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THE ROLE OF GENETIC POLYMORPHISMS OF XENOBIOTIC METABOLIZING ENZYMES IN HUMAN CARCINOGENESIS

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The Role of Genetic Polymorphisms of Xenobiotic Metabolism Enzymes in Human Carcinogenesis

- Epidemiological Perspectives-

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Incidence of selected major cancers in males

Rank	USA*	Japan	Korea**	World
1	Prostate	Stomach	Stomach	Lung
2	Lung	Lung	Liver	Stomach
3	Colon	Colon	Lung	Colon
4	Bladder	Liver	Colon	Mouth
5	Lymph.	Oesoph.	Bladder	Prostate

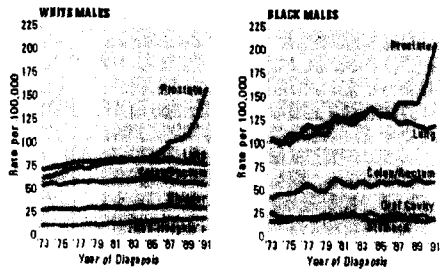
* Caucasian (1987-1991), **1991-1992

Incidence of selected major cancers in females

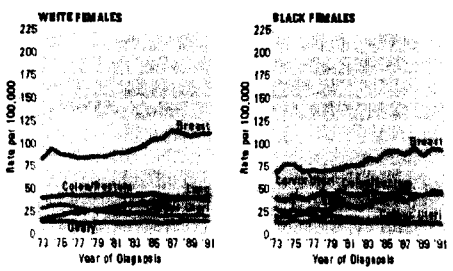
Rank	USA*	Japan	Korea**	World
1	Breast	Stomach	Cervix	Breast
2	Lung	Breast	Stomach	Cervix
3	Colon	Colon	Breast	Colon
4	Corpus Ut	Cervix	Colon	Stomach
5	Ovary	Lung	Liver	Lung

* Caucasian (1987-1991), **1991-1992

Top Five Cancer Incidence Sites: White and Black Males (1973-1991, USA, SEER)

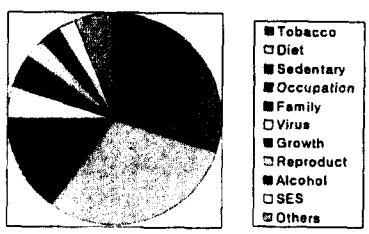


Top Five Cancer Incidence Sites: White and Black Females (1973-1991, USA SEER)

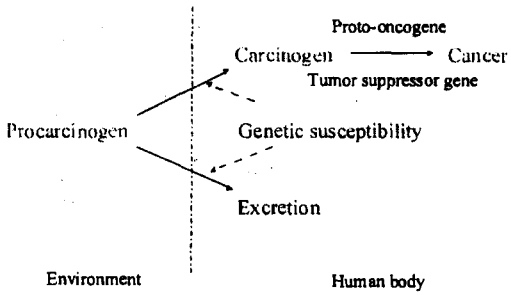


Causes of Human Cancer

- Harvard Report on Cancer Prevention, 1996 -



Cancer & Genetic Susceptibility

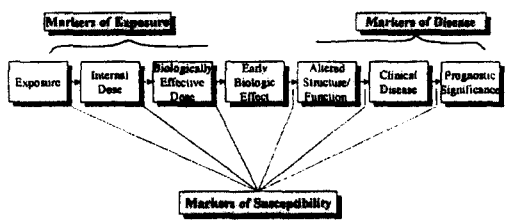


Determinants of Susceptibility

- *carcinogen absorption/activation*
  - physiological factors : pulmonary clearance
  - metabolic phenotypes/genotypes
  - lifestyle confounders : exercise, diet. smoking
- DNA damage processing
  - repair/misrepair (XP, Fanconi's)
- inherited alterations in oncogene/suppressor genes
  - genetic susceptibility (RB, FAP, Li-Fraumeni)

Molecular Epidemiological Research

-NRC, *Environ Health Perspect* (1987)-



Genes and Cancers of Current Investigation

- Mol Epi Lab, SNUCM -

	CYP 1A1	CYP 2E1	GST M/T	NAT 1/2	COMT	EH
Lung	○	○	●	○		○
Breast	○		●	●	●	
Bladder		○	●	●		

(●:analyzed, ○:analyzing)

Breast Cancer - risk factors-

- known risk factors such as family and reproductive history accounts for only 30% of the disease (Cosma et al. 1993).

