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Association of habitual smoking and drinking with single nucleotide polymorphism (SNP) in 40 candidate genes: data from random population-based Japanese samples

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Abstract Basic information on the association between lifestyle factors and candidate genes is valuable for genetic–environmental study. We screened the association of habitual smoking or drinking with polymorphism in 40 candidate genes for a total of 153 single nucleotide polymorphisms (SNPs) using a sample of 339 middle-aged, randomly selected Japanese men. Smoking and drinking statuses were elicited during questionnaire-based interviews. Genes were selected based on their possible involvement in genetic–environmental, life-style interactions and constitute the genes expressing xenobiotic metabolism enzymes, DNA repair enzymes, and other stress-related proteins. The *P* values of odds ratios to habitual smoking for CYP17A1, ESR1, EPHX1, GSTT2, ALDH2, NOS2A, OGG1, and SLC6A4 and those of odds ratios to habitual drinking for CYP1B1, ESR1, HSD17B3, GSTM3, COMT, ADH1C, ALDH2, NOS3, and NUDT1 were under 0.05. These variables were included in a stepwise logistic analysis in order to develop a predictive model for smoking or drinking behavior. In the final model, the only significant variables selected for smoking were OGG1, SLC6A4, EPHX1, ESR1, and CYP17A1, and for drinking, ALDH2 and NUDT1. The findings of the present study suggest that polymorphism in associated candidate genes plays a role in the habitual use of tobacco and alcohol among Japanese men.

Keywords Smoking · Drinking · Single nucleotide polymorphism · Candidate gene · Japanese men · Association study

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Introduction

Basic information on whether lifestyle factors and candidate genes are independent of each other is valuable for genetic–environmental study. Such information is essential for future studies to reveal the interaction of lifestyle factors and genetics. Furthermore, an understanding of genes associated with lifestyle factors will contribute to more accurate risk identification and to establishing tailor-made prevention measures.

Two of the most common and important lifestyle factors, cigarette smoking and alcohol drinking, are related to many diseases, including lung cancer, cardiovascular disease, and other chronic diseases. Hence, genetic influence on the use of tobacco has been strongly implicated by cross-sectional studies in twins, association studies, and numerous other genetic epidemiology data (Carmelli et al. 1992; Hussain et al. 2001; Pianezza et al. 1998; Sabol et al. 1999; Yoshida et al. 2001). It is also recognized that the use of tobacco is often accompanied by alcohol consumption (Hopfer et al. 2001). Alcohol consumption is found to be as heritable as smoking (Heath et al. 1991; McGue et al. 2001), and the contribution of ALDH2 and ADH1C genes to habitual alcohol drinking has been well known. However, current

knowledge on genetic polymorphism related to smoking or drinking behavior is far from sufficient. Information on associated genetic factors could provide a more rational basis for developing smoking or drinking cessation programs, including identification of persons at high risk and the introduction of suitable interventions to prevent smoking-related or drinking-related diseases.

We examined the relationship between the smoking and drinking habits of 339 Japanese men and their genotypes in 153 single nucleotide polymorphisms (SNPs) in 40 selected genes. The genes were selected based on possible involvement in genetic–environmental, life-style interactions and constitute the genes for xenobiotic metabolism enzymes, DNA repair enzymes, and other stress-related proteins. This population-based association study will provide a list of genes independent of habitual smoking and drinking, which may be included in a future case study. It will also establish the role of genetic influence on smoking and drinking behavior and thus contribute to the future development of genotype-based prevention of smoking-related and drinking-related diseases.

Subjects and methods

Subjects

The selection of subjects is described in detail by Tsugane et al. (1992a, b). Briefly, from 1989 to 1991, using a random sampling method employing resident registration rolls, we recruited men aged 40–49 years from five areas having a population of approximately 100,000 people. Between 170 and 195 subjects were selected from each area. The selected individuals were initially invited to participate in the study by e-mail, and a subsequent letter, telephone call, and home visit encouraged participation. A questionnaire-based interview elicited lifestyle details and health check-up information, as well as yielding blood and urine samples. The overall participation rate was 72%, or 634 out of 880.

