# Impact of alcohol consumption with polymorphisms in alcohol-metabolizing enzymes on pancreatic cancer risk in Japanese

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**The putative impact of alcohol on pancreatic cancer (PC) risk remains controversial. Here, we conducted a case-control study in Japanese to assess the impact of alcohol in conjunction with polymorphisms in alcohol-metabolizing enzymes. Cases were 160 patients with pancreatic cancer at Aichi Cancer Center, Nagoya, Japan. Two control groups of 800 age- and sex-matched non-cancer subjects each were independently selected. The impact of alcohol and polymorphisms in *aldehyde dehydrogenase 2* (*ALDH2*) Glu504Lys, *alcohol dehydrogenase* (*ADH*) *1B* His48Arg, and *ADH1C* Arg272Gln on PC risk was examined with multivariate analysis adjusted for potential confounders to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Results showed no independent impact of alcohol or genotype on PC risk except former drinking. To avoid reverse causation, former drinkers were excluded in further analyses. In the analysis of the combined effects of alcohol consumption and genotype, significant impact of alcohol was seen for those subjects with *ALDH2* Lys+ allele, *ADH1B* His/His, or *ADH1C* Arg/Arg (trend $P = 0.077$, 0.003, or 0.020, respectively), each of which is associated with a high concentration or rapid production of acetaldehyde. Analysis of genotype combinations showed that 'ever drinking' with both *ADH1B* His/His and *ALDH2* Lys + was the most potent risk factor for PC relative to 'never drinkers' with both *ADH1B* His/His and *ALDH2* Glu/Glu [OR (95% CI); 4.09 (1.30–12.85)]. These results indicate that alcohol has an impact on PC risk when the effects of alcohol consumption and metabolism are combined. Acetaldehyde may be involved in the mechanisms underlying PC development. (*Cancer Sci* 2009; 100: 296–302)**

The mortality of pancreatic cancer (PC) in Japan is increasing, and is now the sixth leading cause of cancer death. The age-adjusted incidence rates and mortality of PC are 9.1 and 8.4 for men and 5.3 and 4.9 for women, respectively.[1] Because of the difficulty in detecting this cancer in the early operable stage and lack of any curative treatment apart from complete surgical removal, 5-year relative survival rate is only 5.5%.[2] Epidemiological research of PC risk should therefore play an important role in both prevention and decreasing the number of PC deaths.

Lifestyle and other risk factors known to affect the incidence of PC include age, smoking, obesity, diabetes mellitus, chronic pancreatitis, and family history of PC.[3–7] The effect of alcohol consumption on risk has also been investigated in many case-control or cohort studies, but results have been inconsistent.[7–14] In many studies, the impact of alcohol disappeared after adjustment for potential confounders, particularly smoking habits,[12–14] while several groups found a significant impact of alcohol even after adjustment for confounders.[10,11] In our previous report, an impact of alcohol was seen only among former drinkers, and not among current drinkers.[7]

Alcohol is first oxidized to acetaldehyde by the alcohol dehydrogenase (ADH) enzymes, particularly ADH1B and ADH1C. Acetaldehyde is further oxidized to acetate by aldehyde dehydrogenase (ALDH) enzymes, to which ALDH2 is the major contributor. Encoding genes display polymorphisms that modulate individual differences in alcohol-oxidizing capability.[15,16] Regarding ADH1B His48Arg (rs1229984), the 48His allele represents a superactive subunit of ADH1B which has about a ×40 higher maximum velocity (Vmax) than the less active *ADH1B* Arg/Arg form of ADH1B.[15,16] The *ADH1C* 272Arg allele represents a superactive subunit of ADH1C which has a ×2–3 higher Vmax than the *ADH1C* 272Gln allele (rs1693482).[15,16] The *ADH1B* and *ADH1C* genes are located close together in the short arm of chromosome 4, and *ADH1B* His48Arg and *ADH1C* Arg272Gln polymorphisms are considered to be in linkage disequilibrium.[17–19] However, *ADH1B* does not necessarily predict the *ADH1C* locus among Japanese.[20] As for the *ALDH2* Glu504Lys polymorphism (rs671), the 504Lys allele encodes a catalytically inactive subunit.[15,16] Individuals with the *ALDH2* Glu/Lys genotype have only 6.25% of normal ALDH2 504Glu protein, indicating a dominant negative effect of *ALDH2* 504Lys.[21] The *ADH1B* 48His, *ADH1C* 272Arg, and *ALDH2* 504Lys alleles, associated with higher accumulation or rapid production of acetaldehyde, are clustered in Asian populations such as Japanese.[20,22–24] Therefore, these three genetic polymorphisms modify toxic acetaldehyde exposure and are expected to affect cancer risk, especially in Asian populations in whom minor alleles are common.

