Evidence for an Important Role of Alcohol- and Aldehyde-Metabolizing Genes in Cancers of the Upper Aerodigestive Tract

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Abstract

Background: **Incidence and mortality rates of upper aerodigestive tract cancers in Central Europe are among the highest in the world and have increased substantially in recent years. This increase is likely to be due to patterns of alcohol and tobacco consumption. Genetic susceptibility to upper aerodigestive tract cancer in relation to such exposures is an important aspect that should be investigated among populations in this region.**

Methods: **A multicenter case-control study comprising 811 upper aerodigestive tract cancer cases and 1,083 controls was conducted in: Bucharest (Romania), Lodz (Poland), Moscow (Russia), Banska Bystrika (Slovakia), and Olomouc and Prague (Czech Republic). We analyzed six SNPs in three genes related to ethanol metabolism: alcohol dehydrogenase 1B and 1C (*ADH1B*, *ADH1C*) and aldehyde dehydrogenase 2 (*ALDH2*).**

Results: **The *ADH1B* histidine allele at codon 48 was associated with a decreased risk of upper aerodigestive tract cancer; odds ratios (OR) were 0.36 [95% confidence interval (95% CI), 0.17-0.77] for medium/heavy drinkers and 0.57 (95% CI, 0.36-0.91) for never/light drinkers. Moderately increased risks were observed for the *ADH1C* ^{350}Val allele (OR, 1.19; 95% CI, 0.98-1.55) and *ADH1C* ^{272}Gln allele (OR, 1.24; 95% CI, 0.98-1.55). Medium/heavy drinkers who were heterozygous or homozygous at *ALDH2* nucleotide position 248 were at a significantly increased risk of upper aerodigestive tract cancer (OR, 1.76; 95% CI, 1.13-2.75; OR, 5.79; 95% CI, 1.49-22.5, respectively), with a significant dose response for carrying variant alleles (P = 0.0007). Similar results were observed for the *ALDH2* +82A>G and *ALDH2* −261C>T polymorphisms. When results were analyzed by subsite, strong main effects were observed for squamous cell carcinoma of the esophagus for all six variants. Among the 30% of the population who were carriers of at least one *ALDH2* variant, the attributable fraction among carriers (AF_c) was 24.2% (5.7-38.3%) for all upper aerodigestive tract cancers, increasing to 58.7% (41.2-71.0%) for esophageal cancer. Among carriers who drank alcohol at least thrice to four times a week, the AF_c for having at least one *ALDH2* variant was 49% (21.3-66.8%) for all upper aerodigestive tract cancers, increasing to 68.9% (42.9-83.1%) for esophageal cancer.**

Conclusions: **Polymorphisms in the *ADH1B* and *ALDH2* genes are associated with upper aerodigestive tract cancer in Central European populations and interact substantially with alcohol consumption.** (Cancer Epidemiol Biomarkers Prev 2006;15(4):696–703)

Introduction

Cancers of the upper aerodigestive tract, comprising the oral cavity, pharynx, larynx, and esophagus, are common cancers. Taken together, they account for 5.2% of all cancer cases worldwide and 6.4% of all cancer cases in Europe (1). Some of the highest incidence rates are seen in countries of Central and Eastern Europe, where increases of up to an order of magnitude have been reported within a generation (2, 3). Tobacco and alcohol represent important risk factors for these cancers, with evidence of a synergistic interaction (4, 5).

The mechanism for alcohol drinking as a risk factor of upper aerodigestive tract cancers is unclear. Alcohol may act as a solvent for tobacco carcinogens, although it is also possible that acetaldehyde, the metabolite of ethanol, is the primary carcinogen (6). If the latter hypothesis is correct, then one would expect an important role for polymorphisms of alcohol- and aldehyde-metabolizing genes, especially variants that result in greater exposure to acetaldehyde upon consumption of alcohol beverages. Alcohol dehydrogenases (ADH) are enzymes involved in the oxidation of ethanol to acetaldehyde (7). Subsequent oxidation of acetaldehyde to acetate is catalyzed by the enzyme aldehyde dehydrogenase (ALDH). The efficiency in converting ethanol to acetaldehyde, and subsequent conversion to acetate, is largely determined by the *ADH* and *ALDH* gene families, with large potential interindividual differences in acetaldehyde exposure due to the presence of some well-studied common genetic variants with a functional role (8-10). If differences in genetic susceptibility exist, then this could potentially lead to the identification of high-risk groups for upper aerodigestive tract cancer.

For the present study, we have focused on common variants in the *ADH* gene with a proven or likely functional role, namely single nucleotide polymorphisms (SNP) that result in amino acid changes in the encoded enzymes and are associated with altered alcohol metabolism. The functionally important polymorphic sites for *ADH1B* (previously *ADH2*) are Arg^{48}His in exon 3 and Arg^{370}Cys in exon 9 (10). Having a

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Table 1. Selected characteristics of squamous cell carcinoma cases and controls