Smoking and drinking habits

Subjects were asked whether they had ever used tobacco regularly (habitual smoking) and were then classified as ''past smokers,'' ''current smokers,'' or ''never smokers.'' With regard to alcohol drinking, those who did not habitually consume alcohol once or more per month were considered ''nondrinkers;'' otherwise subjects were considered ''drinkers.'' Whether they flush on drinking was recorded as well.

DNA samples and SNPs analyses

A total of 25 ml of blood was drawn by venipuncture, and genomic DNA was extracted from the buffy coat layer using a commercial kit (Wako, Osaka, Japan). Some samples were used in another study (Sugimura et al. 1998) and exhausted before the present study, leaving 339 cases with a representative sample of 0.5 μg or more of DNA. For each gene, 5–8 SNPs were chosen from public databases or published papers, with a total of 289 SNPs being typed for 44 genes using the mass spectroscopy-based technique, Mass ARRAY (Ross et al. 1998). The 44 were selected from genes encoding xenobiotic metabolic enzymes, DNA repair enzymes, and other stress-related proteins. Only SNPs in the Hardy–Weinberg equilibrium with a >0.05 chi-square and SNPs having a minor allele frequency of at least 1% were selected for analysis. Thus, 153 SNPs from 40 genes were finally included. Details of SNP selection, including allele frequency in the 153 SNPs, is described by Yoshimura et al. (2003). The genes included in the present study were cytochrome P450 genes (CYP1A1, CYP1B1, CYP2C9, CYP2C19, CYP2E1, CYP17A1, and CYP19A1), the aryl hydrocarbon receptor gene (AHR), estrogen receptor genes (ESR1, ESR2, and ERRRG), the progesterone receptor gene (PGR), epoxide hydrolase genes (EPHX1, and EPHX2), hydroxysteroid (17-beta) dehydrogenase genes (HSD17B2 and HSD17B3), glutathione S-transferase genes (GSTM2, GSTM3, GSTT2, and GSTP1), N-acetyltransferase genes (NAT1 and NAT2), the catechol-O-methyltransferase gene (COMT), alcohol dehydrogenase genes (ADH1A, ADH1B, and ADH1C), the aldehyde dehydrogenase gene (ALDH2), nitric oxide synthase genes (NOS2A and NOS3), interleukin genes (IL1A and IL1B), repair genes for oxidative DNA damage [OGG1 and NUDT1 (MTH1)], dopamine receptor genes (DRD2, DRD3, and DRD4), the serotonin transporter gene (SLC6A4), the glucocorticoid receptor gene [NR3C1 (GCCR)], the folate metabolizing enzyme gene (MTHFR), and the quinone oxidoreductase gene (NQO1).

Ethical issues

All DNA samples were anonymous and unlinked to specific individual information, i.e., any ID, name, or address. The protocol of the present study was approved by the ethics review committee of the National Cancer Center (protocol number G12-02).

Statistical analysis

First, each SNP was analyzed independently to screen the SNPs possibly related with smoking or drinking. In order to show the ratio of the odds of becoming a smoker or drinker in those exposed to targeted genotype relative to the unexposed individuals, we used an odds ratio as an indicator of the strength of association between genotypes and the smoking or drinking habit. A logistic regression analysis was used to obtain odds ratios, and 95% confidence intervals were calculated using

the standard errors of the logistic regression coefficients. P values were calculated from the χ^2 test. We also calculated the statistical power of the analyses by using the formula of Schlesselman (Schlesselman 1982). We then included all significant SNPs in the above analyses simultaneously in a stepwise logistic regression analysis to select substantially significant ones. We tested the statistical significance once and did not use a correction of multiple testing because we used a multivariate analysis. All computations were performed using the SAS software package Version 8.2 (SAS Institute, Inc., Cary, NC, USA).

