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Shortened telomeres in individuals with abuse in alcohol consumption

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Abstract

Alcohol abuse leads to earlier onset of aging-related diseases, including cancer at multiple sites. Shorter telomere length (TL) in peripheral blood leucocytes (PBLs), a marker of biological aging, has been associated with alcohol-related cancer risks. Whether alcohol abusers exhibit accelerated biological aging, as reflected in PBL-TL, has never been examined.

To investigated the effect of alcohol abuse on PBL-TL and its interaction with alcohol metabolic genotypes, we examined 200 drunk-driving traffic offenders diagnosed as alcohol abusers as per the Diagnostic and Statistical Manual of Mental Disorders [DSM-IV-TR] and enrolled in a probation program, and 257 social drinkers (controls). We assessed alcohol intake using self-reported drink-units/day and conventional alcohol abuse biomarkers (serum γ-glutamyltrasferase [GGT] and mean corpuscular volume of erythrocytes [MCV]). We used multivariable models to compute TL geometric means (GM) adjusted for age, smoking, BMI, diet, job at elevated risk of accident, genotoxic exposures.

TL was nearly halved in alcohol abusers compared to controls (GMs 0.42 vs. 0.87 relative T/S ratio; P<0.0001) and decreased in relation with increasing drink-units/day (P-trend=0.003). Individuals drinking >4 drink-units/day had substantially shorter TL than those drinking 4 drink-units/day (GMs 0.48 vs. 0.61 T/S, P=0.002). Carriers of the common *ADH1B*1/*1* (rs1229984) genotype were more likely to be abusers (P=0.008), reported higher drink-units/day (P=0.0003), and exhibited shorter TL (P<0.0001). The rs698 *ADH1C* and rs671 *ALDH2* polymorphisms were not associated with TL.

The decrease in PBL-TL modulated by the alcohol metabolic genotype ADH1B*1/*1 may represent a novel mechanism potentially related to alcohol carcinogenesis in alcohol abusers.

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INTRODUCTION

The rates of almost any type of cancer increase with age. Alcohol abusers tend to look haggard, and it is commonly thought that heavy drinking leads to premature aging and earlier onset of age-related diseases[1]. Abuse in alcohol drinking is a global health priority[2] that has been associated with cancer at multiple sites [3]. Taken together these data suggest that alcohol abuse may accelerate biological processes related to aging. Nevertheless, the exact mechanisms of how alcohol drinking exerts these effects are not well known, including whether alcohol accelerates biological aging at a cellular level.

All the cells in our body have a biological clock in telomeres, DNA sequences located at the ends of chromosomes that are involved in maintaining genomic stability and regulating cellular proliferation [4]. Telomere length (TL) in proliferating tissues, which can be conveniently measured in peripheral blood leucocytes (PBLs), is longest at birth and shortens progressively as individuals age [5]. Shorter TL has been associated with risk of several age-related diseases and is widely accepted as a marker of biological cellular aging [6]. Epidemiology retrospective [7–12] and prospective [13,14] studies have shown that among subjects of the same age, those with shorter telomeres in PBLs have higher risk of cancer at multiple sites [6–14]. Oxidative stress [15,16] and inflammation [16,17], two mechanisms that accelerate telomere shortening [18,19], have been linked with heavy alcohol consumption (for a review see [20]) as well as with the risk of cancer at multiple sites [21,22]. However, whether alcohol drinking is associated with telomere shortening has never been evaluated.

Mechanisms of alcohol-induced cancer are closely related to the metabolism of ethanol [23]. Alcohol is principally metabolized by the alcohol dehydrogenase (ADH) to acetaldehyde that is further oxidized to acetate by aldehyde dehydrogenase (ALDH2) enzymes, which exist in several polymorphic variants [24]. Among Caucasians, variants in *ADH* genes are common, but very rare in *ALDH2* [24]. Individuals who carry highly active alleles (*ADH1B*2* and *ADH1C*1*) rapidly convert ethanol to acetaldehyde [25]. This leads to acetaldehyde accumulation following alcohol consumption and results in toxic side effects (e.g., flushing syndrome with sweating, accelerated heart rate, nausea, and vomiting) that deter the carriers from acute and chronic alcohol consumption (i.e., individuals with these alleles typically drink little or no alcohol) [26,27] and protect them from alcohol-related cancer [28].

