Genetic Polymorphism of Enzymes Involved in Xenobiotic Metabolism and the Risk of Colorectal Cancer

Chikako Kiyohara

Environmental factors such as smoking cigarette, diets and alcohol may interact with genetic factors, which put one individual at a greater or lesser risk of a particular cancer than another. Advances in molecular biology have allowed many allelic variants of several drug metabolizing enzymes so that individuals with the susceptible genotypes can be determined easily. Many pieces of research have focused on the relationship between the distribution of polymorphic variants of different forms of the metabolic enzymes and colorectal cancer susceptibility because of importance roles of the metabolic enzymes in the activation of many procarcinogens or chemicals. In this respect five groups of the metabolic enzymes, cytochrome P450 (CYP) 1A1/CYP1A2, glutathione S-transferases (GSTs), N-acetyltransferases (NATs), aldehyde dehydrogenase 2 (ALDH2) and methylenetetrahydrofolate reductase (MTHFR), have been discussed here. A positive association between development of colorectal cancer and the mutant homozygous genotype in Mspl polymorphism of CYP1A1 gene has been reported in Japanese in Hawaii. The relation between genetic polymorphisms in GSTs and cancer risk has also taken an interest. At least nine studies have demonstrated the relation between the GST polymorphisms and colorectal cancer. Two of these studies suggested an increased risk of approximately 2-fold among those with the GSTM1 null genotype, while others found no risk increase. None of these studies examined the combined effect of CYP1A1 and GST polymorphisms. Either NAT2 or CYP1A2 alone have been slightly associated with colorectal cancer. When CYP1A2 and NAT2 phenotype were combined, a significant increased risk (odds ratio of 2.8) was seen among well done meat consumers with the rapid-rapid phenotype. Two published studies have found that the risk of colorectal cancer can be enhanced (2-3 fold) in alcohol drinkers with heterozygous genotype of ALDH2 in two Japanese populations recently. Findings from three published studies suggested that the mutant genotype of MTHFR inversely slightly associated with colorectal cancer.

Although some of genetic polymorphisms discussed here have not shown statistically significant increase/decrease in risk, individuals with differing genotypes may have different susceptibilities to colorectal cancer, based on environmental factors. Further studies are needed to identify risk groups more specific and to determine factors of importance in colorectal cancer development. J Epidemiol, 2000; 10: 349-360

colorectal cancer, molecular epidemiology, cytochrome P4501A1, cytochrome P4501A2, glutathione S-transferase, N-acetyltransferase, mitochondrial aldehyde dehydrogenase, methylenetetrahydrofolate reductase
INTRODUCTION

Colorectal cancer is the fourth and the second leading cause of cancer death in male and female Japanese, respectively. Genes that interact with environmental or dietary factors, including cigarette smoking, may play a key role in colorectal carcinogenesis. The attributable risk for metabolic polymorphisms for carcinogens or substances derived from diet with a modest risk increase (e.g., the odds ratio (OR) for the null genotype of glutathione S-transferase (GST) M1 is 2.0) may be higher than that of rare mutations in DNA mismatch repair genes such as hMSH1 and hMSH2 with much higher risk increase (mutation in those gene are frequently found in the germline cells of hereditary nonpolyposis colorectal cancer families). In addition studying gene-environment interactions in relation to risk of cancer may be valuable because positive findings would clearly implicate the substrates with which the gene interacts as disease-causing exposures, clarify cancer etiology and point to preventive dietary and other environmental modifications.

Cigarette smoking, consumption of diets high in red meat and alcohol use probably increase the risk of colorectal cancer. Also cooking meat at high temperatures possibly increase the cancer risk. Compounds in cigarette smoke or diets are modified by drug metabolizing enzymes and some of the metabolites may be the cause of colorectal cancer.

Polycyclic aromatic hydrocarbons (PAHs) and other tobacco-related carcinogens are activated by phase I enzyme cytochrome P450 (CYP) 1A1 and detoxified by phase II enzyme GSTs. The metabolic balance between phase I and phase II enzymes may be of importance to determine genetic susceptibility to colorectal carcinogenesis as well as lung carcinogenesis.