Results

The number of never smokers, past smokers, and current smokers was 55, 71, and 213, respectively. We combined never smokers and past smokers as nonsmokers in order to gain greater investigative power. The number of nondrinkers and drinkers was 71 and 268, respectively. All 153 SNPs in the 40 genes examined are listed in Table 1. Among them, 11 SNPs of five genes (CYP17A1, ALDH2, NOS2A, OGG1, and SLC6A4) in the dominant model and three SNPs of three genes (ESR1, EPHX1, and GSTT2) in the recessive model were found to be associated with habitual smoking in a statistically significant manner (Table 2). Similarly, ten SNPs of five genes (HSD17B3, COMT, ADH1C, ALDH2, and NOS3) in the dominant model and five SNPs of four genes (CYP1B1, ESR1, GSTM3, and NUDT1) in the recessive model were found to be associated with habitual drinking in a statistically significant manner (Table 3). No relationship with smoking and drinking behavior was observed for 32 genes and 31 genes, respectively.

Next, a multivariate analysis was performed using variables including drinking status and the genotype of 14 SNPs listed in Table 2. The odds ratios of five genes for smoking, selected by stepwise logistic regression analysis, are given in Table 4. While a negative association was found in the dominant model between habit-

Table 1 List of 153 SNPs in 40 genes selected for association study

Gene symbol	SNP (rs number)
CYP1A1	rs1048943
CYP1B1	rs10012, rs1056827, rs1056836, rs10916
CYP2C9	rs1505
CYP2C19	rs1322179
CYP2E1	rs3813867, rs2031920, rs2070673
CYP17A1	rs6162, rs6163, rs743572
CYP19A1	rs700518, rs700519, rs4646
AHR	rs2066853, rs713150, rs2074113, rs2237297, rs2237299, rs2282886
ESR1	rs1913474, rs932479, rs2011885, rs974276, rs1062577
ESR2	rs1256054, rs1256049, rs1256027, rs944459, rs2274705, rs1256030
ESRRG	rs1498283, rs1339343
PGR	rs484389
EPHX1	rs1051741, rs1051740, rs6965, rs2292566, rs2234922, rs2292568
EPHX2	rs751141, rs1042032, rs891401, rs2291635, rs1126452, rs747276
HSD17B2	rs2042429, rs1017243, rs1424151, rs996752
HSD17B3	rs2066480, rs375944, rs280654, rs912462, rs2066479, rs867807
GSTM2	rs655315, rs428434
GSTM3	rs1332018
GSTT2	rs1622002, rs2719, rs2267047, rs140186
GSTP1	rs947894, rs762803
NAT1	rs15561
NAT2	rs1801280, rs1799929, rs1799930, rs1495744
COMT	rs4633, rs4680, rs6267, rs2097603, rs2020917, rs2239393
ADH1A	rs931635, rs1229967, rs1229970, rs975833, rs1618572, rs2276332
ADH1B	rs17033, rs1159918, rs1042026
ADH1C	rs1789924, rs1693430, rs2009181, rs2298755, rs3216150
ALDH2	rs671, rs2238151, rs2238152, rs441
NOS2A	rs1060826, rs1060822, rs2072324, rs2297518, rs2297520
NOS3	rs1800783, rs1549758, rs1799983, rs1800780, rs1800779
IL1A	rs17561, rs1800587, rs1800794, rs2071374
IL1B	rs1143627, rs16944, rs1071676, rs1143637, rs1143629, rs1143634
OGG1	rs2075747, rs1052133, rs2072668, rs1801129
NUDT1	rs4866, rs1062492
DRD2	rs1076560, rs1124491, rs6277, rs6275, rs1076563, rs1079596, rs1801028, rs1116313
DRD3	rs6280, rs1800828
DRD4	rs1800955, rs936460, rs752306
SLC6A4	rs1042173, rs2020939, rs2020936, rs1872924, rs25528, rs717742
NR3C1	rs6194, rs258751, rs6196, rs33388, rs33389, rs174050
MTHFR	rs2066470, rs1801133, rs1801131, rs2066471, rs2274976
NQO1	rs1800566

Table 2 Odds ratios (OR) and 95% confidence intervals (CI) of SNPs associated with smoking behavior. Allele A represents major allele; allele a represents minor allele