The aim of the present study was to investigate the effect of alcohol abuse on TL in PBLs, and to elucidate whether such effect is modified by genetic variants in alcohol metabolic genes.

MATERIALS AND METHODS

Study participants

The study population (n=457) was composed by Caucasian males living in Northeastern Italy, including 200 alcohol abusers and 257 controls. Study participants were recruited from November 2008 through September 2009. The alcohol abusers were drunk-driving traffic offenders enrolled in a probation program and referred as outpatients to the forensic toxicology and antidoping ambulatoryof University of Padova. They were defined as alcohol abusers as per the Diagnostic and Statistical Manual of Mental Disorders [DSM-IV-TR] of the American Psychiatric Association, i.e., individuals with a maladaptative pattern of substance use as manifested by recurrent alcohol use in situations in which it is physically hazardous. All of them were found to be driving a vehicle with blood alcohol concentration [BAC] >1.5gr/L. Except for 11 abstainers, controls were social drinkers with variable

alcohol use. Controls were members of a group of white-collar workers, recruited during their periodic check-ups at the Unit of Preventive Health Services, University of Padova as previously described [29]. Alcohol abusers as per DSM-IV-TR were excluded from the control group. Trained interviewers informed all participants of the study objectives and collected personal data including drinking habits, job type, possible elevated non-occupational genotoxic-exposures (smoking, diet, environment), and consumption of vegetables by means of a structured questionnaire, as previously described [29]. The study was approved by the University of Padova's Institutional Review Board. All participants provided written informed consent. Smokers were defined as individuals who reported current active smoking. Nonsmokers were defined as never smokers or former-smokers who had quit smoking at least one year before blood sample collection. Participants donated 5 ml blood sample for TL and genotyping analyses. Blood samples and information from a structured questionnaire were collected from abusers during their follow-up check-up of their probation program, i.e., after at least three 3 months from the beginning of the program. All data were anonymized after collection of personal data and blood samples. DNA samples, isolated using the Promega Wizard genomic DNA purification kit (Promega, Italy), was available for TL analysis in all the 200 abusers and 257 controls. However, DNA was available for genotype analysis in only 149 alcohol abusers and 255 controls.

Usual alcohol intake in both abusers (intake since the beginning of the probation program) and controls (average usual intake) was evaluated based on self-reported questionnaire data and expressed as units of drink/day. Each unit was equivalent to approximately 10–12g alcohol intake. High alcohol intake was defined as > 4 drink-units/day (more than 40g alcohol/day) [23]. The integrated measure of alcohol drinking [(drink-unit per years of exposure (drink-years)] and smoking history (pack-years) for abusers was also collected. Individuals with high dietary intake of genotoxins (in particular polycyclic aromatic hydrocarbons [PAHs]) were those who reported consumption of charcoaled meat or pizza more than once a week; individuals with indoor exposure were those who reported at least one of several exposure sources (i.e., use of fireplace, coal or wood-stove as heating at home; or passive exposure to tobacco smoke) as previously described [29]. We defined participants with a job at elevated risk of accident as those with an occupation in a high-risk category, as classified by the Italian National regulations in matter of alcohol and correlated problems [30].

Serum γ-glutamyltrasferase [GGT] and mean corpuscular volume of erythrocytes [MCV] were measured as conventional biomarkers of alcohol abuse [31]. MCV and GGT, which are increased in individuals with chronic and heavy alcohol drinking, have been shown to return within normal ranges after complete abstinence for 120 days (MCV) or 15–40 days (GGT) [31]. Abusers were also screened through analysis of serum carbohydrate-deficient transferrin (CDT; asialo- plus monosialo- plus disialo-Fe2-transferrin), a traditional biomarker of alcohol abuse that normalizes after 15–30 days of abstinence [31]. CDT analysis was performed on P/ACE MDQ capillary electrophoresis systems (Beckman Coulter) with UV detection at 200 nm (interference filter) and valley to valley integration performed with 32 Karat software (Beckman Coulter). Data were evaluated on the basis of corrected peak areas (peak area divided by detection time). The amounts of single Tf isoforms and CDT (sum of asialo- and disialo-Tf) were calculated as area % in relation to the sum of the corrected peak areas of all detected Tf isoforms.