	Oral, *n* (%)	Pharynx, *n* (%)	Larynx, *n* (%)	Esophagus, *n* (%)	UADT*, *n* (%)	Controls, *n* (%)
Total	168	113	326	176	811	1,083
Country						
Romania	33 (19.6)	7 (6.2)	56 (17.2)	39 (22.2)	142 (17.5)	173 (16.0)
Poland	9 (5.4)	2 (1.8)	178 (54.6)	17 (9.7)	206 (25.4)	209 (19.3)
Russia	106 (63.1)	96 (85.0)	80 (24.5)	62 (35.2)	365 (45.0)	319 (29.5)
Slovakia	20 (11.9)	8 (7.1)	12 (3.7)	0 (0.0)	40 (4.9)	84 (7.8)
Czech Republic	0 (0.0)	0 (0.0)	0 (0.0)	58 (33.0)	58 (7.2)	298 (27.5)
Age (y)						
<40	8 (4.8)	3 (2.7)	5 (1.5)	2 (1.1)	20 (2.5)	34 (3.1)
40-49	31 (18.5)	10 (8.8)	54 (16.6)	23 (13.1)	120 (14.8)	169 (15.6)
50-59	59 (35.1)	49 (43.4)	117 (35.9)	72 (40.9)	303 (37.4)	348 (32.1)
60-69	49 (29.2)	36 (31.9)	96 (29.4)	56 (31.8)	248 (30.6)	336 (31.0)
≥70	21 (12.5)	15 (13.3)	54 (16.6)	23 (13.1)	120 (14.8)	196 (18.1)
Sex						
Female	27 (16.1)	7 (6.2)	40 (12.3)	21 (11.9)	98 (12.1)	252 (23.3)
Male	141 (83.9)	106 (93.8)	286 (87.7)	155 (88.1)	713 (87.9)	831 (76.7)
Smoking status						
Never smoker	22 (13.1)	7 (6.2)	13 (4.0)	16 (9.1)	62 (7.6)	392 (36.2)
Past smoker	13 (7.7)	11 (9.7)	48 (14.7)	24 (13.6)	101 (12.5)	263 (24.3)
Current smoker	133 (79.2)	95 (84.1)	265 (81.3)	136 (77.3)	648 (79.9)	428 (39.5)
Pack-years of smoking						
Never smoker	22 (13.1)	7 (6.2)	13 (4.0)	16 (9.1)	62 (7.6)	392 (36.2)
1-9	6 (3.6)	5 (4.4)	8 (2.5)	10 (5.7)	31 (3.8)	117 (10.8)
10-19	25 (14.9)	15 (13.3)	31 (9.5)	29 (16.5)	105 (13.0)	132 (12.2)
20-29	29 (17.3)	27 (23.9)	57 (17.5)	38 (21.6)	157 (19.4)	167 (15.4)
30-39	43 (25.6)	27 (23.9)	84 (25.8)	40 (22.7)	201 (24.8)	122 (11.3)
≥40	43 (25.6)	32 (28.3)	131 (40.2)	39 (22.2)	249 (30.7)	149 (13.8)
Missing	0 (0.0)	0 (0.0)	2 (0.6)	4 (2.3)	6 (0.7)	4 (0.4)
Alcohol drinking, 1 y before interview						
No drinking	6 (3.6)	0 (0.0)	5 (1.5)	6 (3.4)	17 (2.1)	105 (9.7)
<1/mo	32 (19.0)	15 (13.3)	105 (32.2)	25 (14.2)	184 (22.7)	302 (27.9)
<1/wk	25 (14.9)	17 (15.0)	66 (20.2)	27 (15.3)	139 (17.1)	151 (13.9)
1-2/wk	38 (22.6)	38 (33.6)	67 (20.6)	48 (27.3)	196 (24.2)	218 (20.1)
3-5/wk	18 (10.7)	9 (8.0)	29 (8.9)	22 (12.5)	83 (10.2)	123 (11.4)
Daily	37 (22.0)	13 (11.5)	44 (13.5)	40 (22.7)	139 (17.1)	139 (12.8)
Missing	12 (7.1)	21 (18.6)	10 (3.1)	9 (5.1)	53 (6.5)	45 (4.2)

Abbreviation: UADT, upper aerodigestive tract.
*Upper aerodigestive tract cancers include 28 cases of oral and pharynx-unspecified cases.

histidine at amino acid position 48 constitutes the *2 allele and having a cysteine at amino acid position 370 constitutes the *3 allele (the *1 allele corresponds to the wild-type haplotype, which includes the common alleles at both SNPs). The functionally important polymorphic sites for *ADH1C* (previously *ADH3*) are $Ile^{350}Val$ and $Arg^{272}Gln$; having a valine at codon 350 and glutamine at codon 272 constitutes the *ADH1C**1 allele (8, 9). The *ADH1C**1 and *ADH1B**2 alleles encode for enzymes that result in the ''fast'' metabolism of ethanol. *In vitro* studies have shown that the *ADH1C**1 allele

Table 2. Genotype distribution among controls by alcohol drinking, tobacco smoking, and country (*n* = 1,083)

	ADH1B R48H		*ADH1C* I350V			*ALDH2* +348 C>T		
	Arg/Arg, *n* (%)	Any His, *n* (%)	Ile/Ile, *n* (%)	Ile/Val, *n* (%)	Val/Val, *n* (%)	T/T, *n* (%)	T/C, *n* (%)	C/C, *n* (%)
Alcohol drinking, 1 y before interview								
No drinking	91 (90.1)	10 (9.9)	32 (32.3)	52 (52.5)	15 (15.2)	73 (72.3)	23 (22.8)	5 (5.0)
<1/mo	272 (92.5)	22 (7.5)	98 (34.0)	136 (47.2)	54 (18.8)	202 (67.6)	86 (28.8)	11 (3.7)
<1/wk	133 (89.3)	16 (10.7)	55 (38.7)	64 (45.1)	23 (16.2)	98 (68.1)	44 (30.6)	2 (1.4)
1-2/wk	182 (87.5)	26 (12.5)	73 (36.9)	98 (49.5)	27 (13.6)	141 (66.2)	65 (30.5)	7 (3.3)
3-5/wk	105 (86.8)	16 (13.2)	41 (36.0)	58 (50.9)	15 (13.2)	86 (71.7)	32 (26.7)	2 (1.7)
Daily	119 (88.8)	15 (11.2)	47 (37.3)	61 (48.4)	18 (14.3)	103 (76.9)	30 (22.4)	1 (0.7)
Missing alcohol information	40	4	12	21	11	27	15	3
Missing genotype	32		72			27		
P for χ^2		0.4372			0.8959			0.3154
Pack-years of smoking								
Never smoker	348 (91.1)	34 (8.9)	134 (37.1)	162 (44.9)	65 (18.0)	273 (71.7)	99 (26.0)	9 (2.4)
1-9	100 (86.2)	16 (13.8)	39 (34.5)	55 (48.7)	19 (16.8)	77 (67.0)	34 (29.6)	4 (3.5)
10-19	110 (87.3)	16 (12.7)	38 (31.4)	61 (50.4)	22 (18.2)	9 (19.6)	32 (69.6)	5 (10.9)
20-29	141 (87.6)	20 (12.4)	62 (39.5)	74 (47.1)	21 (13.4)	110 (67.9)	46 (28.4)	6 (3.7)
30-39	108 (92.3)	9 (7.7)	41 (34.7)	64 (54.2)	13 (11.0)	74 (62.2)	41 (34.5)	4 (3.4)
≥40	131 (90.3)	14 (9.7)	42 (30.4)	73 (52.9)	23 (16.7)	101 (68.7)	43 (29.3)	3 (2.0)
Missing smoking information	4	0	2	1	0	4	0	0
Missing genotype	32		72			27		
P for χ^2		0.4435			0.5440			0.8121