IARC judged some kinds of the heterocyclic amines (HCAs) to be possible or probable carcinogens. Humans are exposed to HCAs when they consume fish or meat cooked at very high temperature. HCAs are metabolically activated by CYP1A2 and further activated by N-acetyltransferase (NAT2). Mitochondrial aldehyde dehydrogenase (ALDH2) eliminates most of the acetaldehyde produced during alcohol metabolism, with two alleles ALDH2*1 and ALDH2*2. A mutated ALDH2 homozygotes (ALDH2*1/ALDH2*1) contributes to decreased activity of the enzyme while the wild-type ALDH2*1/ALDH2*2 genotype produced tolerance to alcohol use. It is likely that individuals with the wild-type genotype of ALDH2 gene have decreased risk of alcohol-induced colorectal cancer.

In epidemiological studies, high levels of dietary folate have been inversely associated with colorectal cancer. MTHFR is a key regulatory enzyme in the metabolism of folate. The homozygous mutant genotype of MTHFR gene, which shows decreased enzyme activity, was recently shown to be related to decreased risk of colorectal cancer in three studies.

In this paper, we discuss the relationship between genetic polymorphism of enzymes involved in xenobiotic metabolism and colorectal cancer, with special emphasis on the most investigated genes/enzymes of CYP1A1, CYP1A2, GSTs, NATs ALDH2 and MTHFR.

CYP1A1/GST POLYMORPHISMS AND CIGARETTE SMOKING

Although cigarette smoking has been clearly implicated as a cause of a number of cancers, evidence for an association between cigarette smoking and colorectal cancer appears inconsistent. More recent studies have tended to find cigarette smokers to be at higher risk for colorectal cancer, however. In addition, cigarette smoking has been consistently associated with colorectal adenomas, the precursor of colorectal cancer. The influence of smoking on risk for cancers would most likely be caused by PAHs. These carcinogens can readily reach the mucosa of the colon through the circulatory system. There is also evidence of constituents of cigarette smoke reaching other organs through the circulation. Aromatic-DNA adducts have been identified in human endometrium and breast. If PAHs in cigarette smoke are involved in colorectal carcinogenesis, genetic polymorphisms in the carcinogen metabolizing enzymes are likely to influence the risk of colorectal cancer.

Two phases exist in the metabolism of most chemical carcinogens. In the phase I reaction, the carcinogens exert their effect only after being metabolically activated to intermediate metabolites, which are capable of binding to DNA and causing mutations. The most ubiquitous phase I catalysts are the CYPs. CYP1A1, one form of CYPs, is active toward PAHs such as benzo(a)pyrene (BP). PAH metabolism has been described to take place in the colon. CYP1A1 gene is predominantly expressed in many extrahaepatic tissues. To date, four polymorphic sites in CYP1A1 gene have been reported and among those MspI polymorphism may contribute individual cancer susceptibility as genetic modifiers of cancer risk. MspI polymorphism (T to C transition) in the 3'-flanking region of CYP1A1 gene, which can be classified into 3 genotypes, predominant homozygous alleles (genotype A), heterozygote (genotype B) and homozygous rare alleles (genotype C). Increased inducibility of CYP1A1 enzymatic activity was recently observed among subjects with genotype C. The frequency of genotype C allele is 0.33 in Japanese population, and less than 0.10 in Caucasian populations. Thus, the frequencies of genotype C in Caucasians and Japanese population are less than 1 % and 10 %, respectively. A small case-control study suggested that genotype C was positively associated with in situ colorectal cancer in Japanese in Hawaii (P = 0.008), while no such association was found in Caucasians probably due to the lack of power. The OR for genotype C compared with genotypes A and B combined was...
7.9 (95% confidence interval (CI), 1.4-44.4) in Japanese in Hawaii.

Following phase I reaction, phase II enzymes such as GSTs are responsible for detoxification of activated forms PAH epoxides. GSTs are constitutively found in a wide variety of tissues, with different characteristic patterns of GST isozymes. GSTs also form a superfamily of genes consisting of four distinct families, named alpha, mu, pi and theta. Certain genes within the GSTM, GSTT and GSTP subfamilies (GSTM1, GSTT1 and GSTP1 genes, respectively) are polymorphic in humans. The phenotypic absence of GSTM1 and GSTT1 activity is due to homozygosity for deletion of these genes, termed the null genotype. Two genetic polymorphisms at the GSTP1 locus results from a single base pair substitution in exon 5 (Ile105Val) and exon 6 (Ala114Val). In vitro cDNA expression study suggests that substitution of these amino acid reduces enzyme activity.

