MINI-REVIEW

Gene-environment Interactions and Polymorphism Studies of Cancer Risk in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center II (HERPACC-II)

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Abstract

Genetic polymorphisms may modify the effects of environmental risk factors on cancer occurence. We have recently launched a comprehensive epidemiologic project, HERPACC II (Hospital-based Epidemiologic Research Program at Aichi Cancer Center II), including both lifestyle and polymorphism data, following HERPACC-I which solely concentrated on lifestyle data. As of April 2001, about 3000 samples of DNA are being stored to conduct case-control studies. Genotyping of 46 polymorphisms has been conducted at the laboratory of the Division of Epidemiology and Prevention. Twelve case-control studies and two papers on a new PCR method, PCR-CTPP (polymerase chain reaction with confronting two-pair primers), have been accepted for publication. Significant findings in Japanese were found for 1) gene-environment interaction for esophageal cancer between heavy drinking and *aldehyde dehydrogenase 2 (ALDH2)*, 2) malignant lymphoma risk with *methylenetetrahydrofalate reductase (MTHFR)* and *methionine synthase (MS)*, 3) interactions between smoking and two polymorphisms, *interleukin 1B (IL-1B)* and *myeloperoxidase (MPO)* for *Helicobacter pylori* infection, and 4) smoking habits with *dopamine receptor D2 (DRD2)* and *IL-1B*. Further studies on interactions with polymorphisms will continue to be conducted for Japanese, using larger sizes of samples.

Key Words: lifestyle - genetic polymorphism - cancer risk – case-control study – PCR-CTPP

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Introduction

Epidemiologists have for many years been examining the associations between disease risk and many potential risk factors related to environment exposure and host characteristics. What they learned is that there are many inconsistent findings on associations among different studies. This might be explained partly by random variation, but it is also plausible that the inconsistency is caused by unmeasured characteristics of the study subjects. Genetic traits are considered to be features which could modify the strength of associations, but appropriate tools to examine genetic traits have not been available for epidemiologists until relatively recently.

Now, the PCR (polymerase chain reaction) technique can be used for genotyping also by epidemiologists. This has opened the door to a new field, where measurement of associations with environmental exposures can be conducted with reference to the genotype. Epidemiologic studies with genotype information should allow us to identify the individuals more susceptible to environmental factors. It is well known that smoking elevates the risk of lung cancer, but we do not know well which people are the most sensitive to carcinogens contained in tobacco smoke. Since only a proportion of smokers suffer from lung cancer, genetic traits must play pivotal roles in lung cancer carcinogenesis. Examining genotypes is expected to provide clues to distinguish more susceptible from less susceptible groups,

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and also help resolve biological mechanisms of carcinogenesis and to develop cancer prevention strategies (Perera, 2000). The original main role of epidemiology was, as shown in Figure 1, to measure the association with environmental exposures to prevent diseases. The study objects derived from polymorphism genotyping are: 1) effect modifications by genotype; 2) associations between exposure and genotype; and 3) associations between polymorphisms and disease risk. The elucidation of functional differences among different genotypes and biological mechanisms connecting the function and disease occurrence are not the territories of epidemiology.

Studies on genetic polymorphisms have been conducted at a rapid pace over the last few years (Vineis et al., 1999), not only for cancers but also for a range of other diseases. Since there are a huge number of candidate polymorphisms and studies are needed for each ethnic group, systematic approaches are preferable, not to waste time and manpower. This paper summarizes results of studies on gene-environment interactions from a comprehensive research system established at Aichi Cancer Center, where HERPACC (Hospital-based Epidemiologic Research Program at Aichi Cancer Center) was launched in 1988 (Tajima et al., 2000). In this report, genes and genotypes, but not proteins, are expressed in italics . For example, MPO is an enzyme, and *MPO* is the gene encoding MPO.