Genotype analyses

After DNA isolation with a Promega Wizard genomic DNA purification kit (Promega, Italy), was available for genotype analysis for 149 alcohol abuser and 255 controls. Determination of the *ADH1C *1/*2* (rs 698) Ile350Val in the exon 8 polymorphism was performed following the method based on restriction fragment length polymorphism

previously described [32]. We used primers, identified as 321 and 351, that allow for exclusive amplification of exon 8 of the *ADH1C* gene and generate the Ssp I recognition sequence AATATT as an internal control outside of the tested region. Thus, it is possible to distinguish between the *ADH1C*1* allele with fragments (67 and 63 bp) and the *ADH1C*2* allele with a 130 bp fragment. Alcohol dehydrogenase β subunit (*ADH1B)* polymorphism **1/*2* (rs1229984 Arg47His) and aldehyde dehydrogenase 2 (*ALDH2*) (rs 671 Glu487Lys) were genotyped following the method of Tamakoshi et al. [33] by a duplex polymerase chain reaction (PCR). This method confronting two-pair primers (PCR–CTPP) allows for DNA amplification with one-tube PCR including eight primers, and subsequent electrophoresis in 2% agarose gel. *ADH1B* His and Arg alleles was determined by presence of 280 bp and 219 bp fragments. *ALDH2* Glu and Lys alleles by presence of 119 bp and 98 bp fragments. Quality-control measures were adopted in genotyping, such as validation of results by using the TaqMan-based Real-Time PCR method and blind repeat of 10% of samples.

TL measurement

TL was measured in blood genomic DNA using the multiplex real-time quantitative PCR method described by Cawthon [34]. This method is a modification of previous TL analysis real-time PCR methods that allows for increased reproducibility [34]. This methodmeasures the relative telomere length in genomic DNA by determiningthe ratio of telomere repeat copy number (T) to single copy gene (S)copy number (T/S ratio) in experimental samples relative to a reference pool sample [34]. The single copy gene used in this study was humanβ-globin (*hb*g). The analysis was conducted using a CFX384 real-time PCR detection system (Bio-Rad, Hercules, California, USA). A high-precision MICROLAB STARlet Robot (Hamilton Life Science Robotics, Bonaduz AG, Switzerland) was used for transferring in a 384-well format plate a volume of 5μl reaction mix and 2μl DNA (3 ng/μl). A six points standard curve generated from serially diluted of a pool DNA ranging from 90 ng to 0.37 ng, was inserted in every 384-well plate in this study.

For multiplex real-time PCR we used the primer sets previously described by Cawthon [34]. A primer pair of beta-globin single copy gene (hbgu and hbgd) were combined with the telomere primer pair (telg and telc) in the same reaction mix. The multiplex PCR mix was: iQ SYBR Green Supermix (Bio-Rad) 1×, telg 600nM, telc 600nM, hbgu 250nM, hbgd 250nM, H2O. The thermal cycling profile started with a 95°C incubation for 3 minutes to activate the hot-start iTaq DNA polymerase, then 2 cycles of 15 s at 94°C, 15 s at 49°C, and 32 cycles of 15 s at 94°C, 10 s at 62°C, 10 s at 74°C with signal acquisition, 10 s at 84°C, 10 s at 88°C with signal acquisition At the end of each real-time PCR reaction to verify the specificity of amplified, a melting curve was added from 72°C to 95°C with an increment 0.5°C per step.

All samples were run in triplicate and the average of the three T/S ratio measurements was used in the statistical analyses. To examine the reproducibility of T/S measurement, we repeated the assay for 20 samples in two different days. The between-day coefficient of variation was 3.0%.

Statistical analysis

Statistical comparisons were made between the abusers and controls using the non-parametric Mann-Whitney *U*-test or the Fisher's exact test. Bivariate linear regression was used to assess the influence of age, BMI, vegetable intake, smoking, genotoxic exposure from diet, and jobs with elevated risk of accident on TL (dependent variable) among controls. The dependent variable was always TL which was log-transformed before the analysis to approximate normal distribution. We then classified individuals in usual drink

