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Telomere shortening in alcohol dependence: Roles of alcohol and acetaldehyde

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ABSTRACT

Heavy drinking leads to premature aging and precipitates the onset of age-related diseases. Acetaldehyde (AcH), a toxic metabolite of ethanol, has been implicated in various types of cancer. However, whether alcohol accelerates biological aging at a cellular level is controversial and the mechanism involved is unclear. We addressed these questions by measuring telomere length (TL) in peripheral blood leukocytes of Japanese patients with alcohol dependence (AD) and examined the association between TL, genetic variants of alcohol dehydrogenase (*ADH*)*1B* and aldehyde dehydrogenase (*ALDH*)*2*, and other clinical characteristics. A total of 134 male AD patients and 121 age- and sex-matched healthy controls were evaluated. All patients received endoscopic screening for cancer of the upper aerodigestive tract (UADT). TL was almost 50% shorter in AD patients relative to controls. There were no significant differences in TL between AD patients with and without UADT cancer, and no associations between *ADH1B* and *ALDH2* genotypes and TL. AD patients with thiamine (vitamin B1) deficiency at admission had significantly shorter TL than those with normal thiamine status. Although the exact mechanism underlying the shorter TL in AD patients remain unclear, our findings suggest that alcohol rather than AcH is associated with telomere shortening in AD, which may be accelerated by thiamine deficiency. Future studies should also focus on the association between telomere shortening and TD in the context of oxidative stress.

1. Introduction

Heavy drinking leads to premature aging and precipitates the onset of age-related diseases (Spencer and Hutchison, 1999), including various types of cancer (Baan et al., 2007), hypertension (Briasoulis et al., 2012), decreased gray and white matter volume in the brain (Pfefferbaum et al., 1992), and osteoporosis (Laitinen et al., 1992). However, the mechanistic basis for these effects is not well understood. A shorter telomere length (TL) has been linked to increased risk of several age-related diseases and is widely accepted as a marker of cellular aging (Calado and Young, 2009). An association between heavy drinking and shortened TL in peripheral blood leukocytes (PBLs) has also been reported (Pavanello et al., 2011).

The toxicological effects of alcohol are generally dependent on the duration of exposure and blood alcohol concentration and the levels of its metabolite acetaldehyde (AcH) in body fluids and tissue within that period. The toxicity of AcH is well documented; indeed, it is known to have carcinogenic effects in the upper aerodigestive tract (UADT) (Boccia et al., 2009). It is therefore conceivable that in addition to alcohol, AcH contributes to premature aging and telomere shortening in heavy drinkers.

The primary metabolic pathway for alcohol is its oxidation into AcH by alcohol dehydrogenase (ADH). AcH is subsequently metabolized to acetate, mainly by aldehyde dehydrogenase (ALDH)2. Of the more than 10 ALDH subtypes identified in humans, only ALDH1A1, ALDH1B1, and ALDH2 are known to play a significant role in AcH oxidation (Bosron and Li, 1986). Among these subtypes, mitochondrial ALDH2 is important for human AcH metabolism owing to its low K_m for AcH (Bosron and Li, 1986). The *ALDH2* gene is polymorphic and the *ALDH2*2* allele encodes an inactive form of the enzyme that acts in a semi-dominant manner (Lai et al., 2014). Although *ALDH2*2* is found in 16–35% of East-Asians, including in the Han Chinese, Japanese, Koreans and Vietnamese, it rarely occurs in other ethnic groups, such as in Caucasians, black populations and American Indians (Peng and Yin,

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2009). In the Asian population, the genotype at the polymorphic ALDH2 locus determines the blood AcH concentrations after drinking (Yoshida et al., 1984). The *ALDH2*2* allele arises from a single point mutation in *ALDH2*. The resultant protein, which has a glutamic acid to lysine substitution at residue 504 (rs671), is inactive and cannot metabolize AcH, leading to AcH accumulation after alcohol intake (Enomoto et al., 1991; Hempel et al., 1984; Mizoi et al., 1994; Yoshida et al., 1984) and other adverse reactions such as facial flushing, tachycardia, headache, and nausea (Harada et al., 1981).

