Supporting Information

Direct sequencing of *ADH1B* and *ALDH2* genes

ADH1B rs1229984 and *ALDH2 rs671* polymorphisms were confirmed by direct sequencing. The primers were listed in **Supplemental Table 3**. The PCR was carried out with 50 ng/μL genomic DNA. The PCR condition was performed by initial denaturation at 95℃ for 10min, followed by 35 cycles for at 95℃ for 30 second, 60℃ for 30 second, 72℃ for 30 second and a final extension at 72℃ for 10 min. Sequencing reactions were custom performed by Genomics Biotechnology (Taiwan).

Supplemental Table 1 TaqMan probes used of *ALDH2* and *ADH1B* genes

Genes	**SNP**	**Assay ID**	**Polymorphism**	**Context Sequence [VIC/FAM]**
ALDH2	*rs671*	C_11703892_10	A/G	CGAGTACGGGCTGCAGGCATACACT **[A/G]** AAGTGAAAACTGTGAGTGTGGGACC
ADH1B	*rs1229984*	C_2688467_20	C/T, Transition Substitution	GCCACTAACCACGTGGTCATCTGTG **[C/T]** GACAGATTCCTACAGCCACCATCTA

Supplemental Table 2 Sequences of PCR primers

Genes	Primer name	Sequence (5'→ 3')	Product length (bp)
ALDH2	*ALDH2*-Forward	5'-TCCTGGGAGTGTAACCCATA-3'F	234
	ALDH2-Reverse	5'-CACCAGCAGACCCTCAAG-3'R	
ADH1B	*ADH1B*-Forward	5'-TCTGAATCTGAACAGCTTCTCTT-3'F	194
	ADH1B-Reverse	5'-TCCCTGAGTGTGAATCCTGT-3'R	

Supplemental Table 3 Allele frequencies of TWB control group consisted of 1:4 frequency-matching (TWB-1, N =600) and the residual TWB subjects (TWB-2, N=29,683)

Gene (SNP)	Genotypes	TWB-1 (N = 600)		TWB-2 (N = 29,683)	
		N (%)	HWE[1] *p-value*	N (%)	HWE[1] *p-value*
ADH1B rs1229984	***2/*2**(AA)	311(51.8)	0.1567	16,248(54.7)	0.2837
	***1/*2**(GA)	232(38.7)		11,378(38.3)	
	***1/*1**(GG)	57(9.5)		2,057(6.9)	
ALDH2 rs671	***1/*1**(GG)	341(56.8)	0.9139	15,213(51.3)	0.6796
	***1/*2**(GA)	222(37.0)		12,054(40.6)	
	***2/*2**(AA)	37(6.2)		2,416(8.1)	

[1]HWE, Hardy-Weinberg equilibrium**.**

Supplemental Table 4 Sensitivity analysis of 106 LAD patients and 424 controls from TWB1 with individual-matching under the restriction of age between 30 and 70 years old.

N (%)	LAD (N=106)	TWB-1 (N=424)	*p*-value[1]
Gender			*1.000*
Male	35 (33.0)	140 (33.0)	
Female	71 (67.0)	284 (67.0)	
Age in years			*0.912*
Mean ± SD	58.21±7.26	58.29±7.29	
Median	58.0	58.0	
IQR	54.0-65.0	54.0-65.0	
Education			*1.000*
≤ Elementary school	17 (16.0)	68 (16.0)	
Junior high school	11 (10.4)	44 (10.4)	
Senior high school	41 (38.7)	164 (38.7)	
≥ University	37 (34.9)	148 (34.9)	
Cigarette smoking			*0.819*
Non-smoker	87 (82.1)	358 (84.4)	
Former smoker	8 (7.5)	26 (6.1)	
Current smoker	11 (10.4)	40 (9.4)	
Alcohol drinking			*0.481*
Non-drinker	97 (91.5)	396 (93.4)	
Former drinker	2 (1.9)	11 (2.6)	
Current drinker	7 (6.6)	17 (4.0)	

Abbreviations: N, numbers; LAD, lung adenocarcinoma; TWB, Taiwan Biobank; IQR, interquartile range; SD, standard deviation.
[1]*p-values* were obtained using the Chi-square test or t-test

Supplemental Table 5 Sensitivity analysis of the effect of *ADH1B* and *ALDH2* genotypes of 106 lung adenocarcinoma patients and 424 controls in the conditional logistic regression models.