categories according to frequency of usual alcohol intake (0–1, 2–4, >4 drink-units/day). We examined the relation between TL (dependent variable) and usual drink category (0–1, 2–4, >4) by unadjusted and covariate-adjusted multivariable models to test the effects of possible TL determinants (i.e., age, smoking, BMI, diet, job with elevated risk of accident, genotoxic exposures) on TL and obtained unadjusted and adjusted TL means and 95% Confidence Intervals (CIs). TL of all subjects was log-transformed to approximate normal distribution. Consequently, we present TL data as geometric means and 95% Confidence Intervals (CIs). As alcohol abusers and controls differed in their distribution by age (years), BMI (kg/m^2) and vegetables (servings/week), current smoking (ever/ex or never) and jobs with elevated risk of accident (yes/not), we used multivariable regression models adjusting for all these variables. Age, BMI and vegetables, were fitted as a continuous variables. Current smoking and jobs with elevated risk of accident as categorical variables. To determine whether the genotypes were in Hardy-Weinberg equilibrium, distribution of the observed and expected genotype frequencies were compared using a chi-square test. The interaction terms for drinks x genotype were tested in a multiple linear regression model where the dependent variable was TL and the independent variables were drink-units and genotype, both dichotomously coded (<4 and ≥4 drink- units, 0 and 1; *ADH1B*1/*2* or **2/*2* and *ADH1C*1/*1*, 0*; ADH1B*1/*1 ADH1C*1/*2* or **2/*2*, 1), and the interaction term, which was the product of the first two variables. Statistical significance for the interaction term was tested using a Wald test. Statistical tests were two-sided, and were performed in Stata 9.0 (Stata Corp., College Station, TX).

RESULTS

Study population characteristics and alcohol consumption

The study population included 200 alcohol abusers and 257 controls (Table 1). In average, alcohol abusers were younger than controls (38 vs. 44 years, P<0.001), had moderately lower BMI (25.5 vs 26.0 Kg/m^2, P=0.019), included a higher proportion of current smokers (71% vs. 25%, P<0.001), and were less frequently in jobs at elevated risk of accident (23% vs. 36%, P=0.004). Surprisingly, alcohol abusers reported more frequent vegetable consumption of vegetables than controls (38% vs. 28% reported eating 7 or more servings of vegetables/week, P=0.014). Thirteen percent of the alcohol abusers reported a current consumption (intake since the beginning of the probation program) of more than 4 drink-units/day of alcohol, compared to 2% of the controls (P<0.001). Conversely, 55% of the alcohol abusers reported a usual consumption of 0–1 drink-units/day, compared to 71% of the controls (P<0.001). No correlation between smoking and alcohol was observed in abusers (Spearman's rank correlation Rho=0.072; p=0.308) while the correlation was significant in controls (Spearman's rank correlation Rho=0.161; p=0.0099). MCV, i.e. the conventional biomarker of alcohol abuse with longer half-life, was more frequently above the clinical reference value (≥96 U/L) in abusers (11%) than controls (5%; P=0.021). The proportion of individuals with levels of GGT, i.e., the biomarker with shorter half-life, above the clinical reference value (≥65 U/L) was not significantly higher in abusers (10%) relative to controls (6%). Abusers were further profiled through serum CDT analysis, a short-lived biomarker alcohol abuse. Only four (7%) of the abusers were found positive for CDT.

Telomere length in alcohol abusers and controls

PBL-TL was nearly halved in alcohol abusers (Table 2, geometric mean [GM] 0.43 T/S; range: 0.20–1.11) compared to controls (GM 0.87 T/S; range: 0.30–4.84; P<0.0001). The TL difference was also significant between the 59 nonsmoking alcohol abusers and 192 nonsmoking controls (GMs 0.40 T/S vs. 0.79 T/S; z=8.98, p<0.0001), as well as between the 141 smoking alcohol abusers and 65smoking controls (GMs 0.44 T/S vs. 1.12 T/S z=8.98,

