인체지표를 이용한 분자역학연구의 적용

홍윤철

인하의대 산업의학과

정교하고 민감한 실험실적 방법과 분석역학연구기법이 연결되어 분자역학연구라는 새로운 영역이 만들어지게 되었다. 분자역학연구는 생체용량, 생물학적 유효용량, 조기 생체 영향 및 감수성에 대한 인체지표 등을 활용하여 분자생물학과 전통적인 역학연구를 잇는 가교역할을 한다. 연구에 활용되는 인체지표는 노출에서 시작하여 흡수, 대사, 분포, 대상조직과의 상호작용, 유전적 변화 및 질병까지를 포괄한다.

환경적인 요인은 대부분의 질병에 관여하는 것으로 알려져 있다. 이러한 요인들로는 흡연, 식이, 음주 등의 일상생활에서의 노출뿐 아니라 산업현장 및 일반환경에서의 화학물질까지다양하게 존재한다. 또한 이러한 환경적인 요인에 의한 질병위험은 유전적 또는 후천적인 감수성에 의하여 변화될 수 있다. 분자유전역학은 질병발생에 미치는 이러한 환경적인 요인의 기여도를 밝히는데 중요한 역할을 할뿐 아니라 고민감군을 규명함으로써 예방대책을 수립하는데 중요한 근거를 제시해줄 수 있다.

분자역학연구의 종류는 크게 이행연구(transitional study)와 질병원인규명연구(disease etiology study)로 나눌수 있다. 이행연구는 주로 인체지표와 관련한 연구로서 인체지표의 개발과 특성에 대한 연구이며 질병원인규명연구는 이러한 인체지표들을 이용한 환자-대조군연구, 코호트 연구 등의 역학적 연구를 말한다. 이번 연제에서는 본 연구자가 수행하였던 분자역학연구의 실질적인 예들을 소개하고자 하며 이행연구의 예로 PAH 노출지표인 1-hydroxypyrene, 2-naphthol, 산소성 손상의 조기영향지표인 8-hydroxyguonosine, malondialdehyde, 유전적 감수성지표인 GSTM1, GSTT1 등을 이용하였던 연구들과 질병원인규명연구의 예로 위암 및 뇌혈관질환의 관련성 연구들을 설명하고자한다.

향후, 인체지표들은 환경독성물질에 노출되는 인구집단을 대상으로 한 역학연구에 폭넓게 사용될 것이다. 이를 통하여 분자역학연구는 환경성질환의 예방대책을 수립하는데 있어서 매우 유용한 도구가 될 것이다.

Application of Molecular Biomarkers for Environmental Epidemiology

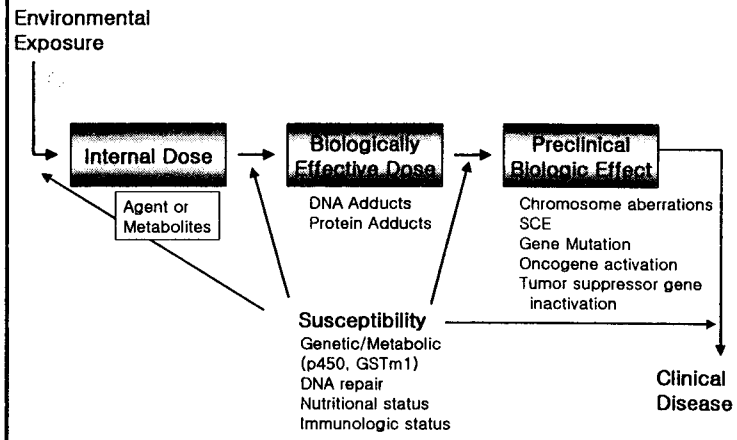
Yun-Chul Hong
Department of Occupational and Environmental Medicine
Inha University College of Medicine

Objectives of Environmental Epidemiology

- **To identify the etiology or the cause of a environmental disease**
- **To determine the extent of environmental disease found in the community.**
- **To study the natural history and prognosis of environmental disease.**
- **To provide the foundation for developing public policy and regulatory decisions relating to environmental problems.**

Molecular Epidemiology

- **Molecular epidemiology**
  bridges
  basic research in molecular biology
  and studies of human disease causation
  by combining laboratory measurement of internal dose, biologically effective dose, biologic effects, and the influence of individual susceptibility with epidemiologic methodologies.