1. Environmental factors
   diet : fatty diet, heterocyclic amines, smoking/alcohol
   environmental estrogens : PCB, DDE
2. Host factors
   family history, reproductive factors(menstruation/pregnancy)
   genetic factors : BRCA1, ***metabolism enzymes***

- large proportion of breast cancer cases cannot be attributed to known risk factors (Helzlsouer et al. 1998).

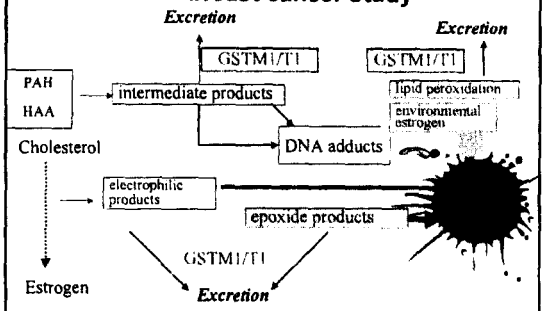
Glutathione-S transferase (GST)

- 17-28 kDa
- four isotypes :alpha, beta, mu, theta
- electrophilic substrates (formed during phase I reaction) + glutathione : hydrophilic metabolites
- major pathway of protection against chemical carcinogens
- free radical scavenging

Human *GSTs*

	# of genes	Location	Substrates
GSTA	>2	6	PAHs, aromatic amines, lipid peroxidation
GSTM	5	1p13	Lipid peroxidation
GSTP	1	11	PAH
GSTT	>2	?	Solvents
Microsomal GST	1	12	

Biologic plausibility of *GSTM1/T1* for breast cancer study



GSTM1/T1 and Breast Cancer

Reference	Cancer Res 1998;58:65-70 Bailey LR *et al.*	JNCI 1998;90:512-8 Helzlsouer KJ *et al.*
Ca/Co	164/162*	110/113
OR (95% CI)		
null *M1*	0.8(0.5-1.2)	2.1(1.2-3.6)
null *T1*	1.1(0.7-1.8)	1.5(0.8-3.0)
both null	0.7(0.4-1.2)	
Significant factors		obese postmenopausal (*GSTM1* null, OR=7.0)

* Caucasian

Objectives

- *distribution of genotypes in healthy Korean*
- *association between different genotypes and breast cancers*
- *gene-environment interaction* between known risk factors and genotypes
- *gene-gene interaction* among genotypes studied

Study Designs

- a hospital based case-control study
  - SNUH, Boramae, Sam-Sung, Asan
  - cases : *histologically confirmed tumors*
  - controls : no cancer or systemic diseases
- interview:
  - reproductive and menstruation factors
  - diet, smoking, alcohol, occupations, etc.
- genotyping of GSTM1 and GSTT1
  - multiplex PCR

Multiplex PCR of *GSTM1 & T1*

GSTT1

ß-globin
GSTM1

Results 1: *GSTs* and Breast Cancer

- 189 cases & 189 age matched controls
- significant risk factors (OR, 95% CI)
  - family history : 3.1 (1.12-8.63)
  - in postmenopausal women
    - BMI >26 vs <20 : 3.1 (1.02-8.86)
  - alcohol : 1.6 (0.98-2.76)
  - ever full-term pregnancy : 0.4 (0.14-0.98)
- smoking, age at menarche : no significance

Results 2 : *GSTs* and Breast Cancer

	Cases (n=189)	Controls (n=189)	OR (95% CI)
GSTM1			
Present	78(42%)	86(47%)	
Null	110(58%)	95(53%)	1.3(0.8-2.0)
GSTT1			
Present	94(50%)	105(58%)	
Null	94(50%)	76(42%)	1.6(1.0-2.5)
GSTM1+GSTT1			
Both present	32(17%)	48(25%)	
Any null	109(58%)	107(57%)	1.5(0.9-2.8)
Both null	48(25%)	34(18%)	2.2(1.1-4.2)

Combination of *GSTM1/T1* genotypes and breast cancer

	Cases N (%)	Controls N (%)	OR(95% CI)*
All women[§]			
No null	32 (17.0)	48(26.5)	1.0 (reference)
One null	108 (57.5)	95(52.5)	1.7 (0.98-3.08)
Two null	48(25.5)	38(21.0)	2.2 (1.13-4.45)
Premenopausal[§]			
No null	17 (14.9)	23(23.7)	1.0 (reference)
One null	66(57.9)	58(59.8)	2.1 (0.94-4.55)
Two null	31(27.2)	16(16.5)	4.4 (1.62-11.93)
Postmenopausal			
No null	15 (20.3)	23(28.8)	1.0 (reference)
One null	42 (56.7)	36(45.0)	1.8 (0.72-4.39)
Two null	12(23.0)	21(26.3)	1.2 (0.42-3.61)

§ P for trend for none, one, two null GST genotypes = 0.03

The ORs were adjusted for age, education, body mass index, age at menarche, age at first pregnancy, age at menopause, duration of breast feeding, and family history of breast cancer.