The highly active *ADH1B*2* allele is prevalent among Asians, particularly in northeastern Asian populations (e.g., Chinese, Japanese, and Koreans). Between 81% and 100% of Japanese harbor at least one *ADH1B*2* allele, with 34%–71% homozygous for the allele (Eng et al., 2007). Carriers of the *ADH1B*2* allele rapidly convert alcohol to AcH, leading to AcH accumulation following alcohol consumption. Therefore, *ADH1B*2* allele has protective effect against alcohol dependence (AD) (Li et al., 2007).

In this study, we examined whether alcohol and/or AcH accelerate cellular aging using TL in PBLs as a biomarker. We focused on AD patients with the *ALDH2*2* allele. In Japan, approximately 15% of alcoholic patients harbor this mutant allele and are thus able to overcome the adverse effects of acetaldehydemia (Yokoyama et al., 2013), given that it plays a more important role than ADH1B in terms of regulating blood AcH level (Peng and Yin, 2009). We also investigated whether telomere shortening is associated with clinical characteristics in AD patients in order to clarify the mechanism underlying the effects of alcohol on aging.

2. Methods and materials

The study was approved by the Ethics Committee of the Kurihama Medical and Addiction Center (2016050). After the details of the study had been carefully explained, all the participants provided written, informed consent.

2.1. Subjects

The patient group consisted of 134 male patients with AD (mean age ± SD: 58.7 ± 9.7 years) who were admitted to the Kurihama Medical and Addiction Center for treatment of AD and who underwent cancer screening. We randomly selected 48 alcoholic patients who were positive for UADT cancer along with 86 age-matched patients who were negative in the cancer screening. We also collected samples from 121 unrelated healthy Japanese volunteers (59.0 ± 10.2 years) as an age-matched non-alcoholic male control group. The drinking habits of the control subjects were assessed using the Kurihama Alcoholism Screening Test (Saito, 1978), and those suspected of AD based on the test results were excluded from the study. Diagnostic and Statistical Manual of Mental Disorders IV criteria were used to diagnose AD.

2.2. Measurement of TL

Genomic DNA was extracted from PBLs using the DNA Extractor WB kit (Wako Chemicals, Osaka, Japan) for TL measurement and *ALDH* genotyping. TL was measured in all samples using the Telo TAGGG Telomere Length Assay kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Briefly, purified genomic DNA was digested with the restriction endonucleases *Hinf*I and *Rsa*I for 2 h at 37 °C. DNA fragments were separated by 0.5% agarose gel electrophoresis (100 V, 1.5 h) and transferred overnight at room temperature to a positively charged nylon membrane by capillary Southern blotting in 20 × saline-sodium citrate (SSC) buffer. The DNA was cross-linked to the membrane by exposure to ultraviolet light for 5 min, and the membrane was washed with 2 × SSC buffer and air dried. Blotted DNA fragments were hybridized overnight at 42 °C to a digoxigenin-labeled probe specific to telomeric repeats and incubated with an alkaline phosphatase-conjugated digoxigenin-specific antibody for 30 min at room temperature. The immobilized probe was visualized using alkaline phosphatase-metabolizing CDP-Star, a highly sensitive chemiluminescent substrate. Signals were analyzed using a charge-coupled device imager. The average terminal restriction fragment length was determined by comparing the bands on the blot to a molecular weight standard using the Gel-Pro Analyzer (Media Cybernetics, Rockville, MD, USA).

2.3. ADH1B and ALDH2 genotyping

ADH1B and *ALDH2* genotypes were determined by polymerase chain reaction–restriction fragment length polymorphism analysis as previously reported (Harada and Zhang, 1993; Xu et al., 1988).

2.4. Clinical diagnosis of liver cirrhosis (LC)

Following alcohol detoxification, patients underwent a routine examination that included physical examination, blood tests, chest and abdominal X-ray, upper gastrointestinal endoscopy, abdominal ultrasound examination, and abdominal computed tomography. A clinical diagnosis of LC was made based on the results of these tests or detection of esophagogastric varices during endoscopic examination.

2.5. Screening for UADT cancer

All AD patients were screened for UADT cancer by physicians according to a regimented cancer-screening program consisting of endoscopy combined with oropharyngeal examination and esophageal iodine staining.