Gene symbol	SNP (rs number)	Reference allele/ variant allele	Amino acid change	Major allele/minor allele	Genotype of nonsmokers			Genotype of smokers			OR (95% CI)aa versus Aa + AA	*P* value	OR (95% CI)aa + Aa versus AA	*P* value
					AA	Aa	aa	AA	Aa	aa				
ESR1	rs1913474	gttc(T/C)aaga	Intron	C/T	36	66	19	59	92	53	1.9 (1.1–3.4)	0.03	1.0 (0.6–1.7)	0.87
EPHX1	rs2292566	ctaa(G/A)attg	Lys → Lys	G/A	52	57	17	104	97	12	0.4 (0.2–0.8)	0.01	0.7 (0.5–1.1)	0.18
GSTT2	rs140186	ttag(G/A)ggat	Locus	G/A	42	46	22	73	96	20	0.5 (0.2–0.9)	0.02	1.0 (0.6–1.6)	0.94
CYP17A1	rs743572	ccac(T/C)gctg	Untranslated region	T/C	29	69	28	72	100	40	0.8 (0.5–1.4)	0.46	0.6 (0.4–1.0)	0.03
ALDH2	rs2238152	caaa(C/A)agat	Intron	C/A	81	42	3	160	48	3	0.6 (0.1–3.0)	0.52	0.6 (0.4–0.9)	0.02
	rs441	tgag(A/G)ccga	Intron	A/G	80	42	3	162	48	3	0.6 (0.1–2.9)	0.51	0.6 (0.3–0.9)	0.02
NOS2A	rs2072324	ttat(C/A)ttct	Intron	C/A	68	45	9	94	98	21	1.4 (0.6–3.1)	0.44	1.6 (1.0–2.5)	0.04
OGG1	rs1052133	caat(C/G)ccgc	Ser → Cys	C/G	29	69	28	72	105	36	0.7 (0.4–1.2)	0.23	0.6 (0.4–1.0)	0.04
	rs2072668	catt(C/G)tgtg	Intron	C/G	29	68	28	71	104	38	0.8 (0.4–1.3)	0.31	0.6 (0.4–1.0)	0.05
	rs1801129	tact(A/G)cggg	Untranslated region	A/G	107	17	1	197	13	1	0.6 (0.0–9.6)	0.71	0.4 (0.2–0.9)	0.02
SLC6A4	rs2020936	agcg(A/G)tctc	Locus	A/G	117	8	1	173	38	2	1.2 (0.1–13.2)	0.87	3.0 (1.4–6.4)	0.008
	rs1872924	gtca(T/C)ctag	Intron	T/C	115	8	1	168	35	2	1.2 (0.1–13.5)	0.89	2.8 (1.3–6.1)	0.003
	rs25528	tggt(T/G)tcgc	Locus	T/G	114	8	1	166	34	2	1.2 (0.1–13.7)	0.90	2.8 (1.3–5.9)	0.003
	rs717742	gcgc(A/T)gaca	Intron	A/T	104	6	1	155	34	2	1.2 (0.1–13.0)	0.88	3.5 (1.5–8.1)	0.006

ual smoking and the minor allele of SNPs in the OGG1 and CYP17A1 genes, a negative association was found in the recessive model for the minor allele of the SNP in EPHX1. In ESR1 and SLC6A4, minor alleles of SNPs were related to an increased risk for habitual smoking in the recessive and dominant models, respectively.

Dividing nonsmokers into never and past smokers indicated that the genotype frequencies containing the minor allele of the SNP in OGG1 (Cys) were similar among current and past smokers but much higher among never smokers. While past smokers have a higher genotype frequency containing the minor allele of the SNP in CYP17A1 than do current smokers, a lower frequency of the genotype containing the minor allele of the SNP in ESR1 was observed among past smokers compared with current smokers.

When the covariates, including smoking status and genes presented in Table 3, were selected using stepwise logistic regression analysis, polymorphisms in the ALDH2 and NUDT1 genes were chosen as the only variants significantly associated with alcohol drinking in the dominant and recessive heritable models for the minor alleles of their SNPs. We further examined the influence of NUDT1 and ALDH2 on alcohol-induced response. While NUDT1 had no association with alcohol-induced flushing, the odds ratio of having the Lys allele of ALDH2 was as high as 33 for alcohol-induced flushing (data not shown).