The homozygous deletion of GSTM1 gene has been shown to occur in approximately 50% of the populations of various ethnic origins, while the homozygous deletion of GSTT1 gene has distributed between 10 and 64% of various ethnic groups. The frequency of the GSTM1 null genotype in Caucasian populations is 30% or less but that in Oriental populations may be similar to the frequency of the GSTM1 null genotype. The GSTP1 polymorphism in exon 6 is less common than that in exon 5. Individuals with homozygous for the 105 valine allele (the mutant allele) are most common among African-Americans (18%) and least common among Japanese (2%) with European-Americans (11%) intermediate between these groups.

Most interest in the possible consequences of GST polymorphisms have focused on the polymorphism at the GSTM1 gene loci. In an earlier study, the GSTM1 null genotype was found to yield a 1.8-fold increased risk for colon cancer. In the second study, an excess of the nulled individuals were seen in colorectal cancer but this was not a significant. When the patients were divided into cancers occurring in the proximal or distal colon, the null genotype became a significant risk factor among the subgroup with distal colorectal tumors (OR, 2.03; 95% CI, 1.06-3.90). Therefore, two of eight showed approximately 2-fold increased risk for colorectal cancer (Table 1). Five published studies have examined the relationship between GSTT1 null genotype and colorectal cancer risk. One of these studies showed the GSTT1 null genotype was related to significantly increased risk colorectal cancer with an OR of 1.9 (95% CI, 1.3-2.8). Two studies have examined the frequency of GSTP1 polymorphism in exon 5; neither study reported any association between GSTP1 polymorphism and susceptibility to colorectal carcinogenesis.

Genetically determined susceptibility to smoking-related cancers may depend on the metabolic balance between phase I and phase II enzymes. BP, one of the most typical PAHs, is metabolized to ultimate carcinogen diol-9,10-oxide by phase I enzyme CYP1A1. Subsequently, the ultimate carcinogen can be metabolized further to innocuous water soluble metabolites through conjugation with glutathione by phase II enzymes GSTs. It is likely that individuals with more reactive phase I enzymes and less efficient phase II enzymes might be at higher risk of cancer than individuals with the opposite combination. Smoking-related risk of colorectal cancer may be more accurately estimated when genetic susceptibility is allowed for as regards both CYP1A1 and GST genotypes. To date, none of these studies examined the combined effect of CYP1A1 and GST polymorphisms.

### Table 1. Relation of GST polymorphisms to colorectal cancer.

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>GSTT1</th>
<th>GSTP1</th>
<th>No. of cases/controls</th>
<th>Combination with smoking</th>
<th>Place of study</th>
<th>Published Year</th>
</tr>
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<tbody>
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<td>1993[2]</td>
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<td>132/200</td>
<td>Australia</td>
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<td></td>
</tr>
<tr>
<td>2.03</td>
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<td>1.9</td>
<td>54/126</td>
<td>Japan</td>
<td>1996[38]</td>
<td></td>
</tr>
<tr>
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<td>NS</td>
<td>218/448</td>
<td>U.K.</td>
<td>1996[39]</td>
<td></td>
</tr>
<tr>
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<td>1567/1889</td>
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<td>1998[41]</td>
<td></td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
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<td>U.S.A.</td>
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<td></td>
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<tr>
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<td>NS</td>
<td>NS</td>
<td>1567/1889</td>
<td>U.S.A.</td>
<td>1999[43]</td>
<td></td>
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<tr>
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<td>NS</td>
<td>196/178</td>
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<td>1999[44]</td>
<td></td>
</tr>
<tr>
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<td>NS</td>
<td>NS</td>
<td>100/155</td>
<td>U.K.</td>
<td>1999[36]</td>
<td></td>
</tr>
</tbody>
</table>

NS indicates that ORs were not significantly different from a value 1.
- not done

a Limited to distal colon.

### CYP1A2/NAT POLYMORPHISMS AND HETEROCYCLIC AMINES

In 1993, IARC judged the HCAs 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo-
zo[4,5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-
phenylimidazo[4,5-b]pyridine (PhIP) to be possible human
carcinogens and 2-amino-3-methylimidazo[4,5-f]quinoline
(IQ) as probably carcinogenic 6. They are formed from
the heating of natural precursors, such as creatine, amino acids and
possibly sugar, in the food 40.