2.6. Determination of blood thiamine levels

Blood samples were collected from AD patient on their first visit to the Kurihama Medical and Addiction Center to determine blood thiamine level by high-performance liquid chromatography as previously reported (Kimura et al., 1980). None of the patients were taking thiamine for treatment of thiamine deficiency (TD).

2.7. Statistical analysis

Statistical comparisons between subjects with AD and non-alcoholic controls were performed with the non-parametric Mann-Whitney *U* test or Fisher's exact test. Multivariate linear regression was used to determine whether TL varies by diagnosis. General linear models relating AD to telomere length adjusted for age, alcohol consumption, and smoking status were generated. Model generation, data analysis, and statistical computations were performed using SAS v.9.2 software (SAS Institute, Cary, NC, USA). For all statistical tests, a two-tailed P value < 0.05 was considered significant. For multiple comparisons, P values were adjusted by Bonferroni correction.

3. Results

3.1. Smoking and drinking habits of the study population

Table 1 shows comparisons of smoking and drinking habits and *ADH1B* and *ALDH2* genotype distributions between patients with AD and control subjects. A higher rate of smoking was observed in patients with AD than in controls (76.9% vs. 24.0%, $P < 0.0001$), although there were no significant differences in the number of cigarettes consumed (21.9 vs. 19.8, $P = 0.18$). Alcoholic subjects had a significantly longer duration of habitual drinking than controls (34 vs. 22 years, $P < 0.0001$), and consumed a larger amount of alcohol per day than controls (117 vs. 26 g pure alcohol/day, $P < 0.0001$).

Table 1
Characteristics and *ADH1B* and *ALDH2* genotype distributions of the study subjects.

	Alcohol-dependent subjects (n = 134)	Controls (n = 121)	P value
Age, mean (range)	58.7 (41–85)	59.0 (40–85)	0.822
Smoking habits			
Smokers, no. (%)	103 (76.9%)	29 (24.0%)	< 0.0001
Ex-smokers, no. (%)	12 (9.0%)	52 (43.0%)	
Non-smokers, no. (%)	19 (14.2%)	40 (33.1%)	
Cigarettes/day[a], mean (range)	21.9 (10–40)	19.8 (10–40)	0.089
Drinking habits and history			
Drinking amount (g alcohol/day), mean (range)	117 (18–330)	26 (0–154)	< 0.0001
Duration of habitual drinking (years), mean (range)	34 (7–65)	22 (0–52)	< 0.0001
ADH1B (rs122994) genotypes	N (%)	N (%)	
*1/*1	54 (40.3)	4 (3.3)	< 0.0001
*1/*2	46 (34.3)	40 (33.1)	
*2/*2	34 (25.4)	77 (63.6)	
ALDH2 (rs671) genotypes			
*1/*1	74 (55.2)	41 (33.9)	< 0.0001
*1/*2	60 (44.8)	65 (53.7)	
*2/*2	0	15 (12.4)	

ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2.
[a] Current smokers only.

3.2. *ADH1B and ALDH2 genotype distributions in AD patients and controls*

ADH1B and *ALDH2* genotype distributions are shown in Table 1. AD patients had higher frequencies of the *ADH1B*1* allele than controls, consistent with a previous report (Higuchi et al., 1995). We found a higher *ALDH2*2* allele frequency in AD patients than an earlier study (Yokoyama et al., 2013) due to the high frequency of individuals with UADT cancer in our AD patient group (Yokoyama et al., 1996). When comparing the control subjects with the general population, a higher *ALDH2*2* allele frequency was found, although the proportion of *ADH1B* was similar (Higuchi et al., 1995).

3.3. *TL and ALDH2 and ADH1B polymorphisms*

TL was about 50% shorter in subjects with AD as compared to control subjects (unadjusted mean TL = 6.88 vs. 12.84 kbp; $P < 0.0001$) (Table 2). This difference remained statistically significant after controlling for age, smoking status, and alcohol consumption using a multivariable regression model (Table 2).