Here, we conducted an age- and sex-matched case-control study to explore the impact of alcohol consumption in conjunction with genetic polymorphisms in alcohol-metabolizing enzymes on PC risk among Japanese.

## Materials and Methods

**Study subjects.** Cases were 160 PC patients with no prior history of cancer who were diagnosed at Aichi Cancer Center Hospital (ACCH), Nagoya, Japan, between January 2001 and November 2005. To avoid spurious associations, we used two independent non-cancer control groups [control 1 (C1), $n = 800$; control 2 (C2), $n = 800$] to give an overall case : control ratio of 1:5. Sex- and age-matched (±2 years) C1 and C2 subjects were independently selected from outpatients who visited ACCH during the same period without a history of any cancer. When results from C1 and C2 were consistent, we pooled controls (C1 + C2) for analysis.

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All subjects were enrolled at first visit to ACCH in the hospital-based Epidemiological Research Program II at ACCH (HERPACC-II) between January 2001 and November 2005. The framework of HERPACC-II has been described elsewhere.[25,26] Briefly, all first-visit outpatients at ACCH aged 20–79 years were asked to fill out a self-administered questionnaire regarding lifestyle items before the development of current symptoms, which was then checked by trained interviewers. Outpatients were also asked to provide a 7-mL blood sample. Approximately 95% of eligible subjects completed the questionnaire and 50% provided blood samples. All data were loaded into the HERPACC database and linked periodically with the hospital cancer registry system to update the data on cancer incidence. Our previous study showed that the lifestyle patterns of first-visit outpatients were in accordance with those in a general population randomly selected from Nagoya, confirming external validity for the study.[27] This study was approved by the Ethics Committee of Aichi Cancer Center Institute. Informed consent was obtained at first visit from all participants.

**Genotyping of *ALDH2*, *ADH1B*, and *ADH1C*.** DNA of each sample was extracted from the buffy coat fraction using a BioRobot EZ1 with an EZ1 DNA blood 350 μL kit or QIAamp DNA blood mini kit (Qiagen K.K., Tokyo, Japan). Polymorphisms of alcohol-metabolizing enzymes *ALDH2* Glu504Lys, *ADH1B* His48Arg, and *ADH1C* Arg272Gln were examined based on TaqMan assays by Applied Biosystems (Foster City, CA, USA). The principle of the TaqMan real-time polymerase chain reaction (PCR) assay system using fluorogenic probes and 5′ nuclease has been described by Livak.[28] All of the assays were done in 96-well PCR plates using a 7500 Fast Real-Time PCR System (Applied Biosystems) coupled with the 7500 Fast System SDS software. Amplification reactions (5 μL) were done in duplicate with 30 ng of template DNA, 2× TaqMan Universal Master Mix buffer (Applied Biosystems), 20× primer and probe mix (Applied Biosystems). Thermal cycling was initiated with a first denaturation step of 20 s at 95°C, and then by 40 cycles of 3 s at 95°C and 30 s at 62°C. Genotyping quality was statistically assessed using the Hardy–Weinberg test in our laboratory; when allelic distributions for controls departed from the Hardy–Weinberg frequency, genotyping was assessed using another method.

**Assessment of exposure.** Daily alcohol consumption in grams was determined by summing the pure alcohol amount in the average daily consumption of Japanese sake (rice wine), shochu (distilled spirit), beer, wine and whiskey, with one cup of Japanese sake (180 mL) considered equivalent to 25 g of ethanol; one large bottle of beer (720 mL) to 25 g; one glass of wine (80 mL) to 10 g; and one shot of whiskey (28.5 mL) to 12.5 g. One drink of shochu, which contains 25% ethanol, was estimated at 108 mL and 27 g of ethanol. Cumulative smoking exposure was evaluated as pack-years, the product of the average number of packs per day and the number of years of smoking. Height and body weight at baseline and weight at age 20 years were self-reported. Current body mass index (BMI) and BMI at age 20 were calculated as current weight and weight at age 20 divided by height squared, respectively, and expressed as kg/m$^2$. Family history of pancreatic cancer was considered positive when at least one parent or sibling had a history of pancreatic cancer.

**Statistical analysis.** All statistical analyses were performed using Stata version 10 (Stata Corp., College Station, TX, US). A *P*-value <0.05 was considered statistically significant. Accordance with the Hardy–Weinberg equilibrium among controls was checked with the $\chi^2$-test to assess discrepancies between expected and observed genotype and allele frequencies. Differences in characteristics between cases and controls were compared with the $\chi^2$-test, and the Mann–Whitney test was used to compare age distribution between two groups. Frequency of alcohol consumption was categorized into the five levels of never, rare, 1–2 times, 3–4 times, and 5–7 times per week. Drinking status was categorized into the three groups of never drinkers, former drinkers, and current drinkers. Drinking experience was categorized into the two groups of never drinkers and ever drinkers. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using an unconditional logistic regression model adjusted for potential confounders. Potential confounders considered in multivariate analysis were age as a continuous variable, sex (male or female), pack-years of smoking (0, 1–20, 21–40, ≥41), current BMI (<20.0, 20.0–22.4, 22.5–24.9, 25–29.9, ≥30.0 kg/m$^2$), BMI at age 20 years (<20.0, 20.0–22.4, 22.5–24.9, 25–29.9, ≥30.0 kg/m$^2$), history of diabetes (yes or no), and family history of PC (yes or no). Among *ALDH2*, *ADH1B*, and *ADH1C* polymorphisms, the two polymorphisms other than that under evaluation were included as confounders when appropriate.