Humans are exposed to HCAs when they consume fish or
meat cooked at very high temperature. Especially, fried meat
is an important source of exposure to HCAs and parameters
influencing the intake are the amount and type of meat ingested,
frequency of consumption, cooking method, cooking tempera-
ture and the length of cooking 40. Given uniform cooking
methods, red meats, such as pork and beef, contain the highest
HCAs, followed by chicken and then fish. Cooked organ
meats and non-meat sources of protein, such as eggs, cheese
and beans, contain very low or undetectable concentrations of
HCAs when using routine methods 45. Gravy is also an impor-
tant source of exposure in countries where gravy and brown
source are prepared from the pan residue after the meat has
been fried 40. HCA intake from gravy contributes up to 30% of
total HCA intake in Swedish population 49. Cooking at low
temperature, less than 200 °C, such as steaming, boiling, stewing
(up to 100 °C), baking, microwaving and roasting (100-200 °C)
can produce little or no HCAs. Cooking at high temperature,
more than 200 °C, frying with fat or oil, broiling, and grilling
(barbecuing) can generate considerable HCAs. Moreover,
formation of MelQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]
quinoxaline(DiMeIQx) and PhIP are temperature dependent
when the same cooking method is employed. For example,
formation of PhIP rises from 0.2 ng/g fired bacon at 175 °C to
4.5 ng/g fried bacon at 225 °C; such a 20-fold increase is also
seen for the others 46. The intake of HCAs from meat, fish and
gravy, in descending order, is PhIP, MelQx, DiMeIQx and,
finally, IQ 40. In long term feeding studies with rats, PhIP,
which is the most abundant HCA in cooked meat 40, has been
shown to cause colon cancer 52,50. A high intake of well-done
meat is a possibly risk factor for human colorectal cancer 5.
This effect could be due to HCAs such as PhIP, MelQx and
DiMeIQx.

HCAs, similar to other xenobiotics, are metabolized by vari-
ous hepatic and extrahepatic enzymes. The initial step of
metabolic activation of HCAs is thought to be N-hydroxy-
lization by hepatic enzyme CYP1A2 7. HCAs are poor substrates
for N-acetylation by N acetyltransferase 2 (NAT2) in the liver.
The N-hydroxy metabolites may serve as substrates for hepatic
conjugation by β-glucuronidases and reabsorption can occur.
Subsequently, NAT2, which is present at high levels in human
colon, can catalyse the O-acetylation of the N-hydroxy hetero-
cyclic amines to form the carcinogenic derivatives 7. Finally
these metabolites readily bind to DNA and initiate the neo-
plastic process. In contrast, for arylamines known to be human
bladder carcinogens, such as 4-aminobiphenyl, N-hydroxy-
lization by CYP1A2 and N-acetylation by NAT2 represent compet-
ing activation and detoxification reactions in the liver. In that
case, the levels of the N-hydroxy metabolites are controlled in
the liver. Subsequently, the proximate carcinogenic N-
hydroxyl arylamines can enter the circulation and be transport-
ted to the target organs such as the bladder 55.

There is a considerable interindividual variation in the activi-
ty of both CYP1A2 and NAT2 56. The reasons for the variabil-
ity of these two enzymes are different. For CYP1A2 activity,
both genetic and environmental factors, about 50% each, are
likely to be responsible for the variability in the enzyme activi-
ty 50. In addition, the polymodal (bi- or trimodal) distribution
of CYP1A2 activity has been reported independently several
laboratories, in which 23-89% of a given population are rapid
CYP1A2 phenotype 50. Various environmental factors, such as
cigarette smoking, certain dietary components, e.g., cruciferous
vegetables and PAHs, as well as HCAs are known to induce the
CYP1A2 activity while oral contraceptive may inhibit the
enzyme activity 50. Despite intensive studies regarding a few
polymorphic variants of the CYP1A2 gene, a clear genetic
source of the variability remains to be demonstrated. CYP1A2
phenotype is highly inducible enzyme system in human tis-
issues following HCAs exposures 50 and one measure of pheno-
type may reflect a relatively recent exposure to inducers or
inhibitors.