There were no differences in TL between alcoholic patients with and without the ALDH2*2 allele; the results were the same after controlling for age, smoking habit, alcohol consumption, and UADT cancer (Table 2). Although AD patients with the *ADH1B*1/*1* genotype had shorter telomeres than those with the *ADH1B*1/*2* or *ADH1B*2/*2* genotypes, this difference was not statistically significant. Control subjects with the *ADH1B*1/*1* genotype tended to have longer telomeres than those with *ADH1B*1/*2* or *ADH1B*2/*2* ($P < 0.10$). However, since only four control subjects had the *ADH1B*1/*1* genotype, this difference may have been simply due to chance. We compared TL for different *ADH1B* and *ALDH2* genotype combinations but did not find any differences (data not shown).

3.4. *TL and clinical characteristics*

We investigated associations between TL and smoking status, onset of habitual drinking and clinical characteristics such as UADT cancer, LC, history of withdrawal seizure and withdrawal delirium, and thiamine (vitamin B1) deficiency in AD patients (Table 3). Although we did not find any association between smoking and telomere shortening, AD patients who started drinking before 20 years of age tended to have shorter telomeres as compared to those who started drinking after the age of 20 years (Table 3). Among the clinical characteristics examined, only TD was associated with shortened TL [TD(+) 6.24 vs. TD(−) 7.10 kbp, $P = 0.018$, age adjusted]. The association between withdrawal delirium and telomere shortening was marginally significant ($P = 0.058$).

4. Discussion

The results presented here demonstrate that male patients with AD had significantly shortened telomeres as compared to age- and sex-matched control subjects. Neither the presence of the *ADH1B*2* allele nor an inactive *ALDH2* allele was associated with TL in patients with AD. Furthermore, our study showed that thiamine deficiency in alcoholic subjects was associated with telomere shortening, and suggested that this can occur by starting to drink early in life (before the age of 20 years). This is the first study demonstrating telomere shortening in PBLs of AD patients.

Previous studies that have examined the relationship between alcohol consumption and TL in the general population have found no associations (Latifovic et al., 2016; Weischer et al., 2014) or have reported associations only in older (≥65 years) individuals (Wang et al., 2017). However, it was reported that Caucasian drunk-driving traffic offenders had shorter TL in PBLs than social drinkers (Pavanello et al., 2011). These authors also reported that the *ADH1B*1/*1* genotype was more prevalent in alcohol abusers and that these individuals reported higher alcohol consumption and had shorter telomeres than those with other *ADH1B* genotypes (Pavanello et al., 2011). Another study comparing TL in the esophageal epithelium showed that patients with AD had significantly shorter telomeres than non-alcoholic control subjects (Aida et al., 2011). The authors speculated that telomere shortening may be associated with the frequent occurrence of squamous cell carcinoma in alcoholic patients.

In this study, we focused on the association between TL and variants of the *ALDH2* and *ADH1B* genes. As stated above, the major enzyme responsible for AcH elimination is ALDH2 (Bosron and Li, 1986). Since

Table 2
Telomere length in patients with alcohol dependence and control subjects and associations between TL and *ADH1B* and *ALDH2* genotypes.

	Alcohol-dependent subjects					Controls				
	Total	*ADH1B* *1/*1	*ADH1B* *1/*2 or *2/*2	*ALDH2*2* (−)	*ALDH2*2* (+)	Total	*ADH1B* *1/*1	*ADH1B*1/*2* or *2/*2	*ALDH2*2* (−)	*ALDH2*2* (+)
Unadjusted telomere length, mean (SE)	6.88 (0.13)*	6.82 (0.19)	6.92 (0.18)	6.84 (0.19)	6.94 (0.18)	12.84 (0.39)	16.72 (1.04)†	12.71 (0.39)	13.58 (0.65)	12.47 (0.48)
Model adjusted for age, mean (SE)	6.87 (0.26)*	6.68 (0.22)	7.02 (0.17)	6.88 (0.18)	6.88 (0.20)	12.86 (0.27)	15.35 (2.03)	12.76 (0.37)	13.67 (0.62)	12.42 (0.44)
Model adjusted for age, smoking, alcohol consumption, and cancer, mean (SE)	6.81 (0.30)*	6.66 (0.22)	7.03 (0.18)	6.87 (0.18)	6.89 (0.20)	13.97 (0.40)	17.32 (2.55)	13.74 (0.45)	14.54 (0.76)	13.51 (0.55)