SNPs, N (%)	LAD (N=106)	TWB-1 (N=424)	Crude OR	95% CI		AOR[1]	95% CI		*P-value*[2]
				LL	UL		LL	UL	
ADH1B -rs1229984									
****2 (A)***	146 (68.9)	613 (72.3)	1			1			
****1 (G)***	66 (31.1)	235 (27.7)	1.17	0.84	1.64	1.18	0.84	1.65	0.344
ADH1B -rs1229984									
****2/*2(AA)***	48 (45.3)	225 (53.1)	1			1			
****1/*2(GA)***	50 (47.2)	163 (38.4)	1.37	0.87	2.17	1.34	0.85	2.12	0.212
****1/*1(GG)***	8 (7.5)	36 (8.5)	1.09	0.47	2.54	1.16	0.49	2.72	0.736
ADH1B -rs1229984									
****2/*2(AA)***	48 (45.3)	225 (53.1)	1			1			
****1/*2+*1/*1(GA +GG)***	58 (54.7)	199 (46.9)	1.33	0.85	2.06	1.31	0.84	2.04	0.230
ALDH2-rs671									
****1 (G)***	138 (65.1)	634 (74.8)	1			1			
****2 (A)***	74 (34.9)	214 (25.2)	**1.57**	**1.13**	**2.19**	**1.58**	**1.13**	**2.21**	**0.007****
ALDH2-rs671									
****1/*1(GG)***	47 (44.3)	238 (56.1)	1			1			
****1/*2(GA)***	44 (41.5)	158 (37.3)	1.40	0.87	2.25	1.33	0.82	2.16	0.242
****2/*2(AA)***	15 (14.2)	28 (6.6)	**2.90**	**1.39**	**6.07**	**3.19**	**1.51**	**6.75**	**0.002****
ALDH2-rs671									
****1/*1+*1/*2(GG+GA)***	91 (85.8)	396 (93.4)	1			1			
****2/*2 (AA)***	15 (14.2)	28 (6.6)	**2.42**	**1.22**	**4.81**	**2.75**	**1.36**	**5.54**	**0.005****

Abbreviations: *ADH1B, alcohol dehydrogenase 1B*; *ALDH2, aldehyde dehydrogenase 2 family member gene*; OR, odd ratio; AOR, adjusted OR; LL, lower limit of 95% CI; UL, upper limit of 95% CI.

[1]Adjusting for smoking status (former smoker *vs*. non-smoker; current smoker *vs*. non-smoker) and alcohol drinking (former drinker *vs*. non-drinker; current drinker *vs*. non-drinker).

[2]*p-values* were bolded under the 0.025 at the significant levels with Bonferroni adjustment

Supplemental Table 6 Characteristics of studies related with ***ALDH2*** **allele frequency** and lung cancer and lung adenocarcinoma

Author/Country/Year	Study design	Cancer type	Allele frequency (N, %)						Crude OR (95% CI)
			Cases			Controls			
			Total	*1	*2	Total	*1	*2	
Minegishi, Japan, 2007	HB Ca/Co	LC	1010	806 (79.80)	204 (25.31)	512	376 (73.44)	136 (36.17)	0.70 (0.55-0.90)
		LAD	660	526 (79.70)	134 (25.48)	512			0.70 (0.54-0.93)
Park, Japan, 2010	HB Ca/Co	LC	1,436	970 (67.55)	466 (48.04)	2,832	1,981 (69.95)	851 (42.96)	1.12 (0.98-1.28)
Shimizu, Japan, 2013	HB Ca/Co	LC	1,144	796 (69.58)	348 (43.72)	930	720 (77.42)	210 (29.17)	1.50 (1.23-1.83)
Our study	TWB-1[1] Ca/Co	LAD	300	198 (66.0)	102 (34.0)	1,200	904 (75.3)	296 (24.7)	**1.57 (1.20-2.07)**
	TWB-2[2] Ca/Co					59,366	42,480 (71.6)	16,886 (28.4)	**1.30 (1.02-1.65)**

Abbreviations: HB, hospital-based; Ca, case; Co, control; LC, lung cancer; LAD, lung adenocarcinoma.

[1]TWB-1 using 1:4 frequency-matching of age group (30-60; >60 years), sex, and education level (four levels: ≤ elementary school, junior high school, senior high school, and ≥ university) from the original 30,283 subjects of TWB dataset.

[2] TWB-2 consists of the remaining subjects from the original 30,283 participants in the TWB dataset, after the exclusion of the 600 subjects of TWB-1.