Interaction between the combination of GST genotypes and alcohol consumption

Combination of GSTM1 & GSTT1	Never drinker OR (95% CI)	Ever drinker OR (95% CI)	
All women			
No null	1.0 (reference)	1.0 (reference)	p-trend in
One null	1.7 (0.94-3.14)	1.7 (0.59-5.08)	ever-drinker
Two null	1.6 (0.79-3.25)	4.2 (1.01-17.31)	<0.05
Premenopausal			
No null	1.0 (reference)	1.0 (reference)	p-trend in
One null	1.5 (0.60-3.52)	1.8 (0.51-6.22)	ever-drinker
Two null	2.0 (0.70-5.70)	5.3 (1.03-27.76)	<0.05
Postmenopausal			
No null	1.0 (reference)	1.0 (reference)	
One null	1.8 (0.77-4.24)	1.8 (0.19-16.49)	
Two null	1.2 (0.46-3.28)	2.0 (0.11-35.81)	

Interaction between the combination of GST genotypes and status of first delivery

Combination of GSTM1 & GSTT1	FFTP-age<30 yr OR (95% CI)	FFTP-age>=30 yr, nullipara OR (95% CI)	
All women			
No null	1.0 (reference)	1.0 (reference)	p-trend in
One null	1.8 (1.00-3.35)	1.9 (0.48-7.26)	<0.05
Two null	1.7 (0.85-3.46)	8.8 (0.88-86.60)	
Premenopausal			
No null	1.0 (reference)	1.0 (reference)	
One null	1.7 (0.72-3.84)	1.4 (0.23-8.30)	
Two null	2.5 (0.94-6.83)	not estimated	
Postmenopausal			
No null	1.0 (reference)	1.0 (reference)	p-trend in
One null	2.0 (0.85-4.88)	2.0 (0.19-20.61)	<0.05
Two null	1.1 (0.39-3.05)	4.5 (0.25-80.57)	

N-acetyltransferases (NAT)

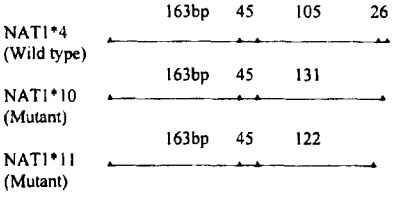
- chromosome 8p22
- transferring an acetyl group from cofactor acetyl coenzyme A to the aromatic amines
- two human isozymes :
  - NAT2 : "polymorphic NAT"
  - NAT1 :
    - human polymorphims were recently recognized
    - functional significance not yet clear

NAT Polymorphisms and Breast Cancer

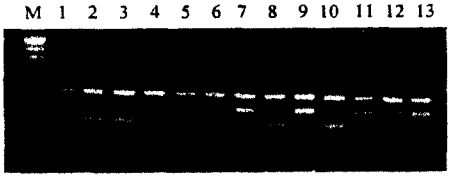
- Millikan (1998) : NAT1/2 & breast ca
  - 493 cases & 473 controls
  - neither NAT1 nor NAT2 alone significant
  - in postmenopausal smoking women
    - NAT1*10 : OR, 9.0 (95% CI, 1.9-41.8)
    - NAT2 rapid : OR, 7.4 (95% CI, 1.6-32.6)
- Chern (1997)
  - 139 cases & 133 controls
  - OR, 1.9(1.0-3.7) in postmenopausal women

Genotyping of NAT1

- DNA extraction with Qiagen extraction kit
- nested PCR and RFLP (MboII)



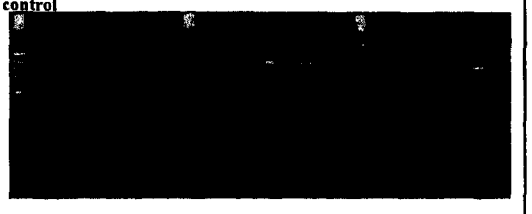
NAT1 : Nested PCR & RFLP(Mbo II)



M : molecular size marker
NAT1*4/4 : lane 5,6,
NAT1*10/4 : lane 1,2,3,4,8,10,12
NAT1* 10/10 : lane 7,9,11,13