p<0.0001). In bivariate linear regression analysis, TL of abusers (n=200) was inversely associated with age (p=0.006) and moderately positively associated with smoking (p=0.06), but not with BMI, vegetable intake, genotoxic exposure from diet, and jobs with elevated risk. In abusers the correlations between the integrated measure of drinking (drink-years) and smoking (pack-years) on PBL-TL showed that drink-years were moderately associated (p<0.07) with TL shortening, while pack-years were positively associated with TL (p=0.018). TL of controls (n=257) was also positively associated with smoking (cigarettes/day) (p=0.005), but not with age, BMI, vegetable intake, smoking, genotoxic exposure from diet, and jobs with elevated risk. GMs adjusted by age, BMI, current smoking, vegetables, and job at elevated risk of accident were 0.42 (0.19–1.10) in alcohol abusers and 0.87 (0.29–4.86) in controls (p<0.001). Fifty-nine (30%) of the alcohol abuser had TL lower than the 5th percentile (0.38 T/S) of the controls (unadjusted P<0.0001; adjusted P=0.0005) [Table 2]. In the entire study population, PBL-TL decreased in relation with increasing alcohol drinking [Table 3]. Tests for trend for shortened TL across all drink categories were statistically significant in both unadjusted (P-trend=0.004) and adjusted (P-trend=0.003) analyses. In particular, subjects drinking >4 drink-units/day had substantially shorter TL (unadjusted GM 0.48 vs. 0.63 T/S, P=0.004; adjusted GM 0.48 vs. 0.61 T/S, P=0.002). PBL-TL did not show significant differences between light (0–1 drink-units/day) and moderate (2–4 drink-units/day) alcohol drinkers (Table 3). However, TL was not associated with usual drinking categories when abusers and controls were evaluated separately (Table 3).

Telomere length and alcohol metabolic polymorphisms

ADH1B and *ADH1C* frequencies were Hardy Weinberg equilibrium (Table 4) and in line with those found in Caucasians by others authors in a larger Caucasian population [28]. As expected, none of the subjects carried the *ALDH2* rs671 polymorphism, which is very rare among Caucasian subjects. Carriers of the *ADH1B*2* (rs1229984) [*Arg/His* or *His/His*] allele were protected from being abusers [OR=0.28; 95% CI 0.14–0.55; Table 4], drank less drink-units/day and showed longer TL (especially among controls and all subjects) (Table 5). Conversely carriers of the *ADH1B*1* genotypes were more likely to be abusers, drank more units and showed shorter TL in the entire study population and among controls (Table 5). Statistical test for the interaction term drink x genotype *ADH1B* was significant among controls, as well as in all subjects (P<0.0001), and borderline significant (P=0.054) among abusers. The rs698 *ADH1C* were neither associated with TL nor with the number of drink-units, and did not show a statistical interaction with alcohol abuse in determining TL.

DISCUSSION

In the present work, we found that alcohol abusers had significant shorter TL in PBLs compared to controls, taking into account several other potential determinants of telomere shortening. PBL-TL decreased with the amount of drinking when all study participants were considered together, particularly in subjects drinking >4 drink-units/day.

TL is widely considered a clock of biological age at the cellular level. Epidemiology studies have shown that among subjects of the same age, those with shorter telomeres in PBLs have higher risk of age-related diseases, such as cancer [6–14]. Our results show that abuse in alcohol drinking is associated with shortened telomeres suggesting a premature aging at the cellular level as reflected in telomere shortening in PBLs. Drinking more than 4 units of drinks/day of wine, has been clearly established as an independent risk factor for cancer [35] while light alcohol drinking was not associated with cancer risk [35]. In our study, we found shorter telomeres particularly among those individuals who consumed heavy amounts of alcohol (e.g., more than 4 drinks/day) when data from all participants were evaluated together. To the best of our knowledge this is the first study relating shorter telomeres to abuse in alcohol drinking. Other studies, that examined the relation between alcohol

drinking and TL in cancer patients [36,37] or in older individuals [38], have found no [36,38] or marginal [37] associations between alcohol intake and TL. However, the influence of alcohol drinking on TL in abusers, who are individuals with harmful and unhealthy heavy irregular pattern of drinking, has never been evaluated [39]. Previous studies have suggested that telomeres are shortened by other risk factors for age-related diseases, such as phsychological stress [16], smoking [36,40,41], obesity [40], chronic inflammation [18,19], male gender [6,14], and exposure to particulate air pollution [42] and PAHs [43]. Chances that shorter TL could depend on factors other than the variables of concern were minimized in the present study because the study participants were all males and of similar ethnicity. In addition, we used multivariable models to adjust for potential confounding factors, including age, BMI, current smoking, vegetables, job at elevated risk of accident, possible genotoxic exposures from diet, as well as from indoor pollutants. No subjects were affected by chronic diseases, including liver cirrhosis [44]. In alcohol abusers or controls separately, however no associations between units of drink and TL were found. Taken together, these results suggest that the condition of being an alcohol abuser, rather than the amount of drinking, is associated with shorter TL. Irregular patterns of alcohol drinking, such as those found among alcohol abusers, have been linked with increased cancer risk [35] as opposed to the favorable effects of moderate and regular alcohol consumption. It is possible, however, that psychological stress [16], as well as other conditions or lifestyle factors associated to being an alcohol abuser or driving under influence might have contributed in the reduction of PBL-TL that we observed in alcohol abusers.