NOTE: The *ADH1C* R272Q, *ALDH2* 82A>G, and *ALDH2* −261C>T genotypes were also not associated with the frequency of drinking per week or the pack-years of smoking.

increases ethanol oxidation by ~2.5-fold compared with *ADH1C**2, whereas the *ADH1B**2 and *ADH1B**3 allele increases ethanol oxidation by 40-fold and 90-fold compared with *ADH1B**1, respectively (11, 12). *ADH1B* and *ADH1C* are located at only 16 kb of distance on chromosome 4, and linkage disequilibrium between *ADH1C**1 and *ADH1B**2 has been shown in several populations (13). *ADH1B* has not been studied for upper aerodigestive tract cancer in European populations. Studies in Asian populations have been reported, which, although they were of small sample size, have consistently shown that the *ADH1B**1 allele is associated with an increased risk of esophageal cancer (14-17).

Studies in populations of European origins have focused on the gene *ADH1C*, although there has been little evidence of a strong effect on upper aerodigestive tract cancer risk (7). In a pooled analysis of seven published case-control studies, including 1,325 cases and 1,760 controls, no increased risk of head and neck cancer for the *ADH1C* valine homozygote at codon 350 or heterozygote genotypes were observed (7). Subsequent published studies on the *ADH1C* genotype have mostly reported null results, with studies in Brazil (18) and Iowa (19) reporting no overall association with head and neck cancer, and a study in Japan reporting no association with esophageal cancer (17). Peters et al. (20) reported an increased risk of laryngeal cancer for carrying the *ADH1C* Val^{350} allele, although no increase in overall head and neck cancer risk was observed.

The *ALDH2* gene, located on chromosome 12, contains a nearly inactive *ALDH2* $Gln^{487}Lys$ SNP (the Lys allele is also known as *ALDH2**2), resulting in homozygote carriers who are unable to oxidize acetaldehyde and heterozygote carriers who do so inefficiently (21). The *ALDH2**2 allele is frequently observed in Asian populations but nearly all Europeans are homozygous for the *ALDH2**1 allele (7). Studies in Japan have consistently reported an increased risk of oral, pharyngeal, laryngeal, and esophageal cancers linked to the *ALDH2**2 allele (14-17, 22-26), whereas one study in Thailand observed no overall association with esophageal cancer (27). Because this variant is almost absent in the European population, we have analyzed three variants, +82A>G, +348 C>T, and −261C>T, which have not been previously reported for any outcome.

Here, we report on a large study of upper aerodigestive tract cancer in Central Europe, with a particular focus on the joint roles of *ADH1B, ADH1C*, and *ALDH2* polymorphisms. Our primary hypothesis was that variants that may be responsible for fast metabolism of ethanol to acetaldehyde, and slow subsequent metabolism to acetate, would result in a higher

Table 3. *ADH/ALDH* genotypes and the risk of head and neck SCC stratified by alcohol drinking

	Overall			By times/wk of drinking*					
	Cases	Controls	OR† (95% CI)	Never/light drinkers (≤2 times/wk)			Medium/heavy (≥3 times/wk)		
				Cases	Controls	OR‡ (95% CI)	Cases	Controls	OR‡ (95% CI)
ADH1B R48H									
Arg/Arg (slow)	719	877	1.00	492	689	1.00	202	228	1.00
Arg/His + His/His (fast)	47	108	0.47 (0.32-0.70)	37	76	0.57 (0.36-0.91)	13	31	0.36 (0.17-0.77)
P for trend			0.0002			0.0186			0.0060
P for interaction									0.3342
ADH1C I350V									
Ile/Ile (slow)	243	331	1.00	151	261	1.00	82	91	1.00
Ile/Val	366	464	1.13 (0.89-1.43)	264	358	1.29 (0.96-1.72)	98	120	0.99 (0.63-1.53)
Val/Val (fast)	151	150	1.38 (1.01-1.88)	105	120	1.48 (1.02-2.15)	37	33	1.48 (0.80-2.73)
Ile/Val + Val/Val			1.19 (0.95-1.49)			1.33 (1.02-1.76)			1.09 (0.71-1.65)
P for trend			0.0480			0.0292			0.3169
P for interaction									0.5238
ADH1C R272Q									
Arg/Arg (slow)	241	350	1.00	150	277	1.00	82	95	1.00
Arg/Gln	352	453	1.16 (0.91-1.47)	254	348	1.32 (0.99-1.77)	93	119	0.99 (0.64-1.54)
Gln/Gln (fast)	144	139	1.49 (1.08-2.05)	102	111	1.69 (1.15-2.46)	33	30	1.42 (0.76-2.66)
Arg/Gln + Gln/Gln			1.24 (0.98-1.55)			1.41 (1.07-1.86)			1.08 (0.71-1.63)
P for trend			0.0161			0.0054			0.3911
P for interaction									0.2987
ALDH2 +82A>G									
A/A	450	673	1.00	312	507	1.00	127	189	1.00
A/G	266	286	1.25 (0.99-1.57)	181	237	1.13 (0.86-1.49)	72	67	1.57 (1.01-2.43)
G/G	38	30	1.63 (0.94-2.82)	24	24	1.70 (0.88-3.29)	15	4	4.38 (1.32-14.53)
A/G + G/G			1.29 (1.03-1.60)			1.18 (0.91-1.54)			1.75 (1.14-2.66)
P for trend			0.0153			0.1144			0.0031
P for interaction									0.0890
ALDH2 +348 C>T									
T/T	462	689	1.00	321	522	1.00	130	193	1.00
T/C	267	270	1.29 (1.03-1.62)	184	223	1.21 (0.92-1.58)	75	62	1.76 (1.13-2.75)
C/C	34	29	1.63 (0.92-2.89)	20	25	1.28 (0.65-2.55)	13	3	5.79 (1.49-22.52)
T/C + C/C			1.32 (1.06-1.65)			1.22 (0.94-1.58)			1.96 (1.27-3.01)
P for trend			0.0091			0.1501			0.0007
P for interaction									0.0328
ALDH2 −261C>T									
T/T	463	686	1.00	322	518	1.00	128	195	1.00
T/C	266	269	1.30 (1.03-1.63)	184	222	1.20 (0.92-1.58)	74	62	1.76 (1.13-2.74)
C/C	34	29	1.66 (0.93-2.95)	20	25	1.29 (0.65-2.58)	13	3	5.79 (1.49-22.49)
T/C + C/C			1.33 (1.07-1.66)			1.21 (0.93-1.57)			1.96 (1.27-3.01)
P for trend			0.0074			0.155			0.0006
P for interaction									0.0249