Supplemental Table 7 Allele frequencies of *ADH1B rs1229984* and *ALDH2 rs671* genotypes among various ethnic groups.

Regions	Populations	*ADH1B, rs1229984*			*ALDH2, rs671*			References
		N	*1 (%)	*2 (%)	N	*1 (%)	*2 (%)	
America	Mexican	97	100.0	0.0	101	99.5	0.5	[1]
South America	Colombian	148	91.55	8.45	148	100.0	0.0	[2]
Europe	Danish	1,188	99.2	0.80	1,188	99.96	0.04	[3]
South Asia	Indian	108	99.54	0.46	108	98.15	1.85	[4]
Southwest Asia	Turkish	81	99.38	0.62	81	100.0	0.0	[5]
Southeast Asia	Vietnamese	235	31.49	68.51	235	79.57	20.43	[6]
East Asia	Chinese	9,339	31.27	68.73	9,339	78.74	21.26	[7]
	Han Chinese in Beijing	206	29.13	70.87	206	83.98	16.02	[8,9]
	Southern Han Chinese	**210**	**24.29**	**75.71**	**210**	**72.86**	**27.14**	[8,9]
	Korean	796	24.81	75.19	796	83.79	16.21	[10]
	Japanese	914	22.05	77.95	1,935	76.54	23.46	[11]
	Taiwanese	**30,283**	**25.58**	**72.44**	**30,283**	**70.14**	**27.88**	**Our study**

Supplemental Table 8 Summarized the frequency of *ADH1B* and *ALDH2* genes in Taiwan population from literature review (focus on case control study, only shown the control groups)

References/Author/Year	Database sources	Total numbers (N)	Age (years ± SD)	Female (%)	*ADH1B, rs1229984* N (%)			HWE *P* value	*ALDH2, rs671* N (%)			HWE *P* value
					***2/*2**	***1/*2**	***1/*1**		***1/*1**	***1/*2**	***2/*2**	
[12] Chung, 2014	NTUH [3]	276	57.6±10.4	5.8	125 (47.9)	111 (42.5)	25 (9.6)	0.960	128 (49.0)	113 (43.3)	20 (7.7)	0.469
[13] Tsai, 2014	NCKUH [4]	514	53.7±0.4 (SE)	4.3	268 (52.1)	221 (43.0)	25 (4.9)	**0.015**	267 (51.9)	203 (39.5)	44 (8.6)	0.541
[14] Liu, 2016	REVEAL-HBV cohort [5]	3,213	30-65	41.2	1,748 (54.4)	1,229 (38.3)	236 (7.3)	0.325	1,617 (50.2)	1,354 (42.0)	250 (7.8)	0.149
[15] Wu, 2016	Population-based	267	45.2±12.0	NA	152 (56.9)	99 (37.1)	16 (6.0)	0.982	130 (47.8)	116 (42.6)	26 (9.6)	0.986
[16] Huang, 2017	NCKUH [4]	940	54.6±0.3 (SE)	5.3	484 (52.9)	381 (41.6)	50 (5.5)	**0.024**	447 (48.8)	386 (42.2)	82 (9.0)	0.918
[17] Chien, 2019	TGP [6]	427	40.0±17.7	44.3	235 (55)	166 (38.9)	26 (6.1)	0.642	215 (50.4)	172 (40.3)	40 (9.4)	0.512
[18] Huang, 2020	CGMH [7]	244	60±10.5	47.5	129 (52.9)	105 (43)	10 (4.1)	**0.044**	118 (48.4)	105 (43)	21 (8.6)	0.729
[19] Wu, 2021	TWB [8]	2,505	67.1±6.3	49.2	1,363 (54.4)	984 (39.3)	158 (6.3)	0.268	1,267 (50.6)	1,022 (40.8)	214 (8.5)	0.698
[20] Yang, 2021	TWB [8]	1,512	NA	NA	789 (52.2)	611 (40.4)	112 (7.4)	0.673	785 (52.1)	603 (40)	119 (7.9)	0.831
[21] Lin, 2021	TWB [8]	16,837	48.3±10.9	51.8	9,159 (54.4)	6,568 (39)	1,110 (6.6)	0.142	8,554 (50.8)	6,832 (40.6)	1,451 (8.6)	0.098
[22] Shan, 2021	NCKUH [4]	940	58.3±0.4 (SE)	59.0	502 (54.6)	334 (36.3)	83 (9)	**0.013**	450 (47.9)	407 (43.3)	83 (8.8)	0.506
Our study	TWB-1[1]	600	61.4±7.8	67.0	311 (51.8)	232 (38.7)	57 (9.5)	0.157	341 (56.8)	222 (37.0)	37 (6.2)	0.914
	TWB-2[2]	29,683	49.3±10.5	67.0	16,248 (54.7)	11,378 (38.8)	2,057 (6.9)	0.284	15,213 (51.3)	12054 (40.6)	2,416 (8.1)	0.680

Abbreviations: HWE, Hardy-Weinberg equilibrium; NA, not available.