*P < 0.0001, patients with alcohol dependence vs. controls.
†P < 0.10, controls with *ADH1B*1/*1* vs. those with *ADH1B*1/*2* or *ADH1B*2/*2*.
TL, telomere length; ADH1B, alcohol dehydrogenase 1B; ALDH2, aldehyde dehydrogenase 2; SE, standard error.

the *ALDH2*2* allele which encodes an inactive enzyme is a major determinant of high AcH levels after drinking, AD patients who are carriers of this allele can serve as a model to examine the causal role of AcH exposure in telomere shortening. The combination of *ADH1B* and *ALDH2* genotypes can be similarly useful, especially in Japanese patients with AD. Since *ADH1B*2* carriers metabolize alcohol more rapidly than those lacking this allele, the combination of *ADH1B*2* and an inactive *ALDH2* allele can lead to AcH accumulation. Indeed, Japanese AD patients with the *ADH1B*2* allele plus the *ALDH2*1/*2* genotype had the highest levels of the AcH-DNA adduct N2-ethylidene-dG in leukocytes, suggesting that AcH is carcinogenic (Yukawa et al., 2012); these individuals also had a high blood AcH/ethanol ratio (Yokoyama et al., 2010). Since AcH induces oxidative stress and inflammation and is genotoxic (Albano, 2006; Seitz and Mueller, 2015), we speculated that AcH accumulation can cause telomere shortening. However, this was not supported by our findings that neither the *ALDH2*2* allele nor the combination of *ADH1B* and *ALDH2* genotypes were associated with TL in AD patients.

Telomeres are triple-guanine-containing sequences that are highly susceptible to damage by oxidative stress (von Zglinicki, 2002). As such, reactive oxygen species generated by the metabolism of alcohol by cytochrome P450 2E1—the main hepatic alcohol-inducible cytochrome—could cause a reduction in TL by inducing double-strand breaks and/or by interfering with the DNA replication fork (Pavanello et al., 2011).

Our results revealed an association between telomere shortening and TD, which is common among alcoholic patients due to decreased thiamine synthesis and absorption (Martin et al., 2003). TD can lead to functional impairment of peripheral nerves and of neurons in the central nervous system, for instance in the case of Wernicke-Korsakoff syndrome. Although the precise molecular mechanism is unclear, it is possible that it involves neuronal death caused by oxidative stress (Desjardins and Butterworth, 2005). Thiamine is essential in carbohydrate metabolism; TD results in a mitochondrial dysfunction and oxidative stress. Higher intake of nutrients with antioxidative properties such as vitamins C and B12 and folate is positively associated with TL (Lee et al., 2017). To our knowledge, although there is no previous report that shows vitamin B1 supplementation significantly improves telomere shortening, it is possible that thiamine may have a positive effect on telomere length. Thus, although it is unclear how TD can lead to telomere shortening, an impairment of mitochondria and consequent oxidative stress may be responsible. On the other hand, TD may simply reflect a lack of other vitamins such as folate, whose deficiency can cause global telomere attrition (Bull et al., 2014).

A limitation of our study is that it does not demonstrate a causal relationship between alcohol and telomere shortening because the study design was cross-sectional. Additional research is needed to determine the mechanistic link between AD and telomere shortening. The same reason limited our scope for exploring the relationships between TL shortening and TD. Another limitation is that we measured TL in PBLs only. On the other hand, PBLs exhibit similar rates of age-dependent TL attrition to other somatic tissues (Daniali et al., 2013), which supports the validity of our results.

In conclusion, this study showed that AD patients have shorter telomeres than healthy controls irrespective of *ADH1B* and *ALDH2* genotypes. These findings suggest that alcohol rather than AcH level is associated with telomere shortening in AD patients. It would be useful to investigate whether long-term abstinence from alcohol and the improvement of nutritional status can suppress telomere shortening. Future studies should also focus on the association between telomere shortening and TD in the context of oxidative stress.