*The results showed similar patterns when stratified by milliliters of ethanol per day (<140 versus ≥140 g/wk) and by years of drinking (<20 versus ≥20 years).
†Adjusted for age, sex, country, pack-years of tobacco smoking, and years of alcohol drinking.
‡Adjusted for age, sex, country, and pack-years of tobacco smoking.

Table 4. *ADH/ALDH* genotypes and the risk of SCC of the oral cavity, pharynx, larynx, and esophagus

	Oral		Pharynx		Larynx		Esophagus	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
ADH1B R48H								
Arg/Arg (slow)	144/635	1.00	100/650	1.00	288/650	1.00	163/792	1.00
Arg/His + His/His	9/87	0.49 (0.23-1.04)	6/89	0.60 (0.24-1.51)	26/89	0.57 (0.34-0.96)	4/95	0.19 (0.07-0.53)
P for trend		0.0619		0.2807		0.0355		0.0016
ADH1C I350V								
Ile/Ile (slow)	49/248	1.00	25/256	1.00	124/256	1.00	42/293	1.00
Ile/Val	67/337	1.08 (0.70-1.66)	54/344	1.52 (0.88-2.63)	136/344	0.77 (0.55-1.08)	92/425	1.61 (1.07-2.43)
Val/Val (fast)	38/116	1.87 (1.10-3.17)	27/116	2.33 (1.21-4.50)	50/116	1.01 (0.64-1.60)	30/134	1.74 (1.02-2.98)
P for trend		0.034		0.0113		0.6620		0.0218
ADH1C R272Q								
Arg/Arg (slow)	48/257	1.00	26/267	1.00	122/267	1.00	42/308	1.00
Arg/Gln	67/332	1.12 (0.73-1.74)	53/337	1.42 (0.82-2.44)	128/337	0.80 (0.57-1.12)	88/415	1.62 (1.07-2.44)
Gln/Gln (fast)	35/109	1.88 (1.10-3.22)	26/109	2.26 (1.17-4.38)	48/109	1.12 (0.70-1.78)	30/122	2.03 (1.18-3.47)
P for trend		0.034		0.0163		0.9572		0.0056
ALDH2 +82A>G								
A/A	95/470	1.00	55/483	1.00	202/483	1.00	82/611	1.00
A/G	50/224	1.12 (0.75-1.67)	44/228	1.61 (1.00-2.57)	95/228	0.88 (0.63-1.23)	69/257	2.11 (1.46-3.05)
G/G	7/23	1.58 (0.60-4.13)	3/23	1.08 (0.29-4.04)	12/23	1.16 (0.52-2.59)	15/28	4.14 (2.03-8.46)
P for trend		0.3538		0.1093		0.7203		<0.0001
ALDH2 +348 C>T								
T/T	100/486	1.00	60/499	1.00	202/499	1.00	83/621	1.00
T/C	50/212	1.14 (0.76-1.71)	43/216	1.50 (0.94-2.39)	95/216	0.93 (0.67-1.30)	71/245	2.29 (1.59-3.30)
C/C	6/21	1.72 (0.61-4.81)	3/21	1.14 (0.30-4.33)	12/21	1.41 (0.62-3.21)	12/27	3.71 (1.73-7.97)
P for trend		0.2957		0.1501		0.8867		<0.0001
ALDH2 −261C>T								
T/T	98/486	1.00	61/496	1.00	202/496	1.00	85/621	1.00
T/C	50/214	1.15 (0.77-1.72)	42/217	1.45 (0.91-2.31)	95/217	0.94 (0.67-1.31)	71/243	2.32 (1.61-3.35)
C/C	6/21	1.75 (0.63-4.89)	3/22	1.13 (0.30-4.31)	12/22	1.43 (0.63-3.25)	12/26	3.85 (1.78-8.36)
P for trend		0.2751		0.1864		0.8505		<0.0001

NOTE: Adjusted for age, sex, country, pack-years of tobacco smoking, and years of alcohol drinking.

exposure to acetaldehyde and increase the risk of upper aerodigestive tract cancer, especially among heavy alcohol consumers.