Trends in alcohol impact were assessed using a score test for average daily ethanol consumption, with scores of 0, none; 1, <30 g; and 2, ≥30 g. Gene–environmental interactions were assessed by the logistic model, which included interaction terms between ethanol consumption and genes with scores of 0, homozygote in major alleles; and 1, others.

## Results

Table 1 shows baseline characteristics of case subjects and the two independent control groups. Men accounted for 70.6% of all subjects. Findings were consistent across both control groups. A significant prevalence of more than 40 pack-years of smoking was seen among case subjects. A history of diabetes was also significantly common in cases as compared to each control group. A current BMI <22.5 was more prevalent in cases, whereas the distribution of BMI at 20 years did not significantly differ between cases and the two controls.

Distributions for alcohol-related characteristics and their adjusted ORs with 95% CIs for PC are shown in Table 2. A significantly increased risk of PC was seen in former drinkers [pooled controls: adjusted OR (95% CI), 4.71 (2.74–8.08)] but not in current drinkers [pooled controls: 1.18 (0.79–1.78)] relative to never drinkers. Adjusted ORs (95% CIs) for frequency of alcohol consumption per week relative to never drinkers among pooled controls were 1.37 (0.70–2.71) for less than once per week (rare drinkers), 0.93 (0.44–1.98) for 1–2 times, 1.99 (1.14–3.45) for 3–4 times, and 1.61 (1.04–2.49) for 5–7 times, showing an increase in OR with drinking frequency (trend $P = 0.026$). To further analyze the impact of alcohol on PC risk, we categorized drinkers into two groups according to average alcohol consumption per day, calculated as the product of pure alcohol consumption of reported alcoholic drinks per day and drinking frequency. We defined drinkers with an intake of <30 g alcohol/day as 'moderate' drinkers and those with an intake of 30 g or more as 'heavy' drinkers. Overall, an impact of alcohol consumption on PC risk was observed among heavy drinkers [pooled controls: adjusted ORs (95% CIs) for alcohol consumption: 1.44 (0.96–2.15) for moderate and 1.92 (1.14–3.21) for heavy drinkers relative to never drinkers], and PC risk increased with alcohol consumption (trend $P = 0.012$). However, if former drinkers were excluded, the impact of alcohol among heavy drinkers disappeared [pooled controls: adjusted OR (95% CI), HR 1.39 (0.79–2.45)].

Adjusted ORs (95% CIs) for PC by genotype distributions of *ALDH2*, *ADH1B*, and *ADH1C* genotype are shown in Table 3. The distribution of these three genotypes was in accordance with expected values according to the Hardy–Weinberg equilibrium. Adjusted ORs (95% CIs) of *ALDH2* Glu/Lys and Lys/Lys were 1.29 (0.91–1.81) and 0.65 (0.32–1.34) relative to *ALDH2* Glu/Glu. In addition to *ALDH2*, the *ADH1B* and *ADH1C* genotypes were also not found to be independent risk factors.