Study Subjects

Study subjects comprised individuals from five sources. 1) Participants of a *Helicobacter pylori (HP)* eradication study, whose enrollment period was between March and December in 1999. 2) Non-cancer female patients, who are recruited at waiting rooms announcing free blood tests to measure cholesterol and triglyceride in the period from June 1999 to April 2000. 3) Cancer patients invited by doctors in charge of their treatment from June 1999. 4) First-visit outpatients since November 2000 for HERPACC-II. 5) examinees of a health checkup run by a local government in August and September 2000, who were asked to allow their residual blood after routine tests to be employed for research purposes. Those aged below 20 years at study enrollment were not included. Table 1 shows the number of participants and obtained numbers of DNA samples, as well as their age range. Since the enrollment of the first-visit outpatients is on going, DNA extraction has not completed for them. Samples of several participants were not available because of failure to draw blood.

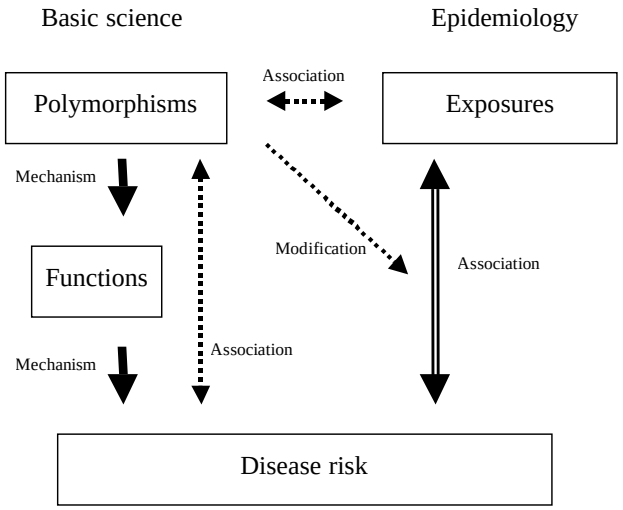

Figure 1. The Role of Epidemiology and Basic Science for Polymorphism Studies

Informed Consent Processes

Participants from all five sources of were individually asked to provide written informed consent for genotyping tests. The names of polymorphisms to be examined were naturally not given, but the differences between cancer genes and genetic polymorphisms were explained to the participants. Since November 2000, an 8-page pamphlet entitled "Oncogenes and Polymorphisms" has been handed to the participants for the sake of supporting their understanding.

For the cancer patients invited by doctors in charge, the announcement was described in the informed consent form that the results of polymorphism tests can be explained if the participants wish to know. Several patients contacted us to ask their genotypes, and they were informed of the results of genotyping. The informed genotypes were *cytochrome p450 17 (CYP17)* for breast cancer patients and *metylenetetrahydrofolate reductase (MTHFR)* and *methionine synthase (MS)* for malignant lymphoma patients. Genotyping for these polymorphisms had been completed before their inquiry.

For the examinees of a regional health checkup, it was explained that the data would become anonymous. We removed the identification from blood and demographic data sets immediately after the residual blood was stored at the Division of Epidemiology and Prevention.

Aichi Cancer Center has an ethical committee to review studies related to genomic tests. Our studies have been approved by the committee for the genetic polymorphism examinations, but not for germ line mutations of hereditary cancers. The approval numbers are 12-23 for *Helicobacter pylori* eradication study, 12-20 for the recruitment of non-cancer female patients and breast cancer patients, 12-13 for the study of case-control study on esophageal cancer, 12-27 for the recruitment of patients with other cancer, 41-2 for the HERPACC-II, and 11-12 for the study on examinees of health checkup.

Genotyping Methods

In the laboratory of the Division of Epidemiology and Prevention, PCR-RFLP (restriction fragment length

polymorphism) and PCR-CTPP (polymerase chain reaction with confronting two-pair primers) (Hamajima et al., 2000a, 2001a) are the main genotyping methods. The former is a well known PCR method which is used worldwide for genotyping. The latter is a newly invented PCR method, which is cheaper and time-saving, and applicable also for the polymorphisms without restriction sites.

Table 2 shows a list of 46 polymorphisms which have been genotyped at the Division of Epidemiology and Prevention, as of April 2001. Once a PCR condition for PCR-CTPP is found, we use PCR-CTPP for genotyping. Roughly speaking, genotyping by PCR-CTPP features double the speed and half the cost of PCR-RFLP, because there is no digestion step.

Statistical Methods

A case-control design was applied to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) by an unconditional logistic model. Adjusted OR means the OR adjusted for sex and age as a continuous variable. Interaction terms were calculated by the logistic model. The term is the ratio of ORs between two subgroups compared; for example, 2 means that OR in one subgroup is twice as high as the OR in the other subgroup.