Materials and Methods

A multicenter case-control study was conducted in six centers from five Central and Eastern European countries: Bucharest (Romania), Lodz (Poland), Moscow (Russia), Banska Bystrika (Slovakia), and Olomouc and Prague (Czech Republic). The overall recruitment period at the centers was from 2000 to 2002. Upper aerodigestive tract cancer cases diagnosed at designated hospitals or cancer clinics and confirmed histologically or cytologically were recruited into the study within 3 months of diagnosis. Of the 906 eligible cases with squamous cell carcinoma (SCC) of the upper aerodigestive tract recruited, blood samples were available for 816 subjects. DNA extractions for five subjects were not successful; thus, 811 cases were genotyped. There were 168 oral SCC cases, 113 pharyngeal SCC cases, 326 laryngeal SCC cases, and 176 esophageal SCC cases. An additional 28 unspecified cases of oral and pharyngeal SCC were included in the overall upper aerodigestive tract cancer case group.

Controls were recruited from inpatients or outpatients in the same hospital as the cases. Only controls with a recent diagnosis from a defined list of diseases unrelated to tobacco and alcohol were included. In Moscow, the controls were frequency matched to the upper aerodigestive tract cancer cases by age, sex, and referral or residence area. In the other centers, controls overlapped with those for a parallel case-control study of lung cancer conducted according to an identical protocol (28-30). Because the lung cancer study was started earlier than the upper aerodigestive tract cancer study, we excluded controls if their interview date was >6 months earlier than the first interview date for the upper aerodigestive tract cancer cases. Written consent for participation was obtained from all study subjects and ethical approval has been obtained for all study centers as well as at IARC. Cases and controls were interviewed with a structured questionnaire on residential and lifestyle history by the same team of interviewers in each center.

Genomic DNA was extracted from blood samples with the use of a QIAamp 96 DNA Blood kit (Qiagen, Hilden, Germany), or with Puregene chemistry (Gentra Systems, Minneapolis, MN) on an Autopure instrument (Gentra Systems). Samples that yielded an insufficient amount of DNA at extraction were subjected to whole genome amplification by use of a phi29-based protocol (GenomiPhi, Amersham Biosciences, Uppsala, Sweden) or reextracted by Puregene chemistry. DNA concentrations were measured by using PicoGreen double-stranded DNA quantification kits (Molecular Probes, Leiden, the Netherlands).

We genotyped six SNPs: *ADH1B* $Arg^{48}His$ (rs1229984; previously $Arg^{47}His$) in exon 3, *ADH1C* $Ile^{350}Val$ (rs698; previously $Ile^{349}Val$) in exon 8, *ADH1C* $Arg^{272}Gln$ (rs1693482; previously $Arg^{271}Gln$) in exon 6, *ALDH2* +82 A>G (rs886205) in the 5′ untranslated region, *ALDH2* +348 C>T (rs440) and *ALDH2* −261C>T (rs441), both in intron 6. Although the two SNPs on *ADH1C* constitute an allele, we have analyzed them separately because they are not in complete linkage disequilibrium with each other. We selected variants in *ALDH2* based on a sequence discovery publication, which reported that these three variants were common in the Caucasian population (31). All polymorphisms were analyzed by the 5 exonuclease assay (i.e., TaqMan assay; ref. 32). Designs of genotyping assays for all SNPs were taken from the website of the SNP500 project.[9] PCR primers and TaqMan probes were synthesized by Applied Biosystems (Foster City, CA).

[9] http://snp500cancer.nci.nih.gov

Table 5. *ADH/ALDH* genotypes and the risk of SCC of the oral cavity, pharynx, larynx, and esophagus, stratified on drinking status

	Oral		Pharynx	
	Light drinkers	Medium drinkers	Light drinkers	Medium drinke
	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)
ADH1B R48H				
Arg/Arg (slow)	1.00	1.00	1.00	1.00
Arg/His + His/His	0.57 (0.24-1.36)	0.25 (0.05-1.23)	0.78 (0.28-2.19)	0.96 (0.17-5.33)
P for interaction		0.4529		0.7945
ADH1C I350V				
Ile/Ile (slow)	1.00	1.00	1.00	1.00
Ile/Val	1.60 (0.93-2.77)	0.50 (0.23-1.09)	2.18 (1.07-4.43)	0.92 (0.29-2.86)
Val/Val (fast)	2.16 (1.10-4.26)	2.12 (0.85-5.29)	3.46 (1.51-7.96)	1.62 (0.37-7.05)
P for trend	0.0213	0.6269	0.0029	0.6269
P for interaction		0.4671		0.3397
ADH1C R272Q				
Arg/Arg (slow)	1.00	1.00	1.00	1.00
Arg/Gln	1.58 (0.92-2.73)	0.55 (0.25-1.18)	2.09 (1.03-4.26)	0.77 (0.24-2.42)
Gln/Gln (fast)	2.31 (1.16-4.57)	1.92 (0.75-4.89)	3.66 (1.58-8.50)	1.42 (0.33-6.09)
P for trend	0.0146	0.7965	0.0022	0.7965
P for interaction		0.2766		0.1924
ALDH2 +82A>G				
A/A	1.00	1.00	1.00	1.00
A/G	0.89 (0.54-1.47)	1.66 (0.80-3.45)	1.55 (0.87-2.76)	0.98 (0.31-3.06)
G/G	1.65 (0.46-5.87)	8.12 (1.40-47.12)	1.96 (0.38-10.12)	—
P for trend	0.9804	0.8074	0.1089	0.8074
P for interaction		0.0397		0.7567
ALDH2 +348 C>T				
T/T	1.00	1.00	1.00	1.00
T/C	0.97 (0.59-1.59)	1.68 (0.81-3.48)	1.87 (1.07-3.29)	1.11 (0.38-3.26)
C/C	1.08 (0.26-4.38)	12.94 (1.72-97.17)	0.89 (0.10-7.62)	—
P for trend	0.9637	0.8986	0.0707	0.8986
P for interaction		0.0360		0.8521
ALDH2 −261C>T				
T/T	1.00	1.00	1.00	1.00
T/C	0.97 (0.59-1.59)	1.64 (0.80-3.38)	1.87 (1.06-3.27)	0.90 (0.29-2.74)
C/C	1.07 (0.26-4.37)	14.03 (1.88-104.55)	0.84 (0.09-7.18)	—
P for trend	0.9526	0.8066	0.0763	0.8066
P for interaction		0.0306		0.6363

NOTE: The results showed similar patterns when stratified by milliliters of ethanol per day (<140 versus ≥140 g/wk) and by years of drinking (<20 versus ≥20 years). Light drinkers, never/light drinkers (≤2 times/wk); medium drinkers, medium/heavy drinkers (≥3 times/wk).
*Adjusted for age, sex, country, and pack-years of tobacco smoking.