In contrast, for NAT2, the variability is primarily due to
genetic variants that determine function, with environmental
factors playing a minor role in the phenotype 50. Alleles
responsible for the variation in NAT2 activity in different eth-
nic groups are well known. The frequency of the NAT2 rapid
acetylator genotype ranges from 41 to 44 % in Caucasians and
from 72 to 92 % in East Asians 41,43,44,61-69). Several studies
have shown that the N-acetyltransferase 1 (NAT1) enzyme can
also acetylate arylamines and HCAs 70,71) but less is known
about the expression of NAT1 and its role in carcinogenesis.
The NAT1 enzyme had frequently been referred to as the
monomorphic, relative to the polymorphic NAT2 enzyme.
Recently some studies have revealed that there is a consid-
erable structural variation in the NAT1 gene 60 and that slow
NAT1 phenotypes exist 71,72). Approximately 30% of popula-
tion with European ancestry carry a variant NAT1*10 allele 71,
which is the rapid allele. Since the relationship between NAT1
and NAT2 polymorphisms appears non-random, a linkage
between NAT2 and NAT1 has been suggested 71.

A recent study suggested the 'current' rapid CYP1A2 pheno-
type is significantly associated with increased risk of colon
cancer or polyp disease, giving an OR of 1.91 (95% CI, 1.20-
2.87) 57). An important role of the CYP1A2 phenotype in colon
carcinogenesis is recently suggested by one study 57 while a
number of studies have verified whether individuals with the
NAT2 rapid acetylator phenotype or genotype are predisposed
to the development of colorectal cancer. So far nineteen stud-
Metabolic Polymorphisms and Colorectal Cancer

Table 2-1. Relation of NAT2 phenotype to colorectal cancer.

<table>
<thead>
<tr>
<th>NAT2</th>
<th>No. of cases/controls</th>
<th>Combination with meat</th>
<th>Place of study</th>
<th>Published Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.37 (1.0-5.9)</td>
<td>43/41</td>
<td>–</td>
<td>U.S.A.</td>
<td>1986&lt;sup&gt;74&lt;/sup&gt;</td>
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<td>3.73 (1.4-9.6)</td>
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<td>–</td>
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<td>1987&lt;sup&gt;75&lt;/sup&gt;</td>
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<tr>
<td>2.48 (1.0-6.0)</td>
<td>43/41</td>
<td>–</td>
<td>U.S.A.</td>
<td>1990&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>NS</td>
<td>109/96</td>
<td>–</td>
<td>Spain</td>
<td>1991&lt;sup&gt;78&lt;/sup&gt;</td>
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<tr>
<td>NS</td>
<td>25/12</td>
<td>–</td>
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<td>1991&lt;sup&gt;79&lt;/sup&gt;</td>
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<tr>
<td>NS</td>
<td>34/205</td>
<td>6.45&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1994&lt;sup&gt;57&lt;/sup&gt;</td>
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<td>1.8 (1.0-3.3)</td>
<td>89/110</td>
<td>1.7 (0.9-3.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Australia</td>
<td>1996&lt;sup&gt;77&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NS indicates that ORs were not significantly different from a value 1.

a Well done preference with rapid CYP1A2/rapid NAT2 versus rare-medium preference with the opposite combination.

b Dose-response effect with increasing exposure.

Table 2-2. Relation of NAT polymorphisms to colorectal cancer.

<table>
<thead>
<tr>
<th>NAT2</th>
<th>NAT1</th>
<th>No. of cases/controls</th>
<th>Combination with meat</th>
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<td>–</td>
<td>36/36</td>
<td>–</td>
<td>Japan</td>
<td>1994&lt;sup&gt;63&lt;/sup&gt;</td>
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<tr>
<td>NS</td>
<td>–</td>
<td>234/329</td>
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<td>–</td>
<td>103/96</td>
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<td>U.K.</td>
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<td>–</td>
<td>U.K.</td>
<td>1999&lt;sup&gt;44&lt;/sup&gt;</td>
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</table>

NS indicates that ORs were not significantly different from a value 1.

–: not done

a The combination of rapid genotype and frequent fried meat consumption versus the remainder combinations.

b The combination of rapid NAT2/rapid NAT1 genotypes and more frequent red meat intake versus the combination of rapid NAT2/rapid NAT1 genotypes and less frequent red meat intake, among men aged 60 years old or older.
meat consumers, the more pronounced OR of 2.79 (95% CI, 1.69-4.47) for the combined rapid-rapid phenotype strongly indicated that the proposed metabolic pathway and the level of exposure to HCAs could play a significant role in the risk for colorectal cancer.