Conflicts of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Table 3
Associations between telomere length and smoking status, onset of habitual drinking, and clinical characteristics in patients with alcohol dependence.

Smoking status	Telomere length			
	Non-smoker (n = 19)	Ex-smoker (n = 12)	Smoker (n = 103)	P value
Unadjusted, mean (SE)	6.83 (0.35)	6.71 (0.44)	6.91 (0.15)	0.902
Age adjusted, mean (SE)	7.12 (0.38)	6.75 (0.44)	6.85 (0.15)	0.774
Onset of habitual drinking (year)	< 20 (n = 34)	20–25 (n = 56)	26 ≤ (n = 44)	P value
Unadjusted, mean (SE)	6.42 (0.26)	7.02 (0.20)	7.06 (0.23)	0.130
Age adjusted, mean (SE)	6.46 (0.26)	6.97 (0.20)	7.10 (0.23)	0.060

Clinical characteristics	Telomere length		
	Positive	Negative	P value
Cancer in the UADT	n = 48	n = 86	
Unadjusted, mean (SE)	6.79 (0.17)	6.93 (0.18)	0.559
Age adjusted, mean (SE)	6.79 (0.22)	6.93 (0.16)	0.598
Liver cirrhosis	n = 13	n = 113	
Unadjusted, mean (SE)	6.28 (0.46)	6.92 (0.14)	0.155
Age adjusted, mean (SE)	6.91 (0.14)	6.36 (0.43)	0.227
History of withdrawal seizure	n = 10	n = 122	
Unadjusted, mean (SE)	6.45 (0.61)	6.91 (0.14)	0.358
Age adjusted, mean (SE)	6.18 (0.50)	6.93 (0.14)	0.147
History of withdrawal delirium	n = 35	n = 97	
Unadjusted, mean (SE)	6.52 (0.22)	7.00 (0.16)	0.112
Age adjusted, mean (SE)	6.45 (0.26)	7.03 (0.15)	0.058
Thiamine deficiency (< 30 ng/ml)	n = 25	n = 76	
Unadjusted, mean (SE)	6.22 (0.27)	7.11 (0.19)	0.015
Age adjusted, mean (SE)	6.24 (0.31)	7.10 (0.18)	0.018

SE, standard error; UADT, upper aerodigestive tract.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpsychires.2018.11.007.