In our study we found a statistically significant association between age and reduction in PBL-TL of alcohol abusers but not in controls. Loss in telomere length is most pronounced in childhood and old age, with a more gradual attrition in mid-life [5]. We have analyzed TL in controls that presented a limited age range (25–62 years) compared to that of abusers (35–75 years). This could limit our capacity to identify an association between age and TL in controls. Study subjects of similar age generally display a large variation in telomere length. Thus, a wider age range, as well as a larger sample size, might be necessary to detect a significant correlation between telomere length and age in healthy subjects such as those in our control group.

Moreover, in our study we found that smoking significantly increases PBL-TL both in abusers and controls. Several studies [8,17,42,45,46], including large investigations such as that conducted by Bischoff et al. [45] and Cassidy et al., 2010 [46], were unable to confirm the negative correlation between PBL-TL and smoking found by others [36,40,41]. The inconsistent results likely reflect a moderate effect of smoking, if any, on PBL-TL that might not be easily detectable. Experimental in-vitro models have shown that during inflammation, which is a central process in mediating health effects from smoking exposure [47], telomere length increase in younger inflammatory T cells [48]. Increased TL we found in current smokers could be attributed to the recruitment of younger inflammatory cells, who have longer TL, from the bone marrow in the bloodstream in response to inflammatory cues [48] such as those associated with smoking [47]. However, the association of alcohol abuse with attrition in PBL-TL in our study was independent of smoking, as demonstrated by analyses stratified by smoking status as well as by multivariable models.

Carriers of the *ADH1B*2* (rs1229984) allele were protected from being abusers, drank significantly less and showed longer TL than those with common wild-type homozygous genotype *ADH1B*1*. Conversely those with the *ADH1B*1* were more likely to be abusers, drank more and showed shorter TL. Alcohol is primarily metabolized by the ADH and ALDH2 enzymes. Among Caucasians, variants in *ADH* genes are common, whereas those in *ALDH2* are very rare. In particular, *ADH1B*2* codes for an enzyme 40-fold more active than

that encoded by the *ADH1B*1* allele, whereas *ADH1C*1* is only 2 or 3 times more active[25], and is associated with large production of acetaldehyde and a corresponding flush syndrome which deters carriers from drinking alcohol [26,27]. Our results are in line with previous studies that found an association between the *ADH1B*2* allele and protection against alcoholism. A large study that analyzed six *ADH* polymorphisms showed that *ADH1B*2* was the genotype that conferred the strongest protection against alcohol-related cancers [28]. Alcohol, if not quickly metabolized to acetaldehyde, may be alternatively metabolized by CYP2E1, the main hepatic alcohol-inducible cytochrome, which is capable of producing large amounts of radical oxygen species (ROS) [49]. The induction of CYP2E1 by methanol increases ROS formation (large amounts of $O2^{-}$ and $H2O2^{\cdot}$ reduced) through the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [23]. Moreover nitric oxide synthase is also induced by alcohol leading to the formation of nitric oxide and highly-reactive peroxynitrite ($ONOO^{-}$) [50]. Lastly, heavy drinking episodes were associated with an unfavourable lipid profile (decrease in high density lipoproteins, increased in low density lipoproteins) that contributes to generate ROS [39]. Through all these pathways, alcohol may create an oxidative microenvironment, suitable for generating specific telomeric damaging agents and, in general, conditions suitable for the development of related pathologies with such as cancer [23]. Telomeres, as triple-guanine-containing sequences, are highly sensitive targets for damage by oxidative stress [15]. It could be hypothesized that high levels of 8-hydroxy-2′-deoxyguanosine (8-OH-dG) the more abundant species of the altered DNA bases when DNA is oxidatively modified by ROS) may be formed from metabolism of abuse in alcohol drinking. Damaging 8-OH-dG formation can induce a reduction in TL by double-strand breaks and/or interference with replication fork [15].