**ALDH2 POLYMORPHISM AND ALCOHOL USE**

The evidence that alcohol increases the risk of upper aerodigestive tract cancers such as esophageal cancer is convincing 5). High alcohol use probably increases the risk of colorectal cancer and the effect generally seems to be related to total ethanol intake, irrespective of the type of alcohol beverage 5).

Taken orally, ethanol is readily absorbed from the gastrointestinal tract and diffuses rapidly and uniformly throughout the body water. The direct carcinogenic effects of alcohol on several sites of cancer and alcohol-related physical alternations have been attributed to acetaldehyde rather than to alcohol itself. The major pathway for the disposition of ethanol is its oxidation in the liver to acetaldehyde and hydrogen. Other tissues such as kidneys, stomach and intestine oxidize ethanol to a small extent 80, 81). Although ethanol is also oxidized in the large intestine to acetaldehyde via colonic bacteria 82), the bacterial capacity of oxidation is low 80). Two principal enzymes, the cytosolic alcohol dehydrogenase (ADH) and the major non-ADH pathway CYP2E1 enzyme, are responsible for the oxidation of ethanol. The further oxidation of acetaldehyde to acetate, which is converted to carbon dioxide via the citric acid cycle, is catalyzed by the ALDH2. Most of the acetaldehyde generated during alcohol metabolism is eliminated by ALDH2 9).

Considerable interindividual variations as well as ethnic differences in alcohol metabolism rate have been reported 80). Protein structural studies have revealed that a point mutation in exon 12 is responsible for the inactivation of ALDH2 isozyme 85). The presence of a single ALDH2*2 allele results in the deficient ALDH2 enzymatic activity 80). Those with the wild-type homozygote (ALDH2*1/ALDH2*1) have great tolerance to alcohol and can drink alcoholic beverages. Those with the mutant homozygote (ALDH2*2/ALDH2*2) are highly intolerant to alcohol and consequently do not drink because the alcohol-flush reaction in the result of excessive acetaldehyde accumulation and the unpleasant symptoms tend to reduce alcohol consumption. The heterozygotes have blood acetaldehyde concentrations ~6 times higher than the wild-type homozygotes 87). Takeshita et al. reported that the mean amount of alcohol consumption in the heterozygotes is about one half that in the wild-type homozygotes 86). As the genotype of ALDH2 serves as an indicator of acetaldehyde exposure after alcohol consumption 80, 81), alcohol drinking may be more harmful for the heterozygotes than the wild-type homozygotes.

Oriental populations of Mongoloid origin show up to 50% isozyme deficiency, whereas none of the Caucasoid and the Negroid populations screened have this isozyme abnormality 86). In a Japanese population, frequencies of wild-type homozygotes (ALDH2*1/ALDH2*1) and carriers with the mutant allele ALDH2*2 are 56 and 44%, respectively 89).

A positive association between upper aerodigestive tract cancers and ALDH2 polymorphism has been described 91, 92). The levels of blood acetaldehyde related to the ALDH2 genotypes may be associated with the risk of colorectal cancer as well as upper aerodigestive tract cancers 85, 86). Two case-control studies 85, 86) in Japan reported an increased risk of colorectal cancer with the ALDH2*1/ALDH2*2 as compared with subjects with the ALDH2*1/ALDH2*1 genotype (Table 3). The first study 86) conducted among alcoholics showed a significantly increased OR of 3.35 in the presence of the ALDH2*2 allele. All subjects with ALDH2*2 allele were ALDH2*1/ALDH2*2 heterozygotes and the daily alcohol consumption did not differ between colon cancer and cancer-free alcoholics. An increased risk of colon cancer was also observed for the carriers with