To ensure quality control, DNA samples from case patients and control subjects were randomly distributed on each PCR plate, and all genotyping was conducted by personnel who were blinded to the case or control status. We randomly selected 10% of the study subjects (i.e., both case patients and control subjects) and reanalyzed their DNA samples for each polymorphism to examine the reliability of the genotyping assays. The concordance was >99% for three variants (*ADH1C* $Arg^{272}Gln$, *ADH1C* $Ile^{350}Val$, *ALDH2* −261 C>T), and 100% for the other three variants (*ADH1B* $Arg^{48}His$, *ALDH2* +82 A>G, and *ALDH2* +348 C>T). We also assessed Hardy-Weinberg equilibrium in the controls for each genotype and did not observe any departures from Hardy-Weinberg equilibrium.

Unconditional logistic regression was used to estimate odds ratios for *ADH* and *ALDH* genotypes with the SAS program (version 8.02), after adjusting for potential confounders, such as age, sex, country, tobacco smoking, and alcohol drinking. We examined the genotype distribution by country and observed variation across countries, consistent with previous reports (7). Thus, we adjusted for country to address potential population stratification. We do not expect population stratification within each country because the population in these countries is generally homogenous. To take into account the potential modifying effect of the interaction between tobacco and alcohol, we included an interaction term for pack-years of smoking and years of drinking in the logistic regression models; this analysis did not change the observed associations or the inferences. The ''slow'' alleles for the *ADH* polymorphisms and the common genotype for the *ALDH2* polymorphisms were taken as the reference group. We conducted stratified analysis for never/light drinkers (≤2 times/wk) and medium/heavy drinkers (≥3 times/wk). Trend tests for ordered variables were done by treating the categorical variable as a continuous predictor in the logistic regression model.

Interactions were assessed by comparing the fit of a regression model, including terms for each of two factors alone to that of a model including an interaction term between the two factors. The haplotype frequencies were estimated by STATA software using the E-M algorithm. Linkage disequilibrium between SNPs was tested by the likelihood ratio comparing the log-linear model, including an interaction term between the two loci, and a model without the interaction term, which assumes independence between the two loci. Finally, we calculated population-attributable fractions for *ADH* and *ALDH* genotypes for upper aerodigestive tract cancer and esophageal cancer, based on the adjusted odds ratios (OR), with the following equations (33): (*a*) $AF_{exposed} = (OR - 1) / OR$; (*b*) $AF_{population} = (A_{1+} / M_{1+})$ [(OR − 1) / OR], where A_{1+}/M_{1+} is the proportion exposed among the cases; (*c*) $PF_{exposed} = 1 - OR$; and (*d*) $PF_{population} = (A_{1+} / M_{1+})(1 - OR)$.

Results

Selected characteristics of the cases and controls are presented in Table 1. The largest number of cases and controls were from

Table 5. *ADH/ALDH* genotypes and the risk of SCC of the oral cavity, pharynx, larynx, and esophagus, stratified on drinking status (Cont'd)

Larynx		Esophagus	
Light drinkers	Medium drinkers	Light drinkers	Medium drinkers
OR* (95% CI)	OR* (95% CI)	OR* (95% CI)	OR* (95% CI)
1.00	1.00	1.00	1.00
0.70 (0.39-1.28)	0.48 (0.18-1.27)	0.25 (0.07-0.85)	—
	0.7934	—	
1.00	1.00	1.00	1.00
0.85 (0.57-1.26)	0.65 (0.33-1.28)	1.98 (1.16-3.38)	1.57 (0.79-3.14)
1.15 (0.69-1.93)	0.94 (0.29-3.09)	1.85 (0.92-3.67)	2.25 (0.87-5.81)
0.7958	0.4305	0.0398	0.0784
	0.3234		0.9361
1.00	1.00	1.00	1.00
0.88 (0.59-1.31)	0.69 (0.35-1.37)	1.90 (1.11-3.23)	1.66 (0.82-3.36)
1.33 (0.79-2.24)	1.00 (0.30-3.30)	2.20 (1.11-4.37)	2.56 (0.95-6.93)
0.4697	0.5483	0.0122	0.0505
	0.1825		0.8529
1.00	1.00	1.00	1.00
0.94 (0.64-1.37)	0.70 (0.34-1.45)	1.74 (1.08-2.78)	2.62 (1.38-4.98)
1.59 (0.63-4.03)	2.67 (0.42-17.11)	3.91 (1.53-9.98)	9.76 (2.62-36.38)
0.7721	0.8376	0.0011	<0.0001
	0.7056		0.1621
1.00	1.00	1.00	1.00
0.95 (0.64-1.39)	0.90 (0.43-1.89)	1.97 (1.23-3.14)	2.74 (1.45-5.18)
1.44 (0.57-3.60)	7.03 (0.94-52.52)	2.78 (0.98-7.89)	11.13 (2.62-47.36)
0.8194	0.4245	0.0016	<0.0001
	0.3044		0.1094
1.00	1.00	1.00	1.00
0.95 (0.65-1.40)	0.88 (0.42-1.82)	1.93 (1.21-3.07)	2.96 (1.57-5.60)
1.45 (0.58-3.64)	7.13 (0.96-53.08)	2.95 (1.04-8.39)	10.74 (2.53-45.68)
0.7977	0.4630	0.0015	<0.0001
	0.3130		0.0781

Russia (365 cases/319 controls), followed by Poland (206 cases/209 controls). The age distribution was fairly similar among cases and controls, but there was a higher proportion of women in the control group compared with the cases. Tobacco smoking and alcohol drinking habits were more common among the cases, as expected.