Since these were case-control studies with prevalent cases, the ORs were examined according to the time interval between diagnosis and study entry. There were no substantial differences in the ORs.

Results of Case-control Studies

Our first paper on polymorphism was published in September 2000 (Hamajima et al., 2000b). As of April 2001, 12 case-control studies and 2 reports on PCR-CTPP methodology (Hamajima et al., 2000a, 2001a) have been accepted for publication. Following is the summary of the results from the 12 case-control studies.

1) Breast cancer

The *cytochrome p450 17 gene (CYP17)* encodes 17α hydroxylase and 17,20-lyase, which are essential enzymes for estrogen biosynthesis. A *T*-to-*C* polymorphism of *CYP17* at 34 base pairs upstream of the translation initiation site is reported to be associated with breast cancer risk (Feigelson et al., 1997). Our case-control study showed no association with the polymorphism; OR=0.97 (95%CI, 0.58-1.64) for the *T/C* genotype and 0.81 (0.39-1.68) for the *C/C* genotype relative to the *T/T* genotype (Hamajima et al., 2000b). A review on *CYP17 T-34C* reported that any association with this polymorphism may be weak, if it exists (Dunning et al., 1999).

We also conducted a case-control study for a *catechol-O-methyltransferase (COMT) Val158Met* polymorphism with low enzyme ability to inactivate 2-hydroxy estradiol and 4-hydroxy estradiol for the *Met* allele, but the association was

Table 1. DNA Sources for Genetic Polymorphism Studies in Division of Epidemiology and Prevention, Aichi Cancer Center

Source	Participants	Available DNA			Age
		Males	Females	Total	
HP eradication study	283				
Non-cancer	245	118	123	241	39-69 years
Cancer patients	38	17	21	38	45-69 years
Non-cancer female outpatients	63	0	63	63	24-69 years
Cancer patients	1,050	531	510	1,041	
Esophagus	102	86	16	102	40-76 years*
Stomach	150	106	44	150	32-82 years*
Colorectum	149	85	63	148	24-78 years*
Pancreas	10	7	3	10	31-79 years*
Lung	193	116	77	193	26-81 years*
Breast	244	0	238	238	26-70 years*
Cervix/Ovary	19	0	19	19	35-82 years*
Prostate	62	62	0	62	53-85 years*
Lymphoma	111	60	49	109	19-79 years*
Head and Neck	10	9	1	10	50-77 years*
First-visit patients as of April 2001	1,297	DN	DN	DN	DN
Examinees of health checkup	468	127	338	465	32-85 years
Total	3,161	793**	1,055**	1,848**	

*: Age at diagnosis. **: First-visit patients were excluded from the total.
DN: Data not yet summarized because the recruitment is on going.

found to be limited; adjusted OR=1.46 (0.90-2.36) for *Val/Met* and 0.99 (0.49-2.02) for *Met/Met* (Hamajima et al., 2001b). Although a study in Taiwan showed a significant OR elevation (3.55, 1.15-13.37) for the *Met/Met* genotype relative to the other genotypes (Huang et al., 1999), five case-control studies, including the Taiwan study and our study (three of Caucasians and two of Orientals) gave inconsistent results, as a review on *COMT* reported for the three Caucasian studies (Dunning et al., 1999).

The third study was on β2 and β3 adrenoceptor genes *(BAR2 and BAR3)*, which are reportedly associated with obesity (Large et al., 1997; Arner et al., 1999). Signals

Table 2. Polyporphisms Genotyped at the Division of Epidemiology and Prevention of Aichi Cancer Center Research Institute as of April 2001