Examples of Markers of Internal Dose

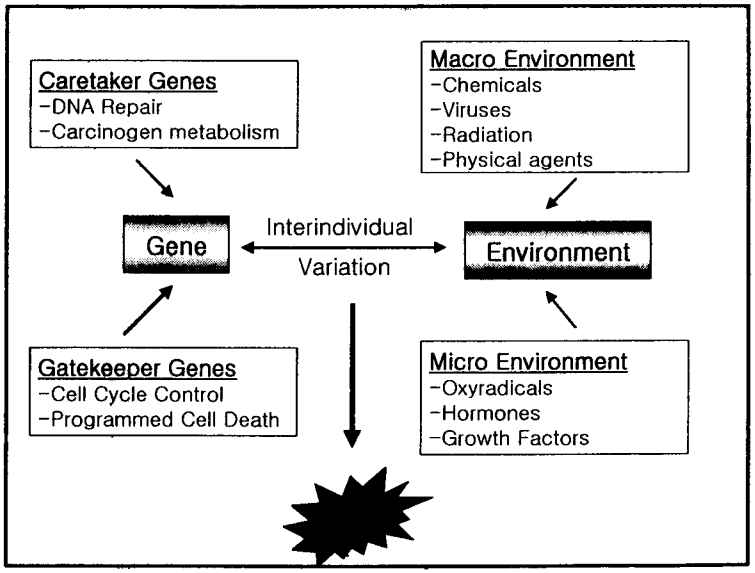
Biomarker	Source of Exposure	Biologic Sample	Measurement
Cotinine	Cigarette smoke	Serum, urine, saliva	Higher levels in smokers compared to nonsmokers
Vitamine levels	Diet	Serum	Serum levels of Vitamines A,E,C, and D linked with risk for various cancers
Selenium	Diet	Hair, toe nails	Levels of selenium linked with risk for lung cancer
Levels of benzene And benzene Metabolites	Cigarette smoke	Urine, breath concentrations	Higher levels of benzene and metabolites in smokers compared to nonsmokers
Levels of lead-210	Cigarette smoke	Bone, soft tissues	Higher levels of lead-210 in smokers compared to nonsmokers
Aflatoxin	Contaminated food	Urine	Higher levels of aflatoxin in urine of exposed compared to nonexposed
Bacterial mutations	Cigarette smoke	Cervical fluids	Samples from smokers more likely to be mutagenic in Ames test
Mean cell volume HDL, ALP	Alcohol	Serum	Marker levels correlate with Alcohol consumption level

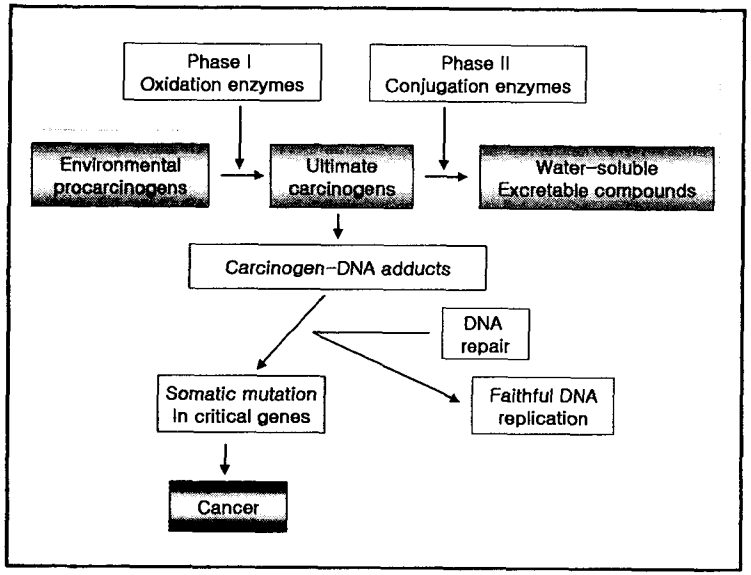
Examples of Markers of Biologically Effective Dose