In Table 2, we explored whether the genotype of the individual may affect alcohol drinking or tobacco smoking behaviors, by examining the distribution of these habits among controls. Significant differences in the distribution of tobacco and alcohol habits were not observed with the *ADH1B* $Arg^{48}His$, *ADH1C* $Ile^{350}Val$, and *ALDH2* +348 C>T genotypes. The *ADH1C* R272Q, *ALDH2* +82A>G, and *ALDH2* −261C>T genotypes were not associated with the frequency of drinking per week or the pack-years of smoking either (these genotypes are in linkage disequilibrium with the genotypes in the tables and had similar distributions).

Odds ratios for the *ADH* and *ALDH* genotypes and the risk of upper aerodigestive tract cancer overall and stratified by the times of alcohol consumption per week are presented in Table 3. Subjects who were homozygous for histidine and heterozygote were combined because of the small number of subjects who were homozygous for histidine. The *ADH1B* histidine allele was associated with a decreased risk of upper aerodigestive tract cancer overall, with potentially a greater protective effect among medium and heavy drinkers [OR, 0.36; 95% confidence interval (95% CI), 0.17-0.77] than among never/light drinkers (OR, 0.57; 95% CI, 0.36-0.91), although this difference was not significant. The *ADH1C* valine allele was associated with a moderately increased risk of upper aerodigestive tract cancer (OR, 1.38; 95% CI, 1.01-1.88), which was of similar magnitude for light and medium/heavy drinkers. A similar effect was observed for the *ADH1C* glutamine allele at codon 272.

Individuals who were heterozygous or homozygous for any of the *ALDH2* variant alleles were at a significant increased risk of upper aerodigestive tract cancer, with a significant dose response for possession of two variant alleles (P = 0.015, 0.009, and 0.007 for *ALDH2* +82, +348, and −261, respectively; Table 3). This increase in risk was less evident among never/light drinkers and became more prominent among medium/heavy drinkers. The OR for each of the homozygous variants among heavy drinkers ranged between 4.38 (1.32-14.53) for *ALDH2* +82 to 5.79 (1.49-22.5) for both *ALDH2* +348 and −261. The interaction between alcohol consumption and these three genes was significant for both *ALDH2* +348 and −261.

When results were analyzed by subtype, strong main effects were observed for squamous cell carcinoma of the esophagus for all six variants (Table 4). This was most apparent for *ADH1B* His (OR, 0.19; 95% CI, 0.07-0.53, P = 0.002) and *ALDH* +82 G/G (OR, 4.14; 95% CI, 2.03-8.46, $P < 0.0001$). Borderline protective effects were observed for the other cancer sites for *ADH1B* His, although associations were not clear for these sites with the three *ALDH* variants. An increased risk with both *ADH1C* variants was observed for oral, pharynx, and esophageal cancer, although not for larynx cancer.

When the analysis was further stratified on the frequency of drinking, the increase in risk for the three *ALDH2* sequence variants among medium/heavy drinkers and esophageal cancer was ~3-fold for heterozygous variants and 10-fold for homozygous variants ($P < 0.0001$ for all three genes; Table 5). Increased risks were also observed with these three polymorphisms and esophageal cancer among light drinkers. Strong increases in risk of >10-fold were also seen for each of the homozygous variants and oral cancer among medium/heavy drinkers, with no increase in risk among never or light drinkers. When we stratified on the milliliters of ethanol per

day (<140 versus ≥140 g/wk) and also on years of alcohol drinking (<20 versus ≥20 years), the results were similar for these sites and for head and neck cancer overall (data not shown).

Linkage disequilibrium was detected among the three *ADH1B* and *ADH1C* loci ($P < 0.001$ for all combinations) and among the three *ALDH2* loci ($P < 0.001$ for all combinations). When common haplotypes between *ADH1B* and *ADH1C* were calculated (not shown in tables), three haplotypes comprised over 99.5% of all possible variants, these being a ''combined slow'' haplotype (*ADH1B* Arg + *ADH1C* 350 Ile + *ADH1C* 272 Arg), a ''fast *ADH1B*'' haplotype (*ADH1B* His + *ADH1C* 350 Ile + *ADH1C* 272 Arg), and a ''fast *ADH1C*'' haplotype (*ADH1B* Arg + *ADH1C* 350 Val + *ADH1C* 272 Gln). When compared with the most common combined slow haplotype, the fast *ADH1B* haplotype was associated with a significant decreased risk of upper aerodigestive tract cancer (OR, 0.5; 95% CI, 0.38-0.81), whereas the fast *ADH1C* genotype had no effect (OR, 1.12; 95% CI, 0.97-1.29). This would suggest that the primary effect of *ADH* genes is a protective effect of the *ADH1B* fast metabolizing allele, not modified by *ADH1C*. We also assessed potential gene-gene interactions, but none were detected.

For *ALDH2* haplotype analysis, we observed that the common haplotypes among our study population were (+82 A and +348 T and −261 T), followed by (+82 G and +348 C and −261 C). With the most common haplotype as the reference group, the OR for the haplotype with the variant allele at all three loci was 1.41 (95% CI, 1.20-1.67). A small frequency of another haplotype was observed (+82 G and +348 T and −261 T) but was not associated with increased risks (OR, 1.31; 95% CI, 0.62-2.45).