Gene	Polymorphism	Method	Reference
ALDH2	*Glu487Lys*	PCR-CTPP	Matsuo et al, 2001a
BAR2(ADRB2)	*Gln27Glu*	PCR-RFLP	Takezaki et al, 2001, Huang et al, 2001
		PCR-CTPP	Hamajima et al, 2000b
BAR3(ADRB3)	*Trp64Arg*	PCR-RFLP	Takezaki et al, 2001, Huang et al, 2001
CCND1	*A867G*	PCR-RFLP	
CD36	*Pro90Ser*	PCR-RFLP	
	A1160C	PCR-CTPP	
COMT	*Val158Met*	PCR-RFLP	Hamajima et al, 2001b
COX2	*C-765G*	PCR-CTPP	Hamajima et al, 2001c
	C-163G	PCR-CTPP	Hamajima et al, 2001c
	C-62G	PCR-CTPP	Hamajima et al, 2001c
	G10T	PCR-CTPP	Hamajima et al, 2001c
	Glu488Gly	PCR-CTPP	Hamajima et al, 2001c
	Val511Ala	PCR-CTPP	Hamajima et al, 2001c
CYP1A1	*MspI*	PCR-RFLP	
CYP17	*T-34C*	PCR-RFLP	Hamajima et al, 2000a
CYP19	*Trp39Arg*	PCR-CTPP	
DRD2	*TaqIA*	PCR-CTPP	
	MboI	PCR-CTPP	
ERCC2(XPD)	*Lys751Gln*	PCR-RFLP	
FcyRIII	*47Leu/Arg/His*	PCR-RFLP	
	Phe158Val	PCR-RFLP	
GSTM1	*Present/Null*	PCR	
GSTP1	*Ile462Val*	PCR-CTPP	
GSTT1	*Present/Null*	PCR	
IGF1	*G2502T*	PCR-CTPP	
IL-1A	*C-889T*	PCR-CTPP	Hamajima et al, 2001d
IL-1B	*C-511T*	PCR-RFLP	Hamajima et al, 2001d
	C-31T	PCR-RFLP	Hamajima et al, 2001d,f
		PCR-CTPP	Hamajima et al, 2001d
IL-1RN	*VNTR* at intron 2	PCR	Hamajima et al, 2001d
IL-1R1	*C-116T*	PCR-CTPP	
IL-6	*17Del/Ins*	PCR-CTPP	
L-myc	*G2886T*	PCR-CTPP	
MPO	*G-463A*	PCR-RFLP	Matsuo et al, 2001b, Hamajima et al, 2001e
MTHFR	*C677T*	PCR-RFLP	Matsuo et al, 2001c
	A1298C	PCR-RFLP	Matsuo et al, 2001c
MS	*A2756G*	PCR-RFLP	Matsuo et al, 2001c
MTRR	*Ile22Met*	PCR-RFLP	
NQO1	*Pro187Ser*	PCR-CTPP	
OGG1	*Ser326Cys*	PCR-CTPP	
p53	*Arg72Pro*	PCR-CTPP	
Se (FUT2)	*Se/sej/se5*	PCR-CTPP	Hamajima et al, 2001a
TNF-A	*G-308A*	PCR-CTPP	
TNF-B	*A252G*	PCR-CTPP	
XRCC1	*Arg399Gln*	PCR-RFLP	
XRCC4	*T323C*	PCR-RFLP	
	G921T	PCR-RFLP	

PCR-RFLP: polymerase chain reaction – restriction fragment length polymorphism, PCR-CTPP: polymerase chain reaction with confronting two-pair primers

through the receptors stimulate lipolysis and thermogenesis. Since obesity is a risk factor of postmenopausal breast cancer, the association between the polymorphisms (*Gln27Glu* of *BAR2* and *Trp64Arg* of *BAR3*) and breast cancer risk was examined (Huang et al., 2001). Unexpectedly, the alleles related to obesity (*Glu* and *Arg*) showed a non-significant decreased risk of breast cancer; adjusted OR=0.65 (0.37-1.16) for individuals harboring the *Glu* allele, and 0.83 (0.54-1.29) for individuals harboring the *Arg* allele. For the women with first childbirth at age 24 years or younger, a significant decrease in the breast cancer risk was observed; adjusted OR=0.34 (0.12-0.97). There were no differences in the OR between premenopausal and postmenopausal women.