**Table 1. Characteristics of case and control subjects**

| | | Cases (%) ($n$ = 160) | Control 1 (%) | | Control 2 (%) | |
|---|---|---|---|---|---|---|
| | | | ($n$ = 800) | $P$-value | ($n$ = 800) | $P$-value |
| Age | Median (range) | 60 (28–78) | 60 (27–79) | 0.927 | 60 (26–79) | 0.881 |
| Sex | | | | | | |
| | Men | 113 (70.6) | 565 (70.6) | 1.000 | 565 (70.6) | 1.000 |
| | Women | 47 (29.4) | 235 (29.4) | | 235 (29.4) | |
| Pack-years of smoking | | | | | | |
| | 0 | 56 (35.0) | 340 (42.5) | 0.013 | 350 (43.8) | 0.009 |
| | 1–20 | 21 (13.1) | 132 (16.5) | | 125 (15.6) | |
| | 21–40 | 31 (19.4) | 155 (19.4) | | 152 (19.0) | |
| | ≥41 | 51 (31.9) | 159 (19.9) | | 157 (19.6) | |
| | Unknown | 1 (0.6) | 14 (1.8) | | 16 (2.0) | |
| History of diabetes | | | | | | |
| | No | 126 (78.8) | 734 (91.8) | <0.001 | 737 (92.1) | <0.001 |
| | Yes | 34 (21.3) | 66 (8.3) | | 63 (7.9) | |
| Current BMI (kg/m$^2$) | | | | | | |
| | <20.0 | 33 (20.6) | 118 (14.8) | 0.051 | 115 (14.4) | 0.075 |
| | 20.0–22.4 | 51 (31.9) | 215 (26.9) | | 207 (25.9) | |
| | 22.5–24.9 | 42 (26.3) | 265 (33.1) | | 274 (34.3) | |
| | 25.0–29.9 | 30 (18.8) | 188 (23.5) | | 179 (22.4) | |
| | ≥30.0 | 4 (2.5) | 8 (1.0) | | 18 (2.3) | |
| | unknown | 0 (0.0) | 6 (0.8) | | 7 (0.9) | |
| BMI at age 20 years (kg/m$^2$) | | | | | | |
| | <20.0 | 50 (31.3) | 264 (33.0) | 0.129 | 244 (30.5) | 0.109 |
| | 20.0–22.4 | 58 (36.3) | 330 (41.3) | | 368 (46.0) | |
| | 22.5–24.9 | 33 (20.6) | 146 (18.3) | | 130 (16.3) | |
| | 25.0–29.9 | 11 (6.9) | 38 (4.8) | | 38 (4.8) | |
| | ≥30.0 | 2 (1.3) | 1 (0.1) | | 3 (0.4) | |
| | Unknown | 6 (3.8) | 21 (2.6) | | 17 (2.1) | |
| Family history of pancreatic cancer | | | | | | |
| | No | 152 (95.0) | 770 (96.3) | 0.459 | 770 (96.3) | 0.459 |
| | Yes | 8 (5.0) | 30 (3.8) | | 30 (3.8) | |

**Table 2. Odds ratios (ORs) of pancreatic cancer by alcohol-related characteristics**

| | Cases | Pooled controls | | Control 1 | | Control 2 | |
|---|---|---|---|---|---|---|---|
| | $n$ | $n$ | ORs[†] (95% CI) | $n$ | ORs[†] (95% CI) | $n$ | ORs[†] (95% CI) |
| Drinking status | | | | | | | |
| Never drinkers | 47 | 602 | 1.00 (reference) | 304 | 1.00 (reference) | 298 | 1.00 (reference) |
| Former drinkers | 33 | 75 | 4.71 (2.74–8.08) | 43 | 4.29 (2.39–7.72) | 32 | 5.24 (2.84–9.64) |
| Current drinkers | 80 | 923 | 1.18 (0.79–1.78) | 453 | 1.25 (0.81–1.92) | 470 | 1.11 (0.73–1.70) |
| | | | Trend $P$ = 0.755 | | Trend $P$ = 0.572 | | Trend $P$ = 0.977 |
| Drinking frequency per week | | | | | | | |
| None | 47 | 602 | 1.00 (reference) | 304 | 1.00 (reference) | 298 | 1.00 (reference) |
| <1 time | 12 | 119 | 1.37 (0.70–2.71) | 62 | 1.39 (0.68–2.85) | 57 | 1.32 (0.64–2.70) |
| 1–2 times | 9 | 126 | 0.93 (0.44–1.98) | 69 | 0.87 (0.40–1.90) | 57 | 0.96 (0.43–2.13) |
| 3–4 times | 23 | 160 | 1.99 (1.14–3.45) | 70 | 2.28 (1.26–4.16) | 90 | 1.67 (0.93–2.98) |
| ≥5 times | 69 | 589 | 1.61 (1.04–2.49) | 295 | 1.69 (1.07–2.69) | 294 | 1.56 (0.99–2.45) |
| Unknown | 0 | 4 | NA[‡] | 0 | NA[‡] | 4 | NA[‡] |
| | | | Trend $P$ = 0.026 | | Trend $P$ = 0.013 | | Trend $P$ = 0.074 |
| Alcohol consumption per day | | | | | | | |
| 0 g | 47 | 602 | 1.00 (reference) | 304 | 1.00 (reference) | 298 | 1.00 (reference) |
| <30 g | 77 | 745 | 1.44 (0.96–2.15) | 371 | 1.50 (0.98–2.31) | 374 | 1.37 (0.90–2.08) |
| ≥30 g | 36 | 254 | 1.92 (1.14–3.21) | 125 | 1.99 (1.15–3.46) | 129 | 1.79 (1.04–3.08) |
| | | | Trend $P$ = 0.012 | | Trend $P$ = 0.012 | | Trend $P$ = 0.032 |

[†]ORs were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, and family history of pancreatic cancer.
[‡]NA indicates not available because of the absence of subjects in this category.