References

Aida, J., Yokoyama, A., Izumiyama, N., Nakamura, K., Ishikawa, N., Poon, S.S., Fujiwara, M., Sawabe, M., Matsuura, M., Arai, T., Takubo, K., 2011. Alcoholics show reduced telomere length in the oesophagus. J. Pathol. 223 (3), 410–416.
Albano, E., 2006. Alcohol, oxidative stress and free radical damage. Proc. Nutr. Soc. 65 (3), 278–290.
Baan, R., Straif, K., Grosse, Y., Secretan, B., El Ghissassi, F., Bouvard, V., Altieri, A., Cogliano, V., 2007. Carcinogenicity of alcoholic beverages. Lancet Oncol. 8 (4), 292–293.
Boccia, S., Hashibe, M., Galli, P., De Feo, E., Asakage, T., Hashimoto, T., Hiraki, A., Katoh, T., Nomura, T., Yokoyama, A., van Duijn, C.M., Ricciardi, G., Boffetta, P., 2009. Aldehyde dehydrogenase 2 and head and neck cancer: a meta-analysis implementing a Mendelian randomization approach. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research. cosponsored by the American Society of Preventive Oncology 18 (1), 248–254.
Bosron, W.F., Li, T.K., 1986. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. Hepatology (Baltimore, Md 6 (3), 502–510.
Briasoulis, A., Agarwal, V., Messerli, F.H., 2012. Alcohol consumption and the risk of hypertension in men and women: a systematic review and meta-analysis. J. Clin. Hypertens. 14 (11), 792–798.
Bull, C.F., Mayrhofer, G., O'Callaghan, N.J., Au, A.Y., Pickett, H.A., Low, G.K., Zeegers, D., Hande, M.P., Fenech, M.F., 2014. Folate deficiency induces dysfunctional long and short telomeres; both states are associated with hypomethylation and DNA damage in human WIL2-NS cells. Cancer Prev. Res. 7 (1), 128–138.
Calado, R.T., Young, N.S., 2009. Telomere diseases. N. Engl. J. Med. 361 (24), 2353–2365.
Daniali, L., Benetos, A., Susser, E., Kark, J.D., Labat, C., Kimura, M., Desai, K., Granick, M., Aviv, A., 2013. Telomeres shorten at equivalent rates in somatic tissues of adults. Nat. Commun. 4, 1597.
Desjardins, P., Butterworth, R.F., 2005. Role of mitochondrial dysfunction and oxidative stress in the pathogenesis of selective neuronal loss in Wernicke's encephalopathy. Mol. Neurobiol. 31 (1–3), 17–25.
Eng, M.Y., Luczak, S.E., Wall, T.L., 2007. ALDH2, ADH1B, and ADH1C genotypes in Asians: a literature review. Alcohol Res. Health : the journal of the National Institute on Alcohol Abuse and Alcoholism 30 (1), 22–27.
Enomoto, N., Takase, S., Yasuhara, M., Takada, A., 1991. Acetaldehyde metabolism in different aldehyde dehydrogenase-2 genotypes. Alcohol Clin. Exp. Res. 15 (1), 141–144.
Harada, S., Agarwal, D.P., Goedde, H.W., 1981. Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese. Lancet 2 (8253), 982.
Harada, S., Zhang, S., 1993. New strategy for detection of ALDH2 mutant. Alcohol Alcohol (Suppl. 1a), 11–13.
Hempel, J., Kaiser, R., Jornvall, H., 1984. Human liver mitochondrial aldehyde dehydrogenase: a C-terminal segment positions and defines the structure corresponding to the one reported to differ in the Oriental enzyme variant. FEBS Lett. 173 (2), 367–373.
Higuchi, S., Matsushita, S., Murayama, M., Takagi, S., Hayashida, M., 1995. Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. Am. J. Psychiatry 152 (8), 1219–1221.
Kimura, M., Fujita, T., Nishida, S., Itokawa, Y., 1980. Differential fluorimetric determination of picogram levels of thiamine, thiamine monophosphate, diphosphate and triphosphate using high-performance liquid chromatography. J. Chromatogr. 188 (2), 417–419.
Lai, C.L., Yao, C.T., Chau, G.Y., Yang, L.F., Kuo, T.Y., Chiang, C.P., Yin, S.J., 2014. Dominance of the inactive Asian variant over activity and protein contents of mitochondrial aldehyde dehydrogenase 2 in human liver. Alcohol Clin. Exp. Res. 38 (1), 44–50.
Laitinen, K., Lamberg-Allardt, C., Tunninen, R., Harkonen, M., Valimaki, M., 1992. Bone mineral density and abstention-induced changes in bone and mineral metabolism in noncirrhotic male alcoholics. Am. J. Med. 93 (6), 642–650.
Latifovic, L., Peacock, S.D., Massey, T.E., King, W.D., 2016. The influence of alcohol consumption, cigarette smoking, and physical activity on leukocyte telomere length. Cancer epidemiology, biomarkers & prevention : a publication of the american association for cancer research. cosponsored by the American Society of Preventive Oncology 25 (2), 374–380.
Lee, J.Y., Shin, C., Baik, I., 2017. Longitudinal associations between micronutrient consumption and leukocyte telomere length. J. Hum. Nutr. Diet. : the official journal of the British Dietetic Association 30 (2), 236–243.
Li, H., Mukherjee, N., Soundararajan, U., Tarnok, Z., Barta, C., Khaliq, S., Mohyuddin, A., Kajuna, S.L., Mehdi, S.Q., Kidd, J.R., Kidd, K.K., 2007. Geographically separate increases in the frequency of the derived ADH1B*47His allele in eastern and western Asia. Am. J. Hum. Genet. 81 (4), 842–846.
Martin, P.R., Singleton, C.K., Hiller-Sturmhofel, S., 2003. The role of thiamine deficiency in alcoholic brain disease. Alcohol Res. Health : the journal of the National Institute on Alcohol Abuse and Alcoholism 27 (2), 134–142.
Mizoi, Y., Yamamoto, K., Ueno, Y., Fukunaga, T., Harada, S., 1994. Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenases in individual variation