Discussion

Among 153 SNPs in the 40 genes, we found that SNPs in five genes (EPHX2, ESR1, hOGG1, SLC6A4, and CYP17A1) were related to habitual smoking and SNPs in two genes (ALDH2 and NUDT1) with habitual drinking. No common allele responsible for both habits was found among the 40 genes investigated in the present study. We also found that 32 genes were independent of smoking and 31 genes were independent of habitual alcohol consumption. However, due to the limitation of sample size, the statistical power was generally not large enough to detect an association that might actually exist for some genes. Further study is required to confirm the results.

A previous Japanese study noted that polymorphism of the SLC6A4 gene in the 5'-flanking region influences smoking behavior. Individuals with the homozygous S (a 44-bp deletion) genotype were less inclined to smoke and/or could more easily stop smoking than L allele (a 44-bp insertion) carriers (Ishikawa et al. 1999), although opposite data also exist (Lerman et al. 1998). In the present study, the presence of the minor allele rs717742 SNP of the same gene appears to confer a more than three-fold increased risk for smoking. Moreover, a significantly increased frequency of the minor allele of the SNP in current smokers compared with that in past smokers suggests that individuals with the minor allele may experience difficulty quitting. The location of the

Table 3 Odds ratios (OR) and 95% confidence intervals (CI) of SNPs associated with drinking behavior. Allele A represents major allele; allele a represents minor allele

Gene symbol	SNP (rs number)	Reference allele/ variant allele	Amino acid change	Major allele/ minor allele	Genotype of nondrinkers			Genotype of drinkers			OR (95% CI)aa versus Aa + AA	*P* value	OR (95% CI)aa + Aa versus AA	*P* value
					AA	Aa	aa	AA	Aa	aa				
CYP1B1	rs1056836	ccca(C/G)tgaa	Leu → Val	C/G	45	20	5	194	68	5	0.2 (0.1–0.9)	0.02	0.7 (0.4–1.2)	0.17
ESR1	rs932479	tatc(C/T)ctca	Intron	T/C	22	41	7	70	132	64	2.9 (1.2–6.5)	0.01	1.3 (0.7–2.3)	0.39
	rs1062577	attc(T/A)tttt	Untranslated region	T/A	30	25	13	126	102	26	0.5 (0.2–1.0)	0.05	0.8 (0.5–1.4)	0.42
GSTM3	rs1332018	atgt(C/A)gggt	Untranslated region	A/C	41	19	4	149	83	2	0.1 (0.0–0.7)	0.01	1.0 (0.6–1.8)	0.95
NUDT1	rs4866	tgca(C/T)gtcc	Val → Met	C/T	55	11	3	212	49	1	0.1 (0.0–0.8)	0.01	0.9 (0.5–1.8)	0.82
HSD17B3	rs2066479	cagc(G/A)gtgc	Gly → Ser	G/A	31	33	7	154	96	17	0.6 (0.2–1.5)	0.31	0.6 (0.3–1.0)	0.04
COMT	rs4680	tggc(G/A)tgaa	Val → Met	G/A	40	24	6	115	124	29	1.3 (0.5–3.2)	0.58	1.8 (1.0–3.0)	0.03
ADH1C	rs1789924	gtta(T/C)gaag	Locus	C/T	32	10		162	15				0.3 (0.1–0.7)	0.01
	rs1693430	aaat(T/C)ggtg	Intron	C/T	32	9		160	15				0.3 (0.1–0.8)	0.01
	rs2298755	cttt(G/C)acaa	Intron	C/G	37	11		172	18	1		0.62	0.4 (0.2–0.8)	0.02
	rs3216150	aaaa(A/-)tcac	Intron	*/A	38	11		169	18	1		0.61	0.4 (0.2–0.9)	0.02
ALDH2	rs671	cact(G/A)aagt	Glu → Lys	G/A	26	29	9	199	58	3	0.1 (0.0–0.3)	< 0.001	0.2 (0.1–0.4)	< 0.0001
	rs2238152	caaa(C/A)agat	Intron	C/A	58	13		183	77	6		0.20	2.0 (1.0–3.9)	0.03
	rs441	tgag(A/G)ccga	Intron	A/G	58	13		184	77	6		0.20	2.0 (1.1–3.9)	0.03
NOS3	rs1800780	gtcc(T/C)gggt	Locus	C/T	19	41	9	114	105	40	1.2 (0.6–2.7)	0.62	0.5 (0.3–0.9)	0.01