2) Esophageal cancer

A strong gene-environment interaction was observed between heavy alcohol drinking and *aldehyde dehydrogenase 2 (ALDH2)* polymorphism for esophageal cancer (Matsuo et al., 2001a). The sex-age-adjusted OR of heavy drinking (75ml or more per day and 5 days or over per week) was 7.84 (95% CI, 2.77-22.2) for individuals with the *Glu/Glu* genotype and 49.6 (14.5-169.4) for those with the *Glu/Lys* or *Lys/Lys*, providing 6.84 (2.39-19.6) for the interaction term. The finding indicated that individuals with the low enzyme activity are much more susceptible to the adverse effects of heavy alcohol drinking.

Myeloperoxidase (MPO) is an enzyme in lysosomes of phagocytes, catalyzing the reaction of chloride and hydrogen peroxide to yield hypochlorous acid that generates hydroxy radicals in the presence of superoxide. The *A* allele *MPO G-463A* polymorphism (a low enzyme activity allele) has been reported to reduce risk of cancer of the lung and larynx (Schabath et al., 2000; Cascorbi et al., 2000). We therefore conducted a case-control study for esophageal cancer, finding that the low activity allele reduced the risk in those aged more than 60 years; adjusted OR=0.15, 95%CI, 0.03-0.76 (Matsuo et al., 2001b).

3) Colorectal cancer

Cyclooxygenase (COX) is a rate limiting enzyme to convert arachidonic acid to prostaglandins, which has two isoforms, COX1 and COX2. While the *COX1* gene is constitutively expressed in a variety of tissues, *COX2* is induced by growth factors and cytokines. Overexpression of *COX2* has been reported in colorectal cancer, and epidemiologic studies elucidated that nonsteroidal anti-inflammatory drugs with a COX inhibitory activity reduce colorectal cancer risk (Smalley et al., 1999). To date, twenty-two polymorphisms have been reported for *COX2* (Accession No. NT001817.3). The association with six polymorphisms, *G-765C, C-163G, C-62G, T10G, Glu488Gly,* and *Val511Ala,* was examined in a case-control study (Hamajima et al., 2001c). All study subjects were found to have *C/C* at –62,*Glu/Glu* at codon 488, and *Val/Val* at codon 511, and there were no differences in the genotype frequencies for *G-765C* (4.6% in controls and 5.4% in cases), *C-163G* (completely linked to *T10G*), and *T10G* (3.0% and 3.4%, respectively) between cases and controls, indicating that the six polymorphisms are not associated with colorectal cancer risk for Japanese. A recent affected sibling-pair study on the association with *COX2* locus demonstrated no linkage to non-familial colon cancer using two highly polymorphic microsatellite markers *D1S191* and *D1S2848* (Wiesner et al., 2001). This suggests that there is no further need to examine the other polymorphisms of COX2 because the linkage study covers the association with all of them.

As stated above, β-adrenoceptors are related to lipid metabolism. Accordingly, the associations with colorectal cancer risk were examined (Takezaki et al., 2001). No significant associations were found for the whole subjects, but subgroup analysis for those with body mass index 22.5 or over showed a significant OR elevation of colon cancer for individuals with the Arg allele relative to those without the allele. Adjusted ORs of current smoking and drinking (5 times or over per week) were significantly elevated for the *Trp/Trp* genotype (adjusted OR=3.98, 1.71-9.26, and 5.84, 2.44-14.0, respectively) but not for the groups of *Trp/Arg* and *Arg/Arg* combined.

4) Malignant lymphoma

Metylenetetrahydrofolate reductase (MTHFR) converts 5,10-methyleneTHF to 5-methylTHF, the predominant circulatory form of folate and carbon donor for the remethylation of homocystein to methionine by methionine synthase (MS). Two polymorphisms, *C677T* and *A1298C* of *MTHFR*, have been reported to reduce the enzyme activity in the *T* allele and *C* allele, respectively. Our case-control study showed that individuals with *677 C/C* and *1298 A/A* genotypes were at high risk of malignant lymphoma compared with the others; adjusted OR=2.26 (1.26-4.02). For *MS*, the *A2756G* polymorphism was examined in the same dataset. The *G/G* genotype with a reduced enzyme activity showed a higher susceptibility; adjusted OR=3.83 (1.21-12.1) relative to the other two genotypes, *A/A* and *A/G* (Matsuo et al., 2001c). The significance was not altered when these three polymorphisms were evaluated in combination.