[1]TWB-1 using 1:4 frequency-matching of age group (30-60; >60 years), sex, and education level (four levels: ≤ elementary school, junior high school, senior high school, and ≥ university) from the original 30,283 subjects of TWB dataset.

[2] TWB-2 consists of the remaining subjects from the original 30,283 participants in the TWB dataset, after the exclusion of the 600 subjects of TWB-1.

[3] NTUH=National Taiwan University Hospital.

[4] NCKUH=National Cheng Kung University Hospital.

[5] REVEAL-HBV=Associated Liver Disease/Cancer in HBV in Taiwan.

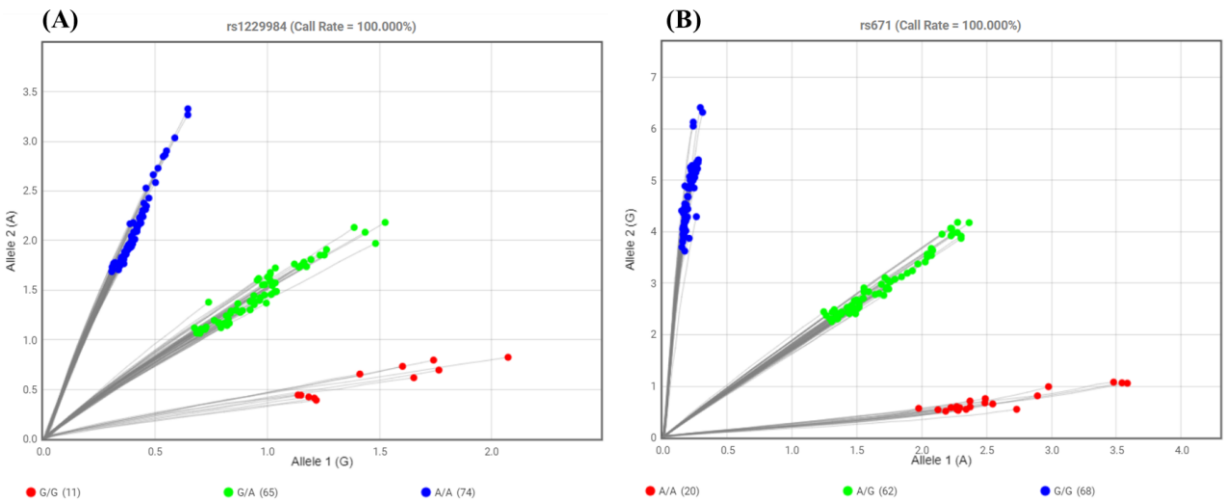
[6] TGP=Taiwanese General Population.

[7] CGMH=Chang Gung Memory Hospital.

