polymorphism in the ALDH2 gene affects its basal and acetaldehyde/ethanol-induced gene expression in human peripheral blood leukocytes and HepG2 cells. *Alcohol Alcohol* **44**, 261-266.

[44] Farres J, Wang X, Takahashi K, Cunningham SJ, Wang TT, Weiner H (1994) Effects of changing glutamate 487 to lysine in rat and human liver mitochondrial aldehyde dehydrogenase. A model to study human (Oriental type) class 2 aldehyde dehydrogenase. *J Biol Chem* **269**, 13854-13860.

[45] Yang Y, Chen W, Wang X, Ge W (2021) Impact of mitochondrial aldehyde dehydrogenase 2 on cognitive impairment in

the AD model mouse. *Acta Biochim Biophys Sin (Shanghai)* **53**, 837-847.

[46] Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, Boland A, Vronskaya M, van der Lee SJ, Amlie-Wolf A, et al. (2019) Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity and lipid processing. *Nat Genet* **51**, 414-430.

[47] Kang S, Gim J, Lee J, Gunasekaran TI, Choi KY, Lee JJ, Seo EH, Ko PW, Chung JY, Choi SM, Lee YM, Jeong JH, Park KW, Song MK, Lee HW, Kim KW, Choi SH, Lee DY, Kim SY, Kim H, Kim BC, Ikeuchi T, Lee KH (2021) Potential novel genes for late-onset Alzheimer's disease in East-Asian descent identified by APOE-stratified genome-wide association study. *J Alzheimers Dis* **82**, 1451-1460.

[48] Shigemizu D, Mitsumori R, Akiyama S, Miyashita A, Morizono T, Higaki S, Asanomi Y, Hara N, Tamiya G, Kinoshita K, Ikeuchi T, Niida S, Ozaki K (2021) Ethnic and trans-ethnic genome-wide association studies identify new loci influencing Japanese Alzheimer's disease risk. *Transl Psychiatry* **11**, 151.

[49] Zhang D, Yang M, Zhou D, Li Z, Cai L, Bao Y, Li H, Shan Z, Liu J, Lv D, Liu Y, Xu C, Ling J, Xu Y, Zhang S, Huang Q, Shi Y, Zhu Y, Lai M (2018) The polymorphism rs671 at ALDH2 associated with serum uric acid levels in Chinese Han males: A genome-wide association study. *Gene* **651**, 62-69.

[50] Wang HJ, Kang PF, Wu WJ, Tang Y, Pan QQ, Ye HW, Tang B, Li ZH, Gao Q (2013) Changes in cardiac mitochondrial aldehyde dehydrogenase 2 activity in relation to oxidative stress and inflammatory injury in diabetic rats. *Mol Med Rep* **8**, 686-690.

[51] Fang T, Cao R, Wang W, Ye H, Shen L, Li Z, Hu J, Gao Q (2018) Alterations in necroptosis during ALDH2-mediated protection against high glucoseinduced H9c2 cardiac cell injury. *Mol Med Rep* **18**, 2807-2815.

[52] Boyum A (1964) Separation of white blood cells. *Nature* **204**, 793-794.