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>No. of cases/controls</th>
<th>Place of study</th>
<th>Published Year</th>
</tr>
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<td>3.4 (1.5-7.5)</td>
<td>46/487</td>
<td>Japan</td>
<td>1998(s)</td>
</tr>
<tr>
<td>2.13 (1.0-4.7)</td>
<td>265/794 (Colon)</td>
<td>Japan</td>
<td>1994(s)</td>
</tr>
<tr>
<td>1.03 (0.5-2.2)</td>
<td>164/794 (Rectum)</td>
<td>Japan</td>
<td>1994(s)</td>
</tr>
</tbody>
</table>

a The heterozygotes versus the wild-type homozygotes.
b Alcoholic men aged 40 years or older.
c Adjusted for age at admission, daily alcohol consumption and amount of cigarette smoking.
d Adjusted for age, daily alcohol consumption, amount of cigarette smoking and dietary factors.
Chromosome breaks appear to be important in nearly all this abnormal base might labilize DNA to strand breaks and increase of the uracil (U) content in DNA and removal of development of deoxynucleotide pool imbalances. This leads to the other hand, methyl (folate) deficiency may also cause imbalances in DNA methylation in colon carcinogenesis. On the other hand, methyl (folate) deficiency may also cause imbalances in DNA methylation in colon carcinogenesis.

Evidence has been accumulated as regards the role for disturbances in DNA methylation in colon carcinogenesis. This imbalance in methylation of DNA is thought to result in abnormal expression of oncogenes and tumor suppressor genes. An interaction between the ALDH2 genotypes and alcohol consumption should be assessed in both studies. If the effect of alcohol consumption is limited to a genetically predisposed subgroup, evaluation of alcohol without genetic information may lead to it being dismissed as a cause of disease because the overall effect may be diluted when susceptible and nonsusceptible individuals are combined.

The relationship between alcohol use and colorectal cancer is less clear in Caucasians than in Orientals of Mongoloid origin. This disagreement may be partly due to the ethnic difference in ALDH2*2 allele frequency. Japanese may be more sensitive to alcohol-induced colorectal carcinogenesis than Caucasians.

Much work remains in elucidation of the underlying mechanisms that link the ALDH2*2 allele and colorectal cancer.

**MTHFR POLYMORPHISM AND FOLATE / ALCOHOL**

Various naturally occurring constituents in vegetables have been studied for their chemopreventive potential against colorectal cancer. Evidence that diets rich in vegetables protect against colorectal cancer is convincing.

Folate, which is rich in fresh leafy or cruciferous vegetables such as spinach, lettuce, cabbage, broccoli, brussels sprouts and cauliflower, has recently been the subject of much research interest. Animal and in vitro cell studies suggested a key role for folate in colon carcinogenesis. In epidemiological studies, high levels of dietary folate or high blood folate levels have been inversely associated with colorectal cancer. Furthermore, diets high in folate particularly in combination with low alcohol use, are associated with decreased risk of colorectal cancer.

Folate is essential for regeneration of methionine, the methyl donor for DNA methylation, and for producing the purines and pyrimidines required for DNA synthesis. Decreased availability of folate may contribute to aberrations in DNA methylation. This imbalance in methylation of DNA is thought to result in abnormal expression of oncogenes and tumor suppressor genes. Evidence has been accumulated as regards the role for disturbances in DNA methylation in colon carcinogenesis. On the other hand, methyl (folate) deficiency may also cause interference with the thymidylate biosynthesis and result in development of deoxynucleotide pool imbalances. This leads to increase of the uracil (U) content in DNA and removal of this abnormal base might labilize DNA to strand breaks. Chromosome breaks appear to be important in nearly all human cancers and are especially common in colorectal cancer.

**MTHFR** is a critical enzyme regulating the metabolism of folate. MTHFR catalyzes the biologically irreversible reduction of 5,10-methyleneTHF to 5-methyl THF, the major circulatory form of folate and carbon donor for the remethylation of homocysteine to methionine. A variant of the human MTHFR gene that results in alanine to valine substitution has been described at bp 677. This is an autosomal recessive mutation. This mutation codes for thermolabile enzyme with reduced MTHFR activity, resulting in elevated plasma homocysteine levels. Individuals with homozygous for this variant have been reported as having 30% of normal enzyme activity and heterozygotes have been reported as having 65% of normal enzyme activity.

The allele frequency of the mutant genotype of MTHFR in Japanese populations is estimated to be 0.33, which is comparable to that in European and American Caucasian populations. In contrast, the mutation has a very low prevalence in African-Americans, for whom the mutation was absent in homozygosity.