In conclusion, our results show that abuse in alcohol drinking is associated with shortened telomeres which suggests a premature aging at the cellular level as reflected in telomere shortening in PBLs. *ADH1B*1* carriers exhibited higher frequency of alcohol abuse, drunk more and present shorter telomeres. Thus, individuals with shorter telomere could be at higher risk of an earlier onset of cancer and this risk may be modulated by the alcohol metabolic *ADH1B*1/*2* genotype. Our findings provide additional biological knowledge that could be used by clinicians to deter subjects from a maladaptative pattern of alcohol use. Future studies are warranted to further determine the mechanisms linking telomere shortening to the risk of alcohol-related cancers.

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Abbreviations

ALT	alanine aminotrasferase
AST	aspartate aminotrasferase
CDT	carbohydrate-deficient transferring
GGT	serum γ-glutamyltrasferase

MCV	mean corpuscular volume of erythrocytes
PBLs	peripheral blood leucocytes
PAHs	polycyclic aromatic hydrocarbons
TL	telomere length

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Table 1

Characteristics of the study population [a].

	Alcohol abusers (N[b]=200)	Controls (N=257)	P value[e]
Age (years)			
Mean (Range)	38(35–75)	44(25–62)	<0.0001
Body mass index (kg/m^2)			
Mean (Range)	25.5(18.9–45.0)	26.0(19.8–38.0)	0.019
Current smoking habits			
Smokers N (%)	141(71%)	65(25%)	<0.0001
[c] Cigs/day Mean (Range)	17.5(0.14–60)	14.6(0.28–40)	0.097
Genotoxic exposure through diet (times/week)			
N(%) of subjects with intake of genotoxin-rich food > once a week	111(56)	168(65)	0.986
Vegetables (servings/week)			
Low (0–3) N(%)	69(35)	112(44)	
Medium (4–6) N(%)	54(27)	73(28)	0.014
High (7 and more) N(%)	77(38)	72(28)	
Exposure indoor pollutant			
Not exposed N(%)	135(68)	198(77)	
Low N(%)	58(29)	51(20)	0.490
High N(%)	7(4)	8(3)	
Subjects with jobs at elevated risk of accident			
N(%)	46(23)	92(36)	0.004
Indicators of alcohol intake and effects			
Usual drinks (drink-units/day)mean (range)	1.93(0.04–10)	1.31(0–5.4)	0.049
Usual drinking category (drink-units/day)			
N(%)0–1	110(55)	182(71)	
N(%)2–4	65(32)	70(27)	<0.0001
N(%)>4	25(13)	5(2)	
MCV (fL) (normal value: 80,0–96,0)			
Mean (Range)	89 (60–102)	89(62–106)	0.187
N(%)[d]≥96.0	22/181(11)	14/255(5)	0.021
GGT(U/L) (normal value: 3–65)			
Mean (Range)	34(5–320)	32(6–172)	0.364
N(%)[d]65	18/182(10)	19(6)	0.356

[a] All the study participants were Caucasian males.

[b] N, number.

[c] Cigs, cigarettes.

[d] Number after/indicate the numbers of subjects we have obtained the informations.

[e] Mann-Whitney U test and Fisher's exact test.

Table 2

Telomere length in alcohol abusers and controls.

	Alcohol abusers	Controls	Statistics	
	n=200	n=257	P value Unadjusted	P-value Adjusted†
Telomere length (T/S)				
Geometric mean (range)*	0.43(0.20–1.11)	0.87(0.30–4.84)	<0.0001	<0.0001
N (%) ≤ 5° percentile TL value of controls (0.38 T/S)	59 (30)	14(5)	<0.0001	0.0005

*Unadjusted geometric means. Geometric means adjusted by age, BMI, current smoking, vegetables, and job at elevated risk of accident were 0.42 in alcohol abusers and 0.87 in controls.