NAT2 : Nested PCR & RFLP

M1(KpnI),M2(TaqI),M3(BamHI)



TaqI polymorphism (M2) : control w/w w/w w/M2 w/w w/w w/w M2/M2
BamHI polymorphism (M3) : control w/M3 w/w w/w w/w w/w w/w w/w
KpnI polymorphism (M1) : control w/w w/w w/M1 w/w w/w w/w w/w

Association between NAT1 genotype and breast cancer

	Cases N (%)	Controls N (%)	OR(95% CI)*
All women			
NAT1*10	**131(73.2)**	**124(74.7)**	**1.0 (reference)**
NAT1*non-10	**48(26.8)**	**42(25.3)**	**1.0 (0.58-1.67)**
Premenopausal			
NAT1*10	**83(74.1)**	**63(70.8)**	**1.0 (reference)**
NAT1*non-10	**29(25.9)**	**26(29.2)**	**0.8 (0.38-1.51)**
Postmenopausal			
NAT1*10	**48(71.6)**	**58(78.4)**	**1.0 (reference)**
NAT1*non-10	**19(28.4)**	**16(21.6)**	**1.6 (0.67-3.99)**

The ORs were adjusted for age, education, body mass index, age at menarche, age at first pregnancy, age at menopause, duration of breast feeding, family history of breast cancer, and menopausal status at baseline.

Gene-host and gene-environment interaction between NAT1 and NAT2 genotypes and breast cancer

	NAT1 wild	NAT1 mutant	NAT2 wild	NAT2 mutant
Family history				
No	1.0	1.1 (0.68-1.84)	1.0	1.1 (0.69-1.66)
Yes	1.0	0.7 (0.10-5.18)	1.0	0.7 (0.14-3.82)
Alcohol consumption				
Never	1.0	1.0 (0.58-1.71)	1.0	1.1 (0.65-1.70)
Ever	1.0	1.9 (0.56-6.67)	1.0	0.9 (0.34-2.15)
Cigarette smoking				
Never	1.0	1.2 (0.67-2.13)	1.0	0.9 (0.55-1.43)
Ever	1.0	5.3 (0.70-39.5)	1.0	0.4 (0.05-2.77)

Catechol-O-methyltransferase (COMT)

- role : inactivation of catechol estrogen by O-methylation
- ubiquitous existence : liver, kidney, brain, RBC,
- polymorphic in human and ethnic difference : wide inter-individual variation of the activity
- molecular epidemiological studies : associated with Parkinsonism, OCD,

Genetic Polymorphism of COMT

: chromosome 22, HSCOMT gene

1947th Nucleotide	158th a.a.	COMT activity
Guanine	**Valine**	**High** ($COMT^H$)
Adenine	**Methionine**	**Low** ($COMT^L$)

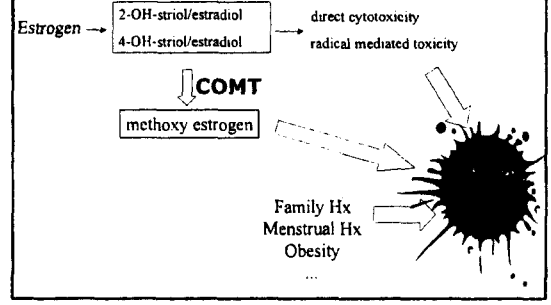
Genotypes
- High activity ($COMT^H$ $COMT^H$)
- Medium activity ($COMT^H$ $COMT^L$)
- Low activity ($COMT^L$ $COMT^L$)

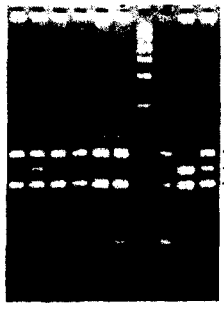
COMT and Breast Cancer

Reference	Cancer Res 1997;57:5493-7 Lavigne A *et al.*	Cancer Res 1998;58:2107-10 Thompson PA *et al.*	Carcinogenesis 1998;19:1943-7 Millikan RC *et al.*
Ca/Co	113/114	281/289	389/379*
OR (95% CI)			
for HL	1.3(0.7-2.6)	1.3(0.9-1.9)	0.8(0.6-1.1)
for LL	1.5(0.7-3.1)	0.8(0.5-1.4)	0.7(0.5-1.1)
Significant factors	obese postmenopausal (for LL, OR=3.6)	obese premenopausal (for HL+LL, OR=5.7)	no significant association

* Caucasian

Is **COMT** a risk factor for ***breast cancer*** ?