SNP of the SLC6A4 gene in the present study is about 10 kb to the polymorphism in the 5'-flanking region of previous studies. The possibility may exist that the two loci are in linkage disequilibrium.

Previous studies indicated that polymorphisms of the genes in the dopaminergic system are candidate genetic markers for habitual smoking; a relationship between the Taq I A or Taq I B RFLP polymorphisms of DRD2 and a predisposition to smoking was suggested, although the results were controversial (Wu et al. 2000; Yoshida et al. 2001). In addition, the DRD4 VNTR polymorphism was noted as another candidate marker for habitual smoking (Shields et al. 1998). No relationship between the selected SNPs of DRD2, DRD3, or DRD4 and habitual smoking was found in the present studies; albeit, the negative findings may narrow the research field to target SNPs in the dopaminergic system for future studies searching for genetic determinants of habitual smoking.

We found that the OGG1 Ser/Cys or Cys/Cys genotypes were associated with a decreased risk for smoking compared with homozygous Ser carriers. However, the OGG1 Cys allele polymorphism is considered to be related to an increased risk for lung cancer and other smoking-related cancers (Goode et al. 2002; Le Marchand et al. 2002). Since smoking is the most potent carcinogenic factor in lung cancer, the negative association of Cys carriers with habitual smoking and the positive association of Cys carriers with lung cancer risk seem controversial. However, a similar paradoxical relationship has been observed between ALDH2 polymorphism, alcohol consumption, and liver cancer incidence. The ALDH2 Lys allele has been related to an elevated risk for liver cancer, and Lys carriers lacking ALDH2 isozyme activity ought to reduce alcohol consumption, a well-known factor in hepatocellular carcinoma (Eriksson 2001; Yokoyama and Omori 2001). However, when those individuals with the Lys allele do drink alcohol habitually, they are at much higher risk for different forms of cancer of the digestive tract, liver, and upper respiratory tract than those without the allele (Munaka et al. 2003; Yokoyama and Omori 2001). The Cys allele may preclude the use of tobacco but may exacerbate the impact of smoking on smoking-related cancer development.

Many previous studies pointed out that the single base mutation from glutamic acid (glutamate) to lysine at residue 504 in ALDH2 was the best-characterized genetic factor influencing alcohol consumption behavior (Goedde et al. 1992; Muramatsu et al. 1995). The findings of the present study are in agreement with the original studies showing Lys allele carriers suffer the alcohol-flush reaction.

None of the previous studies examined whether the EPHX2, ESR1, and CYP17A1 genes contribute to smoking behavior or the NUDT1 gene to alcohol use. We observed an increased frequency of the genotype homozygous for the minor allele of the rs1913474 SNP in ESR1, as well as a decreased frequency of the minor

Table 4 Adjusted odds ratios (OR) and 95% confidence interval (95% CI) of smoking status according to genotype of genes selected by stepwise logistic analysis. Allele A represents major allele; allele a represents minor allele

Gene symbol	SNP (rs number)	Smoking status			OR[a] (95% CI)	
		Never smokers	Past smokers	Current smokers	Current versus nonsmokers	Current versus never smokers
Genotype (Aa + aa) %						
OGG1	rs1052133	89.1 (49/55)	67.6 (48/71)	66.2 (141/213)	0.6 (0.4–1.0)	0.2 (0.1–0.6)
SLC6A4	rs717742	6.1 (3/49)	6.5 (4/62)	18.9 (36/191)	3.5 (1.5–8.2)	3.3 (1.0–11.2)
CYP17A1	rs743572	70.9 (39/55)	81.7 (58/71)	66.0 (140/212)	0.6 (0.4–1.0)	0.8 (0.4–1.6)
Genotype aa %						
EPHX1	rs2292566	12.7 (7/55)	14.1 (10/71)	5.6 (12/213)	0.4 (0.2–0.8)	0.5 (0.2–1.2)
ESR1	rs1913474	20.4 (11/54)	11.9 (8/67)	26.0 (53/204)	1.9 (1.1–3.4)	1.4 (0.7–2.9)

