Session Ⅲ-2

Molecular Epidemiological Study on Environmental and Genetic Risk Factors of Pediatric Cancer in Korea*

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Backgrounds

- second most frequent cause of death among infants or children aged 1 or more (cf. first one is accident and poisoning)
- cured childhood cancer cases need continuous health care
- etiology of childhood cancer remains to be elucidated
- ethnic diversity in genetic composition and environmental exposure warrants epidemiolgical study on childhood cancer in Korean population