of alcohol metabolism. Alcohol Alcohol 29 (6), 707–710.
Pavanello, S., Hoxha, M., Dioni, L., Bertazzi, P.A., Snenghi, R., Nalesso, A., Ferrara, S.D., Montisci, M., Baccarelli, A., 2011. Shortened telomeres in individuals with abuse in alcohol consumption. Int. J. Canc. 129 (4), 983–992.
Peng, G.S., Yin, S.J., 2009. Effect of the allelic variants of aldehyde dehydrogenase ALDH2*2 and alcohol dehydrogenase ADH1B*2 on blood acetaldehyde concentrations. Hum. Genom. 3 (2), 121–127.
Pfefferbaum, A., Lim, K.O., Zipursky, R.B., Mathalon, D.H., Rosenbloom, M.J., Lane, B., Ha, C.N., Sullivan, E.V., 1992. Brain gray and white matter volume loss accelerates with aging in chronic alcoholics: a quantitative MRI study. Alcohol Clin. Exp. Res. 16 (6), 1078–1089.
Saito, S., 1978. KAST (Kurihama alcoholism screening test) and its applications. Jpn. J. Stud. Alcohol 13, 229–237.
Seitz, H.K., Mueller, S., 2015. Alcohol and cancer: an overview with special emphasis on the role of acetaldehyde and cytochrome P450 2E1. Adv. Exp. Med. Biol. 815, 59–70.
Spencer, R.L., Hutchison, K.E., 1999. Alcohol, aging, and the stress response. Alcohol Res. Health : the journal of the National Institute on Alcohol Abuse and Alcoholism 23 (4), 272–283.
von Zglinicki, T., 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. 27 (7), 339–344.
Wang, H., Kim, H., Baik, I., 2017. Associations of alcohol consumption and alcohol flush reaction with leukocyte telomere length in Korean adults. Nutrition research and practice 11 (4), 334–339.
Weischer, M., Bojesen, S.E., Nordestgaard, B.G., 2014. Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. PLoS Genet. 10 (3), e1004191.
Xu, Y.L., Carr, L.G., Bosron, W.F., Li, T.K., Edenberg, H.J., 1988. Genotyping of human alcohol dehydrogenases at the ADH2 and ADH3 loci following DNA sequence amplification. Genomics 2 (3), 209–214.
Yokoyama, A., Muramatsu, T., Ohmori, T., Makuuchi, H., Higuchi, S., Matsushita, S., Yoshino, K., Maruyama, K., Nakano, M., Ishii, H., 1996. Multiple primary esophageal and concurrent upper aerodigestive tract cancer and the aldehyde dehydrogenase-2 genotype of Japanese alcoholics. Cancer 77 (10), 1986–1990.
Yokoyama, A., Tsutsumi, E., Imazeki, H., Suwa, Y., Nakamura, C., Yokoyama, T., 2010. Polymorphisms of alcohol dehydrogenase-1B and aldehyde dehydrogenase-2 and the blood and salivary ethanol and acetaldehyde concentrations of Japanese alcoholic men. Alcohol Clin. Exp. Res. 34 (7), 1246–1256.
Yokoyama, A., Yokoyama, T., Matsui, T., Mizukami, T., Kimura, M., Matsushita, S., Higuchi, S., Maruyama, K., 2013. Trends in gastrectomy and ADH1B and ALDH2 genotypes in Japanese alcoholic men and their gene-gastrectomy, gene-gene and gene-age interactions for risk of alcoholism. Alcohol Alcohol 48 (2), 146–152.
Yoshida, A., Huang, I.Y., Ikawa, M., 1984. Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. Proc. Natl. Acad. Sci. U. S. A. 81 (1), 258–261.
Yukawa, Y., Muto, M., Hori, K., Nagayoshi, H., Yokoyama, A., Chiba, T., Matsuda, T., 2012. Combination of ADH1B*2/ALDH2*2 polymorphisms alters acetaldehyde-derived DNA damage in the blood of Japanese alcoholics. Cancer Sci. 103 (9), 1651–1655.