5) Helicobacter pylori (HP)infection

HP infection is a well-known risk factor for stomach cancer. Studies on the *HP* strain demonstrated that the virulence relates to *vacA* and genes within the cag pathogenicity island (Peek et al., 1999). Just like the research on the characteristics of *HP*, the studies on the host factors related to the susceptibility to *HP* are quite challenging. Associations with HLA types were reported for Japanese (Sakai et al., 1999; Yoshitake et al., 1999). In our laboratory, many polymorphisms have been screened for the association with seropositivity of high-molecular-weight Campylobacter-associated protein (HM-CAP), though the sensitivity of the *HP* infection is a little reduced for Japanese in comparison with the report for Caucasians (Matsuo et al., 2000).

We found that two polymorphisms were associated with

the infection; *interleukin 1B (IL-1B) C-31T* and *MPO G-463A*. Interleukin 1β encoded by *IL-1B* is a multifunctional pro-inflammatory cytokine (Dianarello, 1996), which also inhibit gastric acid secretion. The *IL-1B* polymorphism was reported to be associated with the risk of stomach cancer (El-Omar et al., 2000). The adjusted OR for seropositives among 241 non-cancer patients was 2.32 (1.10-4.92) for the *C/T* genotype and 2.46 (1.06-5.74) for the *T/T* genotype compared with the *C/C* genotype. When analyzed for current smokers, the adjusted ORs were 6.18 (1.34-28.6) and 22.9 (1.97-266), respectively. The interaction between the polymorphism and smoking was statistically significant (Hamajima et al., 2001d).

Since MPO is related to inflammatory processes, the association with *G-463A* polymorphism was also examined (Hamajima et al., 2001e). A low activity allele (*A* allele) showed a non-significant reduced OR for all subjects (adjusted OR=0.69, 0.35-1.35), while it was significant for current smokers (OR=0.19, 0.04-0.96). When compared with smokers with the *G/G* genotype, the other subjects had a lower risk of the infection; OR=0.21 (0.04-0.98) for smokers harboring the *A* allele, 0.39 (0.16-0.92) for non-current smokers with the *G/G* genotype, and 0.37 (0.13-1.03) for non-current smokers harboring the *A* allele. Other polymorphisms not relevant in a biological sense *(CYP17 T-34C, BAR2 Gln27Glu, BAR3 Trp64Arg, COMT Val158Met, MTHFR C677T* and *A1298C, MS A2756G, ALDH2 Glu487Lys, p53 Arg72Pro, XRCC1 Arg399Gln,* and *ERCC2 Lys751Gln)* showed no marked associations.

6) Smoking habit

Smoking is the most important target for cancer prevention. Recent studies indicated that genetic traits may influence smoking behavior (Rossing, 1998). The association with the dopamine receptor D2 gene (DRD2) was examined for non-cancer patients. In our subjects, the sex-age-adjusted OR of being ever-smokers relative to *A1/A1* was 1.65 (0.67-4.04) for the *A1/A2* and 3.68 (1.50-9.05) for the *A2/A2* (Yoshida et al., 2001). The association observed in this study was opposite to that observed for Caucasians. The *A2* allele is rare among Caucasian smokers. We are now attempting to confirm our findings with another group of Japanese subjects.

A significant association between smoking and *IL-1B C-31T* was found for non-cancer outpatients, and a non-significant similar association for health checkup examinees, indicating that the individuals with the *C/T* or *T/T* genotype have a tendency to refrain from smoking; the sex-age-adjusted OR=0.45 (0.21-0.97) relative to the *C/C* among the outpatients and 0.83 (0.43-1.61) among the health checkup examinees (Hamajima et al., 2001f).

Strategy for Polymorphism Selection

Since tremendous numbers of polymorphisms exist, an efficient strategy to identify those associated with risk modification is important. The target polymorphisms can be categorized as follows.

The first group comprises the carcinogen-metabolizing enzymes. Activation and detoxification of carcinogens should theoretically be related directly to the cancer risk. Although the situation is complex, *cytochrome p450s (CYPs)* and *glutathione S-transferases (GSTs)* polymorphisms are likely to affect susceptibility. Since the same substrate can be metabolized by different enzymes, the effect may be attenuated. Therefore, series of polymorphisms related to activation and detoxification enzymes need to be evaluated at the same time. In addition, the contrast may be weakened, if the individuals with a lower level of exposure to hazardous materials are selected as the study subjects, so that larger numbers are required. When the enzyme is specific and exclusive to a carcinogen, exemplified for ALDH2 and aldehyde (Matsuo et al., 2001a), a strong interaction may be observed.