[a]OR was adjusted for alcohol drinking status (never, past, and current drinking)

allele of rs743572 SNP in CYP17A1, among current smokers compared with past smokers. The findings may suggest involvement of the ESR1 and/or the CYP17A1 SNP in smoking, as well as in difficulty in quitting, among homozygous minor allele carriers of the ESR1 SNP and/or major allele carriers of the CYP17A1 SNP. An American study indicated that the ESR1 gene might play a role in anxiety (Comings et al. 1999). Anxiety, perhaps, drives individuals to use tobacco for comfort.

The present population-based study using a random Japanese sample screened genes related to habitual smoking and drinking. The findings provided basic but essential information on the association between candidate genes and smoking or drinking behavior for future case-only study, which could determine the interaction between genetics and behavior. The findings also confirmed that ALDH2 gene polymorphism is associated with habitual alcohol consumption, suggesting for the first time that polymorphism in the OGG1, SLC6A4, EPHX1, ESR1, CYP17A1, and NUDT1 genes may influence tobacco and/or alcohol use among Japanese men. Allelic association is a powerful method for detecting genetic influence on complex traits such as cigarette smoking and drinking; yet it cannot formally identify the functional polymorphisms responsible for the phenotype because the association analysis is based on the linkage disequilibrium between the marker SNPs and the causative genetic variation. Further studies are needed to confirm our findings, as well as to expose the underlying molecular mechanism.

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References

Carmelli D, Swan GE, Robinette D, Fabsitz R (1992) Genetic influence on smoking–a study of male twins. N Engl J Med 327:829–833

Comings DE, Muhleman D, Johnson P, MacMurray JP (1999) Potential role of the estrogen receptor gene (ESR1) in anxiety. Mol Psychiatry 4:374–377

Eriksson CJ (2001) The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25:15S-32S

Goedde HW, Agarwal DP, Fritze G, Meier-Tackmann D, Singh S, Beckmann G, Bhatia K, Chen LZ, Fang B, Lisker R et al (1992) Distribution of ADH2 and ALDH2 genotypes in different populations. Hum Genet 88:344–346

Goode EL, Ulrich CM, Potter JD (2002) Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 11:1513–1530

Heath AC, Meyer J, Jardine R, Martin NG (1991) The inheritance of alcohol consumption patterns in a general population twin sample: II. Determinants of consumption frequency and quantity consumed. J Stud Alcohol 52:425–433

Hopfer CJ, Stallings MC, Hewitt JK (2001) Common genetic and environmental vulnerability for alcohol and tobacco use in a volunteer sample of older female twins. J Stud Alcohol 62:717–723

Hussain SP, Hofseth LJ, Harris CC (2001) Tumor suppressor genes: at the crossroads of molecular carcinogenesis, molecular epidemiology and human risk assessment. Lung Cancer 34 (Suppl 2):S7–S15

Ishikawa H, Ohtsuki T, Ishiguro H, Yamakawa-Kobayashi K, Endo K, Lin YL, Yanagi H, Tsuchiya S, Kawata K, Hamaguchi H, Arinami T (1999) Association between serotonin transporter gene polymorphism and smoking among Japanese males. Cancer Epidemiol Biomarkers Prev 8:831–833

Le Marchand L, Donlon T, Lum-Jones A, Seifried A, Wilkens LR (2002) Association of the hOGG1 Ser326Cys polymorphism with lung cancer risk. Cancer Epidemiol Biomarkers Prev 11:409–412

Lerman C, Shields PG, Audrain J, Main D, Cobb B, Boyd NR, Caporaso N (1998) The role of the serotonin transporter gene in cigarette smoking. Cancer Epidemiol Biomarkers Prev 7:253–255