Biomarker	Source of Exposure	Biologic Sample	Measurement
DNA and protein adducts	Cigarette smoke	Serum	More common in smokers than nonsmokers
Estrogen and androgen levels	Cigarette smoke	Serum, urine	Examine hormone levels in relation to daily smoking levels
Estrogen levels	Alcohol	Serum, urine	Higher levels in alcohol drinkers than nondrinkers

Early Biologic Effect or Response

Compound analyzed	Exposure source	Biologic Sample	Population
Single strand breaks	Styrene	WBC	Workers
Unscheduled DNA synthesis	Propylene oxide	WBC	Workers
Sister chromatid exchange	Various industrial exposures, radiation, air pollution	WBC	Workers, residents
Micronuclei	Organic solvents, heavy metals, cigarette smoke, betel quid	WBC, oral mucosa	Workers
Chromosomal aberrations	Various industrial exposures, radiation, air pollution	WBC	Workers, residents
DNA hyperploidy	Aromatic amines	Bladder and lung cells	Workers
HPRT mutation	Chemotherapeutic agents, radiation	WBC	Patients, workers
GPA mutation	Chemotherapeutic agents, radiation	RBC	Patients, Japanese atom bomb survivors
Mutation in tumor suppressor genes	AFB1	Tumor tissue	Patients
Oncogene activation	PAH, cigarette smoke	Serum	Workers, cancer patients





Molecular Epidemiologic Studies

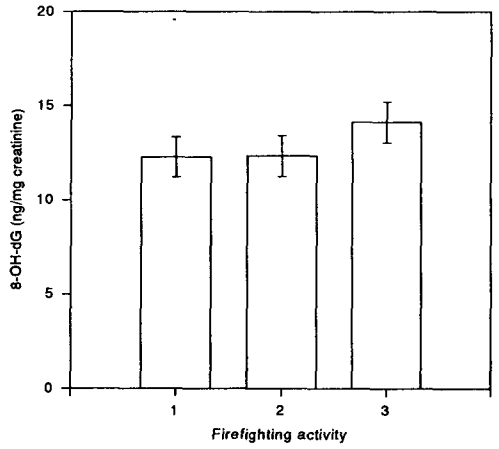
Transitional studies	Disease etiology studies
a. Biomarker development Studies – reliability studies – sample collection & processing studies	a. Case-control study – case definition refined – evaluating exogenous exposure – evaluating genetic susceptibility
b. Biomarker characterization studies – cross-sectional studies – *longitudinal studies*	b. Cohort study
	c. Nested case-control study Case-cohort designs

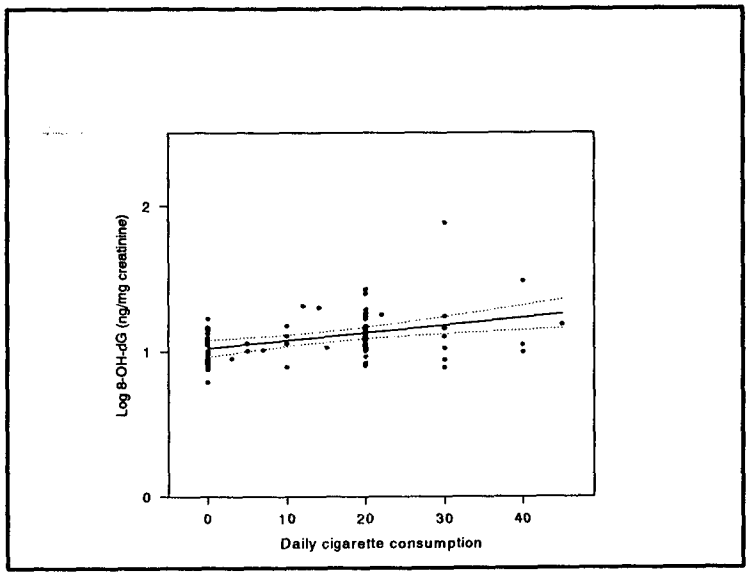
Transitional Study (1)

Influence of genetic susceptibility on the urinary excretion of 8-hydroxydeoxyguanosine of firefighters

Yun-Chul Hong, Hye-Sook Park, Eun-Hee Ha

Occup Environ Med 2000; 57: 370-375





Transitional Study (2)

Variations in urinary 1-hydroxypyrene glucuronide in relation to smoking and modification effects of GSTM1 and GSTT1

Yun-Chul Hong, Jong-Han Leem, Hye-Sook Park, etc.