Epidemiologic evidence, including that from prospective studies done in males, suggested that subjects with the homozygous mutation were consistently shown to have an approximately two-fold decrease in the risk of colorectal cancer compared with the wild-type homozygous and heterozygous genotype combined (Table 4). As shown in Table 4, the decreased risk associated with the mutant genotype was seen only in those consuming little or no alcohol. Alcohol has been hypothesized as having several possible mechanisms that may influence carcinogenesis, including one that may indirectly alter DNA methylation patterns by affecting the intestinal absorption, hepatobiliary metabolism and renal excretion of folate. The second study estimated that ORs for the mutant genotype was 0.8 (95% CI, 0.6-1.1) for males and 0.9 (95% CI, 0.6-1.2) for females as compared with that for the wild-type homozygote and showed a weak protective effect on colon cancer risk among individuals with the mutant homozygous genotype. The magnitude of the association was less than that reported previously.

Individuals with the mutant homozygote have lower levels of plasma 5-methyl THF because the conversion of 5,10-methylene THF to 5-methyl THF, which is essentially irreversible under physiological conditions, is less efficient. Thus they may be less prone to get into imbalances of nucleotide pools during DNA synthesis. Low levels of 5-methyl THF probably result in lower levels of cellular methionine and S-adenosylmethionine, which is required for DNA methylation, and then lead to aberrant DNA methylation. When dietary methyl supply is enough, individuals with the mutant homozygous genotype are at reduced risk of colorectal cancer probably because higher levels of 5,10-methylene THF may prevent alternation of DNA synthesis.

In contrast, when the methyl content in dietary intake is low
Table 4. Relation of MTHFR polymorphism to colorectal cancer.

<table>
<thead>
<tr>
<th>OR 1 (95% CI)</th>
<th>OR 2 (95% CI)</th>
<th>No. of cases/controls</th>
<th>Place of study</th>
<th>Interaction with alcohol</th>
<th>Published year</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6&lt;sup&gt;a&lt;/sup&gt; (0.3-1.1)</td>
<td>0.1&lt;sup&gt;a&lt;/sup&gt; (0.01-0.9)</td>
<td>144/627</td>
<td>U.S.A</td>
<td>P=0.02</td>
<td>1996&lt;sup&gt;(2)&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.9&lt;sup&gt;b&lt;/sup&gt; (0.3-0.8)</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt; (0.3-0.8)</td>
<td>202/306</td>
<td>U.S.A</td>
<td>P&lt;0.01</td>
<td>1997&lt;sup&gt;(3)&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

OR 1: Multivariate-adjusted risk.
OR 2: Risk for the combination of genotype and alcohol use.
NS: P value was not less than 0.05.

<sup>a</sup> The mutant and heterozygous genotypes versus wild-type genotype.
<sup>b</sup> The mutant homozygotes versus the wild-type homozygotes and heterozygotes combined.

or depleted by alcohol consumption, the mutant homozygotes may be less able to compensate, leading to potentially oncogenic alternations in DNA methylation; the protective association of the MTHFR polymorphism is thus eliminated. Since alcohol may deplete 5-methyl THF, individuals with the mutant homozygote would be predicted to be particularly disadvantaged.

Thus far, all of three studies, to greater or lesser degrees, showed a protective effect of the mutant genotype on colorectal carcinogenesis. Dietary supply may be particular critical among the mutant homozygotes of MTHFR gene.

CONCLUSIONS

The application of molecular epidemiology to the study of genetic polymorphism of enzymes involved in xenobiotic metabolism has met with some difficulty. As discuss here, there are numerous conflicting reports on the association between different genotypes such as NAT genotypes and colorectal cancer risk. It is evident that there are large interethnic differences in those genes, as exemplified here by comparing allele frequencies in some populations. In the CYP1A1 locus, the detrimental mutations frequently found in the CYP1A1 gene among Japanese populations cannot be appreciably identified among Caucasian populations. This is also the case for ALDH2 gene. Thus connection of between cancer and a particular polymorphic site in one ethnic group might be of limited value of as a genetic marker for cancer in another ethnic group and extrapolations should be avoided.

It is concluded that, at the present stage, a few of the polymorphic sites stated here can yet be used as biomarkers for increased/decreased colorectal cancer risk. To obtain a better understanding of genetic susceptibility to environmental factors, studies are needed to consider genetic characteristics of the population that may alter genetic susceptibility in combination with high and low risk environmental factors.

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