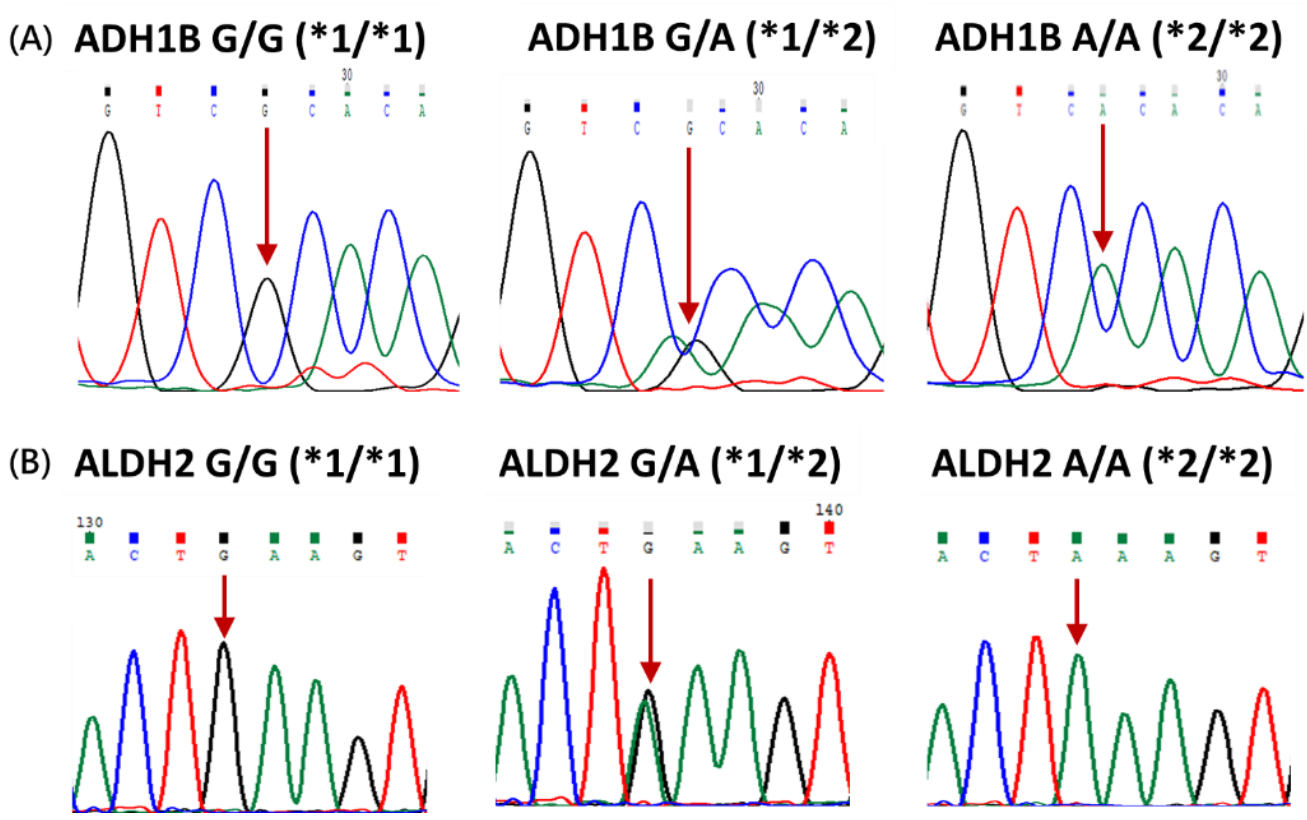
[8] TWB=Taiwan biobank.

Supplemental Table 9 Allele frequencies of *ADH1B* and *ALDH2* genotypes by age in 10-year range of the TWB cohort