McGue M, Iacono WG, Legrand LN, Elkins I (2001) Origins and consequences of age at first drink. II. Familial risk and heritability. Alcohol Clin Exp Res 25:1166–1173

Munaka M, Kohshi K, Kawamoto T, Takasawa S, Nagata N, Itoh H, Oda S, Katoh T (2003) Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. J Cancer Res Clin Oncol 129:355–360

Muramatsu T, Wang ZC, Fang YR, Hu KB, Yan H, Yamada K, Higuchi S, Harada S, Kono H (1995) Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shanghai. Hum Genet 96:151–154

Pianezza ML, Sellers EM, Tyndale RF (1998) Nicotine metabolism defect reduces smoking. Nature 393:750

Ross P, Hall L, Smirnov I, Haff L (1998) High level multiplex genotyping by MALDI-TOF mass spectrometry. Nat Biotechnol 16:1347–1351

Sabol SZ, Nelson ML, Fisher C, Gunzerath L, Brody CL, Hu S, Sirota LA, Marcus SE, Greenberg BD, Lucas FRt, Benjamin J, Murphy DL, Hamer DH (1999) A genetic association for cigarette smoking behavior. Health Psychol 18:7–13

Schlesselman JJ (1982) Case-control studies: design, conduct, analysis. Oxford University Press, New York

Shields PG, Lerman C, Audrain J, Bowman ED, Main D, Boyd NR, Caporaso NE (1998) Dopamine D4 receptors and the risk of cigarette smoking in African-Americans and Caucasians. Cancer Epidemiol Biomarkers Prev 7:453–458

Sugimura H, Wakai K, Genka K, Nagura K, Igarashi H, Nagayama K, Ohkawa A, Baba S, Morris BJ, Tsugane S, Ohno Y, Gao C, Li Z, Takezaki T, Tajima K, Iwamasa T (1998) Association of Ile462Val (Exon 7) polymorphism of cytochrome P450 IA1 with lung cancer in the Asian population: further evidence from a case-control study in Okinawa. Cancer Epidemiol Biomarkers Prev 7:413–417

Tsugane S, Gey F, Ichinowatari Y, Miyajima Y, Ishibashi T, Matsuishima S, Hirota Y, Inami T, Yamaguchi M, Karita K, Kabuto M, Takeshima Y, Todoriki H, Tsuda M, Akabane M, Furuichi Y, Hamada G, Watanabe S (1992a) Cross-sectional epidemiologic study for assessing cancer risks at the population level I. Study design and participation rate. J Epidemiol 2:75–81

Tsugane S, Gey F, Ichinowatari Y, Miyajima Y, Ishibashi T, Matsuishima S, Hirota Y, Inami T, Yamaguchi M, Karita K, Kabuto M, Takeshima Y, Todoriki H, Tsuda M, Akabane M, Furuichi Y, Hamada G, Watanabe S (1992b) Cross-sectional epidemiologic study for assesing cancer risks at the population level II. Baseline data and correlation analysis. J Epidemiol 2:83–89

Wu X, Hudmon KS, Detry MA, Chamberlain RM, Spitz MR (2000) D2 dopamine receptor gene polymorphisms among African-Americans and Mexican-Americans: a lung cancer case-control study. Cancer Epidemiol Biomarkers Prev 9:1021–1026

Yokoyama A, Omori T (2001) Alcohol and oropharyngolaryngeal and digestive tract cancer. Nihon Arukoru Yakubutsu Igakkai Zasshi 36:551–566

Yoshida K, Hamajima N, Kozaki K, Saito H, Maeno K, Sugiura T, Ookuma K, Takahashi T (2001) Association between the dopamine D2 receptor A2/A2 genotype and smoking behavior in the Japanese. Cancer Epidemiol Biomarkers Prev 10:403–405

Yoshimura K, Hanaoka T, Ohnami S, Kohno T, Liu Y, Yoshida T, Sakamoto H, Tsugane S (2003) Allele frequencies of single nucleotide polymorphisms (SNPs) in 40 candidate genes for gene-environment studies on cancer: data from population-based Japanese random samples. J Hum Genet 48:654–658