Toxicology Letters 1999; 108: 217-223

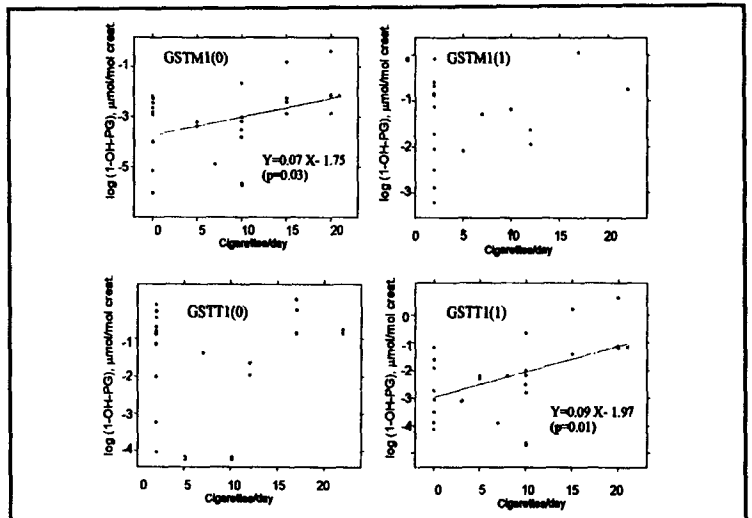


Fig. 2. Regression plot showing the relationship between log 1-OHPG and cigarette smoking according to GSTM1 or GSTT1 subtype.

Transitional Study (3)

Maternal Genetic Effects on Neonatal susceptibility to Oxidative Damage From Environmental Tobacco Smoke

Yun-Chul Hong, Heon Kim, Moon-Whan Im, etc.

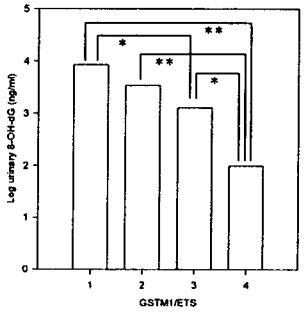
J Natl Cancer Inst 2001; 93: 645-647

Table 1. Urinary concentrations of biomarkers of maternal exposure to ETS and neonatal oxidative injury

Biomarkers	ETS nonexposed mothers GM (95% CI)	ETS Exposed mothers GM (95% CI)
Maternal exposure biomarker		
Nr. of maternal subjects	37	44
Cotinine (μg/g creatinine)	8.61 (4.84 to 9.51)	12.11 (7.34 to 19.98)
1-OHP (μg/g creatinine)	0.13 (0.10 to 0.14)	0.16 (0.12 to 0.21)
2-naphthol (μg/g creatinine)	3.34 (2.27 to 3.56)	4.61 (3.37 to 6.30)
Neonatal oxidative injury biomarker		
Nr. of neonatal subjects	8	12
8-OhdG (μg/L)†	0.93 (0.24 to 1.50)	4.03 (2.13 to 7.61)
MDA (mmol/L)	0.69 (0.42 to 0.83)	0.76 (0.56 to 1.05)

†Statistically significant difference between ETS non-exposed and exposed group with the use of Student's t-test (P = .047).

Figure 1. Comparison of log concentrations of neonatal urinary 8-OHdG among *GSTM1*/ETS subgroups



Group 1(N=8): *GSTM1*(0)/ETS(+)
Group 2(N=4): *GSTM1*(0)/ETS(-)
Group 3(N=4): *GSTM1*(1)/ETS(+)
Group 4(N=4): *GSTM1*(1)/ETS(-)

*: P<0.05
**: P<0.01

Disease Etiology Study (1)