The second is the hormone-metabolizing enzymes and hormone receptors for hormone related cancers. Polymorphisms of *CYP11A1 (side-chain cleavage enzyme), CYP17, CYP19 (aromatase), SDR5A2 (steroid 5α reductase), CYP1A1, COMT, ER (estrogen receptor), PR (progesterone receptor), AR (androgen receptor)* genes are thus targets for study. Against expectation, the influences and modifications by these polymorphisms seem not so clear (Dunning et al., 1999, Hamajima et al., 2000c). Probably, the effect of one enzyme on hormone levels may be masked by other enzyme activities, because the production and destruction of hormones are closely linked to each other and polymorphisms of several enzymes could be involved.

The third is enzymes and receptors related to lipid metabolism for lipid related cancers. There are many candidate genes with polymorphisms, such as *APOA1, APOA2, APOA4, APOB, APOC2, APOC3, APOE, APOH, LPL, CETP, LCAT,* and *Lipoprotein receptors* (Ye et al., 2000). We showed that *BAR2* and *BAR3* polymorphisms potentially affected the breast cancer risk (Huang et al., 2001) and colorectal cancer risk (Takezaki et al., 2001), though the effect may be weak or moderate. Results for *CD36 Pro90Ser* and *A1160C* will soon be obtained for breast and colorectal cancers.

The fourth comprises DNA repair enzymes. Cancer cells are considered to be the result of DNA changes, so that studies on polymorphisms in this group might be expected to produce new important findings. We have so far conducted genotyping of *ERCC2 (excision repair cross-complementing group 2), XRCC1 (X-ray repair cross-complementing group 1), XRCC4 (X-ray repair cross-complementing group 4), OGG1 (8-oxoguanine-DNA glycosylase 1)*. There are still several candidate polymorphisms to be examined.

The fifth is the polymorphisms of enzymes and receptors relating to inflammation and immune responses. Studies on interleukin polymorphisms, expecially *IL-1B C-31T*, generated significant findings in our laboratory. Other interleukins, IL-receptors, TNFs, and other inflammation-related genes remain to be examined. The *A* allele carriers of *TNF-A G-308A* are rare in Japanese (3.3%, n=575,

Kamizono, et al., 2000), but not in Caucasians (32.4%, n=312 from the Australian general population without asthma, Moffatt et al., 1997). Alleles with a sufficiently high frequency to allow analysis should normally be selected. We learned the importance of this from our study on *COX2*, where the minor allele carriers were too few for statistical analysis (Hamajima et al., 2001c).

There are other types of genes which exhibit polymorphisms; examples exist for addiction and dopamine-related enzymes and receptors, smoking habit and nicotine metabolizing enzymes, folate related cancer and MTHFR and MS, high cell turnover and mitotic checkpoint related forms, oncogenes and so forth. Thus there are a great number of possible combinations regarding polymorphisms and carcinogenesis.

Polymorphisms should have functional consequences, and furthermore the difference in function between the alleles should be large enough to cause risk elevation/reduction or modification of the other factors. Usually, polymorphisms documented or suspected to influence the expression and to cause the protein structure affecting the function are selected. Generally speaking, examining the association with disease risk is easier than examining functions. Accordingly, it may be wise to screen the association with the candidate polymorphism, without waiting for results of functional tests.