- **Gel Electrophoresis**
  - 100 V for 35 minutes
  - 3% Metaphor® gel

200 bp
150 bp
114 bp
96 bp
83 bp

Selected characteristics of 181 breast cancer cases and 288 controls

	Premenopausal		Postmenopausal	
	Cases	Controls	Cases	Controls
Age	41.2(6.0)*	38.1(7.8)	57.9(8.9)	58.2(9.0)
Education (over college)	33%	28%	19%*	11%
Age at menarch	15.2(1.5)*	14.6(1.7)	6.0(1.7)	15.9(1.8)
Fullterm pregnancy	93%	87%	96%*	89%
Age at first pregnancy	26.8(4.0)*	25.4(3.0)	25.5(3.5)*	23.8(3.7)
BMI	22.6(2.9)	22.3(3.0)	24.1(3.6)	23.5(3.3)
Family Hx of breast ca.	8%	6%	14%*	5%

Mean (SD), *: P<0.05

COMT and Breast Cancer

	Cases	Controls	OR
HH	88(49%)	170(59%)	1.0
HL+LL	93(51%)	118(41%)	1.5(1.01-2.20)

HH: $COMT^{Val/Val}$
HL: $COMT^{Val/Met}$
LL: $COMT^{Met/Met}$

Interactive effect of COMT genotypes and BMI on breast cancer

	BMI(lean)		BMI(normal)		BMI(obese)	
	Ca/Co	OR*(95% CI)	Ca/Co	OR(95% CI)	Ca/Co	OR(95% CI)
Postmenopausal						
HH	6/13	1.0 (reference)	12/22	1.0 (reference)	15/23	1.0 (reference)
HL+LL	8/10	1.2 (0.26-5.83)	11/14	1.6 (0.53-5.03)	19/19	1.5 (0.60-3.86)
Premenopausal						
HH	9/44	1.0 (reference)	20/35	1.0 (reference)	15/25	1.0 (reference)
HL+LL	21/28	2.0 (0.88-4.70)	18/16	3.3 (1.10-10.06)	16/25	1.0 (0.38-2.43)

*OR was adjusted on age and education of subjects.

The interaction of the combination of *GSTM1/T1* genotypes and COMT polymorphism for breast cancer

		COMT	
	HH-type	HL+LL type	*p for interaction*
O ***All women***			
No null	1.0 (ref.)	3.1 (1.0-9.6)	
One null	3.4 (1.3-8.9)	4.9 (1.9-13.0)	*p=0.04*
Two nulls	5.0 (1.6-15.6)	3.6 (1.2-11.2)	
p for trend	*p=0.006*	*p=0.83*	
O ***Premenopausal***			
No null	1.0 (ref.)	2.2 (0.4-11.0)	
One null	3.6 (1.0-13.1)	5.7 (1.5-22.7)	*p=0.006*
Two nulls	6.7 (1.4-31.7)	11.3 (1.7-76.4)	
p for trend	*p=0.01*	*p=0.04*	
O ***Postmenopausal***			
No null	1.0 (ref.)	3.5 (0.6-20.0)	
One null	3.7 (0.8-17.4)	4.4 (1.0-20.3)	*p=0.68*
Two nulls	3.8 (0.6-23.8)	2.2 (0.4-12.2)	
p for trend	*p=0.1*	*p=0.5*	

Conclusions

- genotypes of risk
  - both *GSTM1* and *GSTT1* null type
  - low activity COMT genotypes
- gene-environment interaction
  - *GSTM1/T1*: alcohol consumption, nulliparous, first pregnancy 30YO
  - *NAT1*: smoking
  - COMT: obese premenopause
- gene-gene interaction
  - both *GSTM1/T1* null & low activity COMT genotypes: 11-fold increases in risk

Future Directions

- to evaluate the *association between previously studied genes with other biologically relevant genes for breast cancer*
  - estrogen metabolism: CYP1B1, CYP17,
  - DNA repair: XRCC1, hOGG1,
  - oncogene/tumor suppressor gene: BRCA1,
- to explore the potential usefulness of high throughput technology: *DNA chips*,
- to validate the genotypes for early detection of high risk individuals of breast cancer

Acknowledgement

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  - Cho SH, Yoo KY (prev med, SNUCM), Shin SG (SNUCM)
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  - breast ca: Noh DY, Choe KJ, Ahn SH(SNUH, Asan)
  - bladder ca: Kim SW, Park MS, Choi H, Choi HY (SNUH, Boramae, Sam-Sung)
  - lung ca: Kim YH, Lee SJ(SNUH)
- interview & genotyping
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