†Adjusted by age, BMI, current smoking, vegetables, job at elevated risk of accident

Table 3

Telomere length according to usual drinking categories

Usual drinking category	Telomere length, T/S			
	Geometric mean	(95% CI)	p-value	p-trend
All Subjects (n=457)				
Unadjusted				
0–1 drink-units/day	0.67	(0.62–0.71)	Ref.	
2–4 drink-units/day	0.63	(0.57–0.69)	0.33	
>4 drink-units/day	0.48	(0.39–0.59)	0.004	0.004
Adjusted*				
0–1 drink-units/day	0.67	(0.63–0.72)	Ref.	
2–4 drink-units/day	0.61	(0.56–0.68)	0.14	
>4 drink-units/day	0.48	(0.39–0.59)	0.002	0.003
Abusers (n=200)				
Unadjusted				
0–1 drink-units/day	0.44	(0.41–0.46)	Ref.	
2–4 drink-units/day	0.43	(0.40–0.46)	0.67	
>4 drink-units/day	0.42	(0.38–0.46)	0.41	0.48
Adjusted*				
0–1 drink-units/day	0.43	(0.41–0.45)	Ref.	
2–4 drink-units/day	0.43	(0.41–0.46)	0.91	
>4 drink-units/day	0.43	(0.39–0.48)	0.95	0.92
Controls (n=257)				
Unadjusted				
0–1 drink-units/day	0.86	(0.79–0.94)	Ref.	
2–4 drink-units/day	0.89	(0.77–1.02)	0.68	
>4 drink-units/day	0.97	(0.58–1.62)	0.66	0.58
Adjusted*				
0–1 drink-units/day	0.88	(0.81–0.96)	Ref.	

Usual drinking category	Telomere length, T/S			
	Geometric mean	(95% CI)	p-value	p-trend
2–4 drink-units/day	0.83	(0.73–0.96)	0.45	
>4 drink-units/day	0.88	(0.53–1.45)	0.99	0.55

* Adjusted by age, BMI, current smoking, vegetables, elevated risk of accident

Table 4

ADH1B, *ADH1C* and *ALDH2* frequencies and OR in abuse drinkers and controls

	Abuse drinkers		Controls		
Genotypes	**Observed number(%)**	**Expected number(%)[a]**	**Observed number(%)**	**Expected number(%)[a]**	**[b] OR(95% CIs)**
ADH1B (rs1229984)					
**1/*1*	136(91)	136(92)	191(75)	192(75)	Ref.
**1/*2*	13(9)	12(8)	61(24)	58(23)	0.28(0.14–0.55)**
**2/*2*			3(1)	4(2)	
ADH1C (rs698)					
**1/*1*	60(40)	61(41)	106(42)	103(41)	Ref.
**1/*2*	70(47)	69(46)	110(44)	116(46)	0.92(0.60–1.42)
**2/*2*	19(13)	20(13)	35(14)	32(13)	
ALDH2 (rs671)					
**1/*1*	149(100)	-	257(100)	-	ND
**1/*2*					
**2/*2*					

[a] According to Hardy –Weinberg

[b] Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) were calculated using the Fisher's Exact method

** P=0.008.

Table 5

users, controls and all subjects according to *ADH1B* (rs1229984) and *ADH1C* (rs698) genotypes.

hol abusers				Controls					All Subjects			
ts/day	Telomere length	Drink- genotype [c]		Drink- units/day	≥4 drink- units/day	Telomere length	Drink- genotype[c]		Drink- units/day	≥4 drink- units/day	Telomere length	Drink- genotype [c]
	GM [b] (range)	r P	N	Median (range)	N (%)	GM (range)	r P	N	Median (range)	N (%)	GM (range)	r P
	0.41 (0.23–0.86)	0.159	191	1.00 (0–5.40)	15 (8)	0.83 (0.32–4.85)	0.34	327	1 (0–10.0)	39 (12)	0.62 (0.23–4.85)	0.35
	0.44 (0.34–0.63)	0.054	64	0.90° (0–4.00)	2 (3)	1.01§ (0.30–4.29)	<0.001	77	0.86## (0–4.00)	2°° (3)	0.88§§ (0.30–4.29)	<0.0001
	0.42 (0.23–0.86)	0.04	145	1.00 (0–5.40)	10 (7)	0.90 (0.33–4.85)	0.02	234	1 (0–10.0)	25 (11)	0.67 (0.23–4.85)	0.01
	0.42 (0.24–0.78)	0.649	106	1.00 (0–5.29)	7 (7)	0.82 (0.30–4.06)	0.729	166	1 (0–9.00)	16 (10)	0.65 (0.24– 4.06)	0.793

ultiple linear regression model where the dependent variable was TL and the independent variables were drink-units and genotype, both
3*1/*2 or *2/*2 and ADH1C*1/*1, 0; ADH1B*1/*1 ADH1C*1/*2 or *2/*2, 1), and the interaction term, which was the product of the first two
lity by Wald test.