As shown in Tables 2 and 3, ORs and trends for alcohol-related characteristics and genotype distributions were consistent across the two control groups. We therefore pooled data for the two control groups in later analyses. In addition, to exclude possibility of reverse causation, former drinkers were excluded in these later analyses on the assumption that alcohol-related diseases due to long exposure of alcohol, such as alcoholic pancreatitis, might influence the reporting of drinking status.


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**Table 3. Odd ratios (ORs) of pancreatic cancer by genotype distribution of *ALDH2*, *ADH1B*, and *ADH1C* genotypes**

| | | Cases | Pooled controls | | Control 1 | | Control 2 | |
|---|---|---|---|---|---|---|---|---|
| | | *n* | *n* | ORs[†] (95% CI) | *n* | ORs[†] (95% CI) | *n* | ORs[†] (95% CI) |
| *ALDH2* | | | | | | | | |
| | Glu/Glu | 74 | 790 | 1.00 (reference) | 404 | 1.00 (reference) | 386 | 1.00 (reference) |
| | Glu/Lys | 77 | 653 | 1.29 (0.91–1.81) | 325 | 1.35 (0.94–1.94) | 328 | 1.22 (0.85–1.75) |
| | Lys/Lys | 9 | 157 | 0.65 (0.32–1.34) | 71 | 0.79 (0.37–1.67) | 86 | 0.55 (0.26–1.17) |
| *ADH1B* | | | | | | | | |
| | His/His | 101 | 975 | 1.00 (reference) | 482 | 1.00 (reference) | 493 | 1.00 (reference) |
| | His/Arg | 55 | 551 | 1.00 (0.70–1.43) | 274 | 0.99 (0.68–1.43) | 277 | 1.00 (0.69–1.45) |
| | Arg/Arg | 4 | 74 | 0.51 (0.18–1.45) | 44 | 0.47 (0.16–1.35) | 30 | 0.55 (0.18–1.63) |
| *ADH1C* | | | | | | | | |
| | Arg/Arg | 140 | 1428 | 1.00 (reference) | 711 | 1.00 (reference) | 717 | 1.00 (reference) |
| | Arg/Gln | 20 | 169 | 1.22 (0.73–2.01) | 86 | 1.20 (0.71–2.05) | 83 | 1.28 (0.75–2.19) |
| | Gln/Gln | 0 | 3 | NA[‡] | 3 | NA[‡] | 0 | NA[‡] |

[†]ORs were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, and family history of pancreatic cancer.
[‡]NA indicates not available because of the absence of subjects in this category.
*ALDH2*, aldehyde dehydrogenase 2; *ADH1B*, alcohol dehydrogenase 1B; *ADH1C*, alcohol dehydrogenase 1C.

**Table 4. Combined impact of alcohol consumption with *ALDH2*, *ADH1B*, or *ADH1C* genotypes on pancreatic cancer risk among case and control subjects, excluding former drinkers**

| Alcohol consumption/day | Cases *n* | Pooled controls *n* | Genotype ORs[†] | (95% CI) | *P*-value | Cases *n* | Pooled controls *n* | Genotype ORs[†] | (95% CI) | *P*-value | Interaction *P*[‡] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *ALDH2* | | | Glu/Glu (normal) | | | | | Lys+ (weak) | | | |
| 0 g | 8 | 143 | 1.00 | (reference) | | 39 | 459 | 1.67 | (0.75–3.75) | 0.211 | |
| <30 g | 27 | 423 | 1.35 | (0.58–3.18) | 0.485 | 28 | 268 | 2.26 | (0.93–5.46) | 0.072 | |
| ≥30 g | 18 | 184 | 1.98 | (0.77–5.13) | 0.159 | 7 | 48 | 3.27 | (1.03–10.44) | 0.045 | |
| | | | Trend $P = 0.284$ | | | | | Trend $P = 0.077$ | | | 0.920 |
| *ADH1B* | | | His/His (rapid) | | | | | Arg+ (slow) | | | |
| 0 g | 28 | 392 | 1.00 | (reference) | | 19 | 210 | 1.17 | (0.61–2.21) | 0.640 | |
| <30 g | 32 | 422 | 1.44 | (0.80–2.57) | 0.221 | 23 | 269 | 1.42 | (0.75–2.70) | 0.286 | |
| ≥30 g | 16 | 114 | 2.99 | (1.39–6.44) | 0.005 | 9 | 118 | 1.30 | (0.54–3.17) | 0.558 | |
| | | | Trend $P = 0.003$ | | | | | Trend $P = 0.722$ | | | 0.096 |
| *ADH1C* | | | Arg/Arg (rapid) | | | | | Gln + (slow) | | | |
| 0 g | 41 | 550 | 1.00 | (reference) | | 6 | 52 | 1.59 | (0.61–4.12) | 0.341 | |
| <30 g | 45 | 613 | 1.31 | (0.80–2.15) | 0.289 | 10 | 78 | 2.52 | (1.11–5.69) | 0.026 | |
| ≥30 g | 21 | 200 | 2.07 | (1.05–4.06) | 0.035 | 4 | 32 | 2.47 | (0.74–8.25) | 0.141 | |
| | | | Trend $P = 0.020$ | | | | | Trend $P = 0.644$ | | | 0.774 |