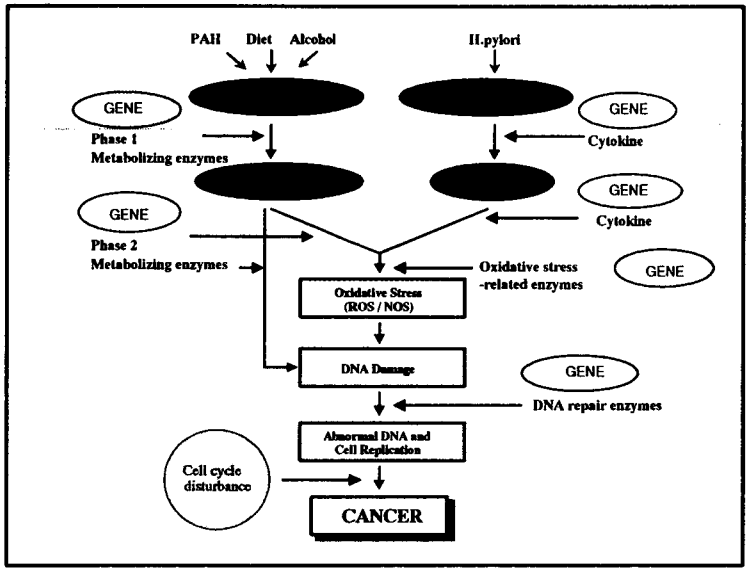
Genetic Epidemiology of Stomach and Liver Cancer

Yun-Chul Hong, Sung-Il Cho, Don-Hang Lee, etc.

21세기프론티어(인간유전체기능연구사업)

Susceptibility Genes Involved in Gastric Carcinogenesis

- Xenobiotic metabolism enzymes
  - GSTs (glutathione S-transferases) M1/T1/P1
  - CYP (cytochrome P-450)1A1, CYP2E1
- Oxidative stress-related enzymes
  - MPO (myeloperoxidase)
  - MnSOD (manganese superoxide dismutase)
- DNA repair genes
  - XRCC1 (X-ray repair cross-complementing 1)
  - hOGG1 (8-hydroxydeoxyguanosine DNA glycosylase/apurinic lyase)



Cancer Lett 2001 Aug 10;169(1):21-6

Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Saadat I

GSTM1 null type : OR=2.3 (95% CI: 1.15-4.95)

Cancer Epidemiol Biomarkers Prev 2000 Jan;9(1):73-80

GSTT1 and GSTM1 null genotypes and the risk of gastric cancer: a case-control study in a Chinese population. Setiawan VW

GSTT1 null type: OR=2.33 (95% CI: 0.75-7.25)

Cancer Epidemiol Biomarkers Prev 2000 Jul;9(7):675-80

Cytochrome P450 2E1 polymorphism in gastric cancer in Brazil: case-control studies of Japanese Brazilians and non-Japanese Brazilians. Nishimoto IN

CYP2E1 RsaI variant type:

OR=0.46 (95% CI: 0.21-1.04) in non-Japanese Brazilians,

OR=0.98 (95% CI: 0.50-1.90) in Japanese Brazilians

Int J Cancer 2000 Nov 15;88(4):601-6

Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. Shen H

XRCC1 26304 CC genotype: OR=1.86 (95% CI=1.09-3.20) for gastric cardia cancer

Jpn J Cancer Res 1998 Aug;89(8):825-8

Infrequent mutations of the hOGG1 gene, that is involved in the excision of 8-hydroxyguanine in damaged DNA, in human gastric cancer. Shinmura K

hOGG1 Cys326 allele: OR increased non-significantly

Disease Etiology Study (2)

Environmental Epidemiology of Cerebrovascular Disease

Yun-Chul Hong

Ecotechnopia

To Know About...

Air pollution and Cerebrovascular disease

- air pollution and exposure biomarker
- relationship between exposure and early biological effective biomarker
- early biological effective biomarker and cerebrovascular disease

Genetic susceptibility

- gene-environmental interaction
- gene-gene interaction

Biomarker distribution across cases and controls

Exposure Biomarkers

PAH Exposure biomarker (1-OHP, 2-Naphthol)

VOC exposure biomarker (Hippuric acid, Muconic acid)

Heavy metal exposure biomarker (Cadmium)

Early Biological Effect Biomarker

Oxidative injury biomarker (8-OH-dG, MDA)

Genetic Susceptibility Biomarkers

CYP1A1, CYP2E1, GSTM1, GSTT1, GSTP1, NAT2, UGT1A6

MPO, MnSOD, GPX