Adjustment for Prognostic Effects in Prevalent Case-control Studies

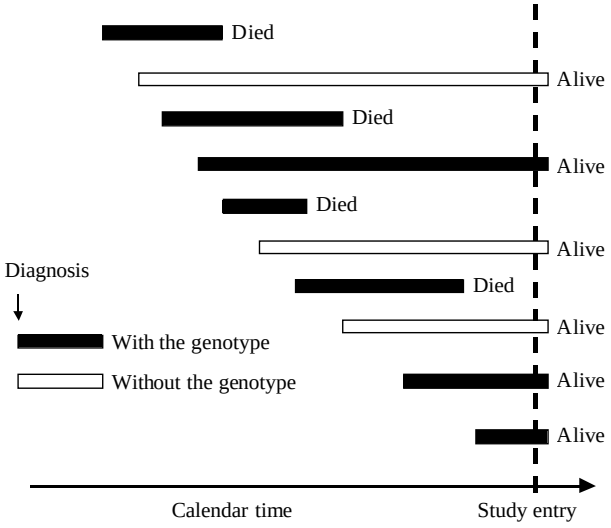

Figure 2. Proportion of a risk-increasing, prognosis-worsening genotype among incident and prevalent cases. The proportion of patients with the genotype is 70% (7/10) among all cases (incident cases), but 50% (3/6) for the cases at study entry (prevalent cases), due to the association with poor prognosis. When the genotype proportion is 50% among controls, the OR for disease occurrence is to be 2.3, but the OR obtained in prevalent case-control study is unity.

The case-control studies described here included not only incident cases but also prevalent cases. Since the ORs derived from prevalent case-control studies are affected by prognostic effects of the factors under study, attention should be paid to this question in interpretation of the results. For the factors increasing disease risk and associated with a poor prognosis, the observed OR is an underestimated value, which means that the true value for disease risk elevation is higher than the observed one. Figure 2 shows ten cases; four died in a short period, and six are alive. Those with a poor prognosis tend to have a high risk genotype. Accordingly, the percentage for the genotype is lower for the alive cases (prevalent cases) than for all cases (incident cases), resulting in underestimation of the OR. For the factors increasing disease risk and associated with better prognosis, the observed OR is an overestimated value, though this may be very exceptional in a biological sense. If the factor is not associated with prognosis, the observed OR expresses the relative risk for disease occurrence. In order to examine the extent of the prognostic effect, stratified analysis is easiest. In our case-control studies, there were no differences in the ORs of genotypes according to the interval from diagnosis (Hamajima et al., 2000b; Hamajima et al., 2001b; Huang et al., 2001). When there is no substantial difference in the OR estimate among the subgroup analysis according to the interval from diagnosis, the estimate reflects the relative risk for disease occurrence. If adjustment is required, an adjusted genotype proportion approach and an incomplete-data case-control design approach can be proposed (Hamajima et al., 2001g)

Concluding Comments

Studies on gene-environment interactions provide more specific risk estimations for environment factors. In the past, epidemiologists had to measure the strength of association as an average for the whole population under study, bearing in mind that the susceptibility might be different among the individuals with different genetic traits. Just like the estimation of lung cancer risk according to smoking status, the risk estimation taking the genotypes into account will be more specific for each genotype group. The probabilistic (stochastic) model will approach the deterministic causal concept by incorporating genetic traits (Rothman et al., 1998). This is a direction that epidemiologists have been seeking.

Our system is one of the best in terms of the productivity of polymorphism risk studies in Japan, as well as the cancer epidemiology in general. There are several advantageous factors for our team. First, our hospital is a large cancer hospital where many cancer patients visit. For example, the number of breast cancer patients is the second or third largest in such an institution in Japan. Second, clinical departments are very cooperative and understand our studies. This is a key element for effective enrollment of patients. The consecutive enrollment of first-visit patients was established at the start of HERPACC-I in 1988 (Tajima et al., 2000).

Accordingly, the shift to HERPACC-II was very smooth. Third, the other divisions of the Research Institute can share the benefits from the system, whose staff assist us and facilitate the research. Fourth, PCR-CTPP was invented in an early stage of the polymorphism project. It speeded up the genotyping. Fifth, manpower has been supplied from other institutes including Nagoya University Graduate School of Medicine. Aichi Cancer Center Research Institute became one branch of the Graduate School in 1998. The last is the timely research funding to establish the laboratory in Division of Epidemiology and Prevention. All these factors have been supporting this project, which will continue to produce data on gene-environment interactions for cancers in Japanese.

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Picture. The Staff for Genotyping in HERPACC-II. Left to right, Dr. K Matsuo, Dr. N Hamajima, and Ms. T Saito in the front row, and Dr. X-E Huang, Ms. M Kato, Ms. M Tani, and Ms. N Takeuchi in the rear row.