[†]Odd ratios (ORs) were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, family history of pancreatic cancer and nonevaluated two polymorphisms.
[‡]Interactions evaluated in the model included age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, family history of pancreatic cancer, alcohol consumption by score (none: 0, <30 g: 1 and ≥30 g: 2), *ALDH2*, *ADH1B*, or *ADH1C* by score (*ALDH2* Glu/Glu: 0, Lys+ : 1, *ADH1B* His/His: 0, Arg+ : 1 and *ADH1C* Arg/Arg:0, Gln+ : 1), and the cross-product of scores.
*ALDH2*, aldehyde dehydrogenase 2; *ADH1B*, alcohol dehydrogenase 1B; *ADH1C*, alcohol dehydrogenase 1C.

To assess the influence of alcohol and acetaldehyde metabolism in PC risk, we evaluated the impact of the combination of *ALDH2*, *ADH1B*, or *ADH1C* genotypes with daily alcohol consumption (Table 4). Overall, no impact of alcohol consumption on PC risk was observed among cases and controls. However, on combination of *ALDH2* genotype and alcohol consumption, adjusted ORs (95% CIs) of moderate and heavy drinkers with the *ALDH2* Glu/Glu or Lys + allele relative to never drinkers with *ALDH2* Glu/Glu were 1.35 (0.58–3.18) and 1.98 (0.77–5.13) for those with *ALDH2* Glu/Glu, and 2.26 (0.93–5.46) and 3.27 (1.03–10.44) for those with *ALDH2* Lys+, and alcohol consumption showed a borderline trend to increased PC risk in *ALDH2* Lys+ (*ALDH2* Glu/Glu, trend-$P = 0.284$, *ALDH2* Lys+, trend-$P = 0.077$). Among those with the *ADH1B* His/His genotype, adjusted ORs (95% CIs) for PC with alcohol consumption were 1.44 (0.80–2.57) for moderate and 2.99 (1.39–6.44) for heavy drinkers, compared with never drinkers, with this trend being significant (trend $P = 0.003$). In contrast, the trend was not significant among those with *ADH1B* Arg+ allele (trend $P = 0.722$). With regard to the *ADH1C* genotype, trends were similar to those for the *ADH1B* genotype (*ADH1C* Arg/Arg, trend $P = 0.020$, *ADH1C* Gln+, trend $P = 0.644$). Interaction of the *ADH1B* genotype with alcohol consumption was marginally significant, suggesting the existence of a gene–environment association between alcohol consumption and alcohol metabolizing enzymes (interaction $P = 0.096$).

The combined impact of *ALDH2* genotype with either *ADH1B* or *ADH1C* genotype and drinking experience on PC risk is further explored in Table 5. Adjusted ORs (95% CIs) of current drinkers with both *ADH1B* His/His and *ALDH2* Glu/Glu or Lys+, relative

**Table 5. Combined impact of *ALDH2* with *ADH1B* or *ADH1C* genotypes and drinking experience on pancreatic cancer risk among case and control subjects**

| | *ALDH2* Glu/Glu (normal) | | | *ALDH2* Lys+ (weak) | | |
|---|---|---|---|---|---|---|
| | Cases *n* | Pooled controls *n* | ORs[†] (95% CI) | Cases *n* | Pooled controls *n* | ORs[†] (95% CI) |
| *ADH1B* His/His (rapid) | | | | | | |
| Never drinkers | 4 | 94 | 1.00 (reference) | 24 | 298 | 2.09 (0.70–6.29) |
| Current drinkers | 24 | 367 | 1.78 (0.58–5.45) | 24 | 169 | 4.09 (1.30–12.85) |
| *ADH1B* Arg + (slow) | | | | | | |
| Never drinkers | 4 | 49 | 1.88 (0.44–7.97) | 15 | 161 | 2.21 (0.69–7.09) |
| Current drinkers | 21 | 240 | 2.15 (0.68–6.80) | 11 | 147 | 1.84 (0.54–6.33) |
| *ADH1C* Arg/Arg (rapid) | | | | | | |
| Never drinkers | 8 | 133 | 1.00 (reference) | 33 | 417 | 1.48 (0.65–3.36) |
| Current drinkers | 35 | 540 | 1.27 (0.55–2.95) | 31 | 273 | 2.29 (0.95–5.51) |
| *ADH1C* Gln + (slow) | | | | | | |
| Never drinkers | 0 | 10 | NA[‡] | 6 | 42 | 2.69 (0.84–8.58) |
| Current drinkers | 10 | 67 | 3.09 (1.07–8.90) | 4 | 43 | 2.07 (0.55–7.78) |