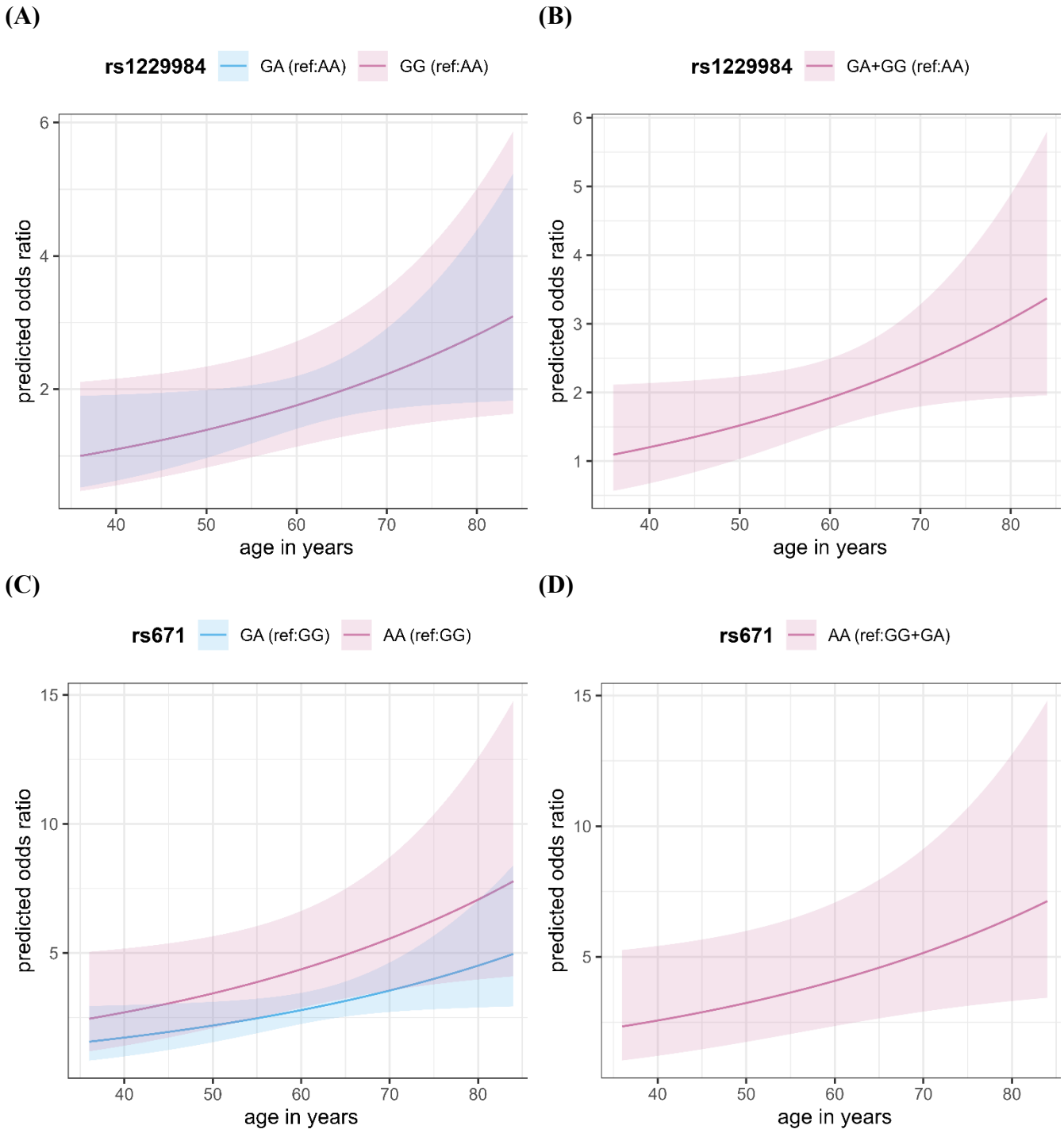
Age range (years)	Total numbers (N=86,322)	*ADH1B, rs1229984* N (%)			*ALDH2, rs671* N (%)		
		***2/*2**	***1/*2**	***1/*1**	***1/*1**	***1/*2**	***2/*2**
30-39	18,502	9,968 (53.88)	7,219 (39.02)	1,315 (7.11)	9,584 (51.80)	7,430 (40.16)	1,488 (8.04)
40-49	20,248	10,977 (54.21)	7,831 (38.68)	1,440 (7.11)	10,442 (51.57)	8,152 (40.26)	1,654 (8.17)
50-59	27,095	14,657 (54.09)	10,505 (38.77)	1,933 (7.13)	14,074 (51.94)	10,896 (40.21)	2,125 (7.84)
60-69	20,142	10,876 (54.00)	7,832 (38.88)	1,434 (7.12)	10,381 (51.54)	8,193 (40.68)	1,568 (7.78)
70-79	335	167 (49.85)	138 (41.19)	30 (8.96)	172 (51.34)	141 (42.09)	22 (6.57)



Supplemental Fig. 1. The allelic discrimination plots of TaqMan genotyping assays included 150 case patients. (A) *ADH1B rs1229984* (n = 150); (B) *ALDH2 rs671* (n = 150). Red and blue dots represent the homozygous genotypes and green circles represent the heterozygous genotypes. (*rs1229984**1/*1: homozygous for G/G; *rs1229984**1/*2: heterozygous for G/A; *rs1229984**2/*2: homozygous for A/A); *rs671* *1/*1: homozygous for G/G; *rs671**1/*2: heterozygous for A/G; *rs671**2/*2: homozygous for A/A).



Supplemental Fig. 2. The representative sequencing data of *ADH1B rs1229984* and *ALDH2 rs671*. (A) *ADH1B rs1229984* (*ADH1B*1/*1*: homozygous for G/G; *ADH1B*1/*2*: heterozygous for G/A; *ADH1B*2/*2*: homozygous for A/A); (B) *ALDH2 rs671* (*ALDH2*1/*1*: homozygous for G/G; *ALDH2*1/*2*: heterozygous for A/G; *ALDH2*2/*2*: homozygous for A/A).



Supplemental Fig. 3. Predicted odds ratios for *ADH1B rs1229984* and *ALDH2 rs671* across age in the 150 lung adenocarcinoma patients and 600 controls from TWB-1. (A-B) *ADH1B rs1229984*; (C-D) *ALDH2 rs671*.

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