Finally, attributable fractions in carriers (AF_c) and in the population (AF_p) were calculated for *ALDH2* +348 C>T (a tag SNP in the Hapmap data) and the prevented fraction in carriers (PF_c) and in the population (PF_p) were calculated for *ADH1B* R48H (Table 6). The AF_p for *ALDH2* +348 C>T was 9.6% for all upper aerodigestive tract cancers and 31.2% for esophageal cancer. Among medium/heavy drinkers, the AF_p was 19.8% and 37.9%, respectively. Among the 30% of the population who were carriers of the *ALDH2* +348 C>T variant, the AF_c was 24.2% for all upper aerodigestive tract cancers, increasing to 58.7% for esophageal cancer. The AF_c among medium/heavy drinkers were 49.0% and 68.9%, respectively. Regarding *ADH1B* R48H, the overall PF_p was 3.3% for all upper aerodigestive tract cancers and 1.9% for esophageal cancer. Among carriers of at least one variant, the PF_c was 53% and 81%, respectively. The PF_p and PF_c were higher among medium/heavy drinkers for all upper aerodigestive tract cancers, although it could not be calculated for esophageal cancer due to a zero value in the cells.

Discussion

We have identified specific variants of the *ALDH2* gene that are associated with a strongly increased risk of upper aerodigestive tract cancer among medium to heavy drinkers, with homozygotes for the rare allele having the highest risk and heterozygotes having an intermediate risk. These variants seem to explain a substantial proportion of upper aerodigestive tract cancer, especially among regular drinkers. However, the attributable risks must be interpreted with caution because our study was not population based. We also detected a protective effect for carriers of the rare allele of *ADH1B* R48H.

Previous studies on the association between sequence variants and upper aerodigestive tract cancer in European populations have focused on the *ADH1C* gene, although results have been inconsistent (7). In the current study, we identified an effect of *ADH1C* I350V homozygosity that is associated with fast ethanol metabolism, although this was no longer apparent in the haplotype analysis incorporating *ADH1B*. One possible explanation for this is that the primary association is with *ADH1B* and the association with *ADH1C* is partially due to linkage disequilibrium. As linkage disequilibrium patterns can differ between populations, this could also explain why different studies have produced inconsistent results. However, an independent effect of *ADH1C* I350V cannot be completely ruled out due to its suggested functionality. This may imply that slow initial metabolism of ethanol to acetaldehyde may be the primary risk factor for upper aerodigestive tract cancer, and not fast initial metabolism as was the prior hypothesis of previous studies and of our study. An alternative, *a posteriori* hypothesis is that fast initial metabolism may lead to a peak in acetaldehyde exposure, inducing alternative mechanisms to clear this peak. On the other hand, more moderate initial metabolism may not induce such mechanisms, resulting in a greater overall exposure. Functional studies would be required to test this possibility.

Another potential hypothesis for the protective effect observed for the *ADH1B* R48H polymorphism is that there is complexity due to multiple substrates. The ADH family is involved in retinol metabolism, with a greater role suggested for ADH4 and ADH1B (34). Thus, dietary intake of vitamin A with the fast metabolizing *ADH1B* allele may protect against upper aerodigestive tract cancers. Our results on the ADH1B R48H variant are consistent with previous reports. Small studies on *ADH1B* in Japanese populations have reported increases in esophageal cancer in individuals who were homozygous for the slow histidine allele at codon 48, on the order of 4- to 6-fold (14, 15). When taking the *ADH1B* histidine allele carriers as the reference, our results show a 5-fold increase in esophageal cancer risk.

Previous studies on *ALDH2* have focused on the *ALDH**2 allele at position 487 that results in a near inactivation of the gene product among homozygote carriers and very limited activity among heterozygote carriers. This leads to a build up of acetaldehyde upon alcohol consumption, resulting in a toxic reaction, including nausea, increased heart rate, and flushing. Several small studies of head and neck cancer in Japanese populations have identified an increased risk for the *ALDH2**2 allele. For the three *ALDH2* variants that we analyzed, functional information is lacking. Two of these variants are intronic, whereas the *ALDH2* +82 variant is located in the 5′ untranslated region. Furthermore, it is unlikely that the current results can be explained by avoidance of alcohol. As can be

Table 6. Attributable and prevented fractions for *ADH1B* and *ALDH2* genotypes

	ADH1B R48H		*ALDH2* +348 C>T	
	$PF_{carrier}$	$PF_{population}$	$AF_{carrier}$	$AF_{population}$
UADT cancer	53.0 (30.0-68.0)	3.3 (1.8-4.2)	24.2 (5.7-38.3)	9.6 (2.2-15.1)
In heavy drinkers	64.0 (23.0-83.0)	3.9 (1.4-5.0)	49.0 (21.3-66.8)	19.8 (8.6-27.0)
Esophageal cancer	81.0 (47.0-93.0)	1.9 (1.1-2.2)	58.7 (41.2-71.0)	31.2 (2.2-37.8)
In heavy drinkers	—	—	68.9 (42.9-83.1)	37.9 (23.6-45.7)

NOTE: Adjusted for age, sex, country, pack-years of tobacco smoking, and years of alcohol drinking.

seen from Table 2, the patterns of alcohol consumption among controls in the different genotype groups for *ADH1B*, *ADH1C*, and *ALDH2* were similar, with no significant evidence of an association between alcohol drinking and genotypes. If there were an association between genotype and behavior, this would add to dilute any observed effect associated with these variants, indicating a potential greater real effect.

Although the current results are also unlikely to be due to chance, in particular the protective effect for the *ADH1B* histidine allele for all upper aerodigestive tract cancers (P = 0.0002), and all three of the *ALDH2* variants for esophageal cancer (dose response P values < 0.001), replication of this analysis in other studies of at least similar size is required. This would help to clarify several aspects of the current analysis, including whether the *ALDH2* variants are primarily restricted to esophageal cancer, and to further assess the attributable fraction of these variants. Lohmueller et al. (35) have previously shown that, even for effects that are subsequently replicated, the first report tends to overestimate the actual effect. Studies in other populations may also help to determine whether these polymorphisms explain the very high rates of upper aerodigestive tract cancer in Central Europe. Subsequent studies may wish to focus not only on the variants included in this report but also other relevant variants for these three genes, including haplotype tagging SNPs in *ADH1B*, *ADH1C*, and *ALDH2*, as recently identified by the HapMap project.

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