[†]Odds ratios (ORs) were adjusted for age, sex, pack-years of smoking, history of diabetes, current BMI, BMI at 20 years, family history of pancreatic cancer, and nonevaluated polymorphisms.
[‡]NA indicates not available because of the absence of subjects in this category.
*ALDH2*, aldehyde dehydrogenase 2; *ADH1B*, alcohol dehydrogenase 1B; *ADH1C*, alcohol dehydrogenase 1C.

to never drinkers with both *ADH1B* His/His and *ALDH2* Glu/Glu were 1.78 (0.58–5.45) and 4.09 (1.30–12.85), respectively. While adjusted ORs (95% CIs) of current drinkers with *ADH1B* Arg+ and *ALDH2* Glu/Glu or Lys+, relative to never drinkers with both *ADH1B* His/His and *ALDH2* Glu/Glu were 2.15 (0.68–6.80) and 1.84 (0.54–6.33), respectively. These findings show that the combination of *ADH1B* His/His with *ALDH2* Lys+ for current drinkers was the most potent risk factor for PC. With regard to combinations of *ADH1C* and *ALDH2* genotypes, PC risk among current drinkers with the combination of *ADH1C* Arg/Arg with *ALDH2* Lys+ allele relative to never drinkers with both *ADH1C* Arg/Arg and *ALDH2* Glu/Glu was marginally significant [adjusted ORs (95% CIs): 2.29 (0.95–5.51)]. The OR (95% CI) for current drinkers with the combination of *ADH1C* Gln+ with *ALDH2* Glu/Glu allele relative to never drinkers with both *ADH1C* Arg/Arg and *ALDH2* Glu/Glu was 3.09 (1.07–8.90).

## Discussion

Here, we found that the risk of PC was increased with alcohol consumption in subjects with the *ALDH2* Lys+ allele, or *ADH1B* His/His or *ADH*1 Arg/Arg genotypes, but not in those with the *ALDH2* Glu/Glu genotype, or *ADH1B* Arg+ or *ADH1C* Gln+ alleles. Combined analysis of *ALDH2* with the *ADH1B* or *ADH1C* genotypes demonstrated a significant impact of alcohol in subjects with both *ALDH2* Lys+ and *ADH1B* His/His relative to those with both *ALDH2* Glu/Glu and *ADH1B* His/His. To our knowledge, this is the first study to examine the combined significance of alcohol consumption and each of the *ADH1B*, *ADH1C*, and *ALDH2* genotypes in PC risk.

The carcinogenic effect of acetaldehyde in various types of cancer has been shown in experimental studies.[29,30] Given that the metabolisms of alcohol and acetaldehyde are strongly influenced by genetic polymorphisms of alcohol-metabolizing enzymes, namely *ALDH2* Glu504Lys, *ADH1B* His48Arg, and *ADH1C* Arg272Gln, evaluation of the effect of these polymorphisms on cancer risk in combination with alcohol consumption is worthwhile. Consistent with previous reports,[12–14] we found that an impact of alcohol consumption on PC risk was not recognized if genotypes of *ALDH2*, *ADH1B*, and *ADH1C* were not taken into consideration. However, stratification of analyses by the respective genotypes revealed a significant or marginally significant impact of alcohol consumption on PC risk among subjects with *ALDH2* Lys+, *ADH1B* His/His, and *ADH1C* Arg/Arg. These findings suggest that among populations in which any of these three genotypes is prevalent, the association between alcohol and PC may be null unless the genotype is included as a potential confounder.

ALDH2 of the 504Lys allele has been shown to be an inactive form exerting a dominant negative effect on alcohol-metabolizing activity *in vitro*.[15,16] Peng *et al.* validated this negative effect in human studies, showing that among subjects with the *ALDH2* Glu/Lys genotype, the peak in acetaldehyde blood concentration and area under the curve (AUC) for acetaldehyde after the intake of 0.5 g/kg ethanol were about 20 and 30 times higher than respective values in subjects with *ALDH2* Glu/Glu.[31] On this basis, pancreatic cells in individuals with the *ALDH2* Glu/Lys genotype would be exposed to a considerably larger amount of acetaldehyde after ingestion of alcohol. This striking difference in acetaldehyde metabolism would also explain why the impact of alcohol was observed only among subjects with the *ALDH2* Lys+ allele. In contrast, among subjects with *ALDH2* Glu/Glu, acetaldehyde peak and AUC were not statistically different between those with *ADH1B* His/His and *ADH1B* Arg/Arg,[31] although the *ADH1B* 48His allele represents a superactive subunit of ADH1B with a higher Vmax than the other allele.[15,16] Considering these previous and present results, we speculate that the rapid production and exposure of acetaldehyde within pancreatic cells exposed to alcohol influences the risk of PC with regard to *ADH1B* and *ADH1C*.

In the additional analyses of the combined impact of *ADH1B* or *ADH1C* with the *ALDH2* genotype, a particular increase in risk was seen among current drinkers who had both *ADH1B* His/His and *ALDH2* Lys+ or both *ADH1C* Arg/Arg and *ALDH2* Lys+. This finding supports our hypothesis that acetaldehyde may be involved in the underlying mechanisms of PC development. This analysis also demonstrated that PC risk among current drinkers with the combination of *ADH1C* Gln+ with the *ALDH2* Glu/Glu allele was significantly increased compared with never drinkers with both *ADH1C* Arg/Arg and *ALDH2* Glu/Glu, which is not consistent with our hypothesis. However, the number of control subjects in our study population with both *ADH1C* Gln+ and *ALDH2* Glu/Glu was too small to rule out chance association. Validation of these results will require studies in a larger number of subjects in other populations.


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In the present study, we separately assessed the impact of *ADH1B* and *ADH1C* on PC risk. Although several reports have found linkage disequilibrium between *ADH1B* rs1229984 and *ADH1C* rs1693482,[17–19] results from a recent large-scale study conducted in Europe strongly support the independent impact of these two loci on aerodigestive tract cancer risk, regardless of linkage disequilibrium.[32] This finding reflects those of our previous study on drinking behavior.[20] Reasonable assessment can therefore be carried out on the individual effects of *ADH1B* and *ADH1C*.

Chronic pancreatitis has been suggested as contributing to PC risk,[5] and several studies have shown that the *ADH1B* His + allele increases the risk of pancreatitis in heavy drinkers.[33,34] With regard to the mechanism of pancreatic cancer, acetaldehyde exposure may increase PC risk by inducing a state of chronic pancreatitis. Information regarding past history of pancreatitis may aid in further understanding the mechanisms underlying pancreatic cancer.

In a previous case-control study, Miyasaka *et al.* reported that although the *ALDH2* Lys$^+$-allele was found to be a risk factor for PC among subjects with both drinking and smoking habits, frequency of drinking habit did not differ significantly between patients and controls, regardless of presence or absence of *ALDH2* Glu/Lys polymorphisms.[35] These findings suggested possible effect modification by smoking in the impact of *ALDH2* polymorphism. However, in our analyses stratified by drinking and smoking, we did not observe this effect (data not shown). This inconsistency may have arisen by chance due to the small sample size of both studies, or may be attributed to residual confounding by smoking or other factors. Another explanation may be due to the selection of controls from different population bases, which might in turn affect allele frequencies as well as prevalence of drinking or smoking habit. Cases and controls in our study were all sampled from the same population base at Aichi Cancer Center. In contrast, the study by Miyasaka *et al*. investigated cases from National Kyushu Cancer Center and sampled controls from a comprehensive population-based longitudinal study conducted in rural areas in Aichi prefecture. Future studies should employ an appropriate study design, possibly a prospective one, with appropriate confounders and a sufficient number of subjects to sustain stratification by smoking and drinking as well as multiple genotypes.

With regard to the methodological background of our study, one important factor was selection of the control base population. We used non-cancer patients at the ACCH for this purpose on the basis that our subjects arose within this population, thereby warranting internal validity. We have previously confirmed the similarity of this population to the general population in terms of a range of exposures of interest, in this case alcohol consumption, thereby warranting external validity.[27] Further, genotype distribution of the *ALDH2*, *ADH1B*, and *ADH1C* polymorphisms in our controls was similar to that in the general population.[36] A second potential source of bias was the medical background of the controls. However, our previous study in women demonstrated that this had only limited impact: more than 66% of non-cancer outpatients at ACCH have no specific medical condition, while the remaining 34% have specific diseases such as benign tumors, non-neoplastic polyps or both (13.1%); mastitis (7.5%); gastrointestinal disease (4.1%); or benign gynecologic disease (4.1%).[37] The situation for men is comparable. Bias from this issue, if present, therefore appears limited. Furthermore, in contrast to standard hospital-based studies, the HERPACC system is less prone to information bias because all data are collected prior to diagnosis. Lastly, we did not apply an adjustment of multiple comparisons in the analysis because we have an *a priori* hypothesis in the present study. Therefore, our findings need to be interpreted cautiously.

In conclusion, alcohol intake has an impact on PC risk when alcohol consumption and genotype polymorphisms of alcohol-metabolizing enzymes are combined. Our finding that the impact of alcohol on PC risk was observed among individuals with *ALDH2* Lys+, *ADH1B* His/His, or *ADH1C* Arg/Arg, associated with a rapid production or high accumulation of acetaldehyde, indicates that acetaldehyde may play a substantial role in the underlying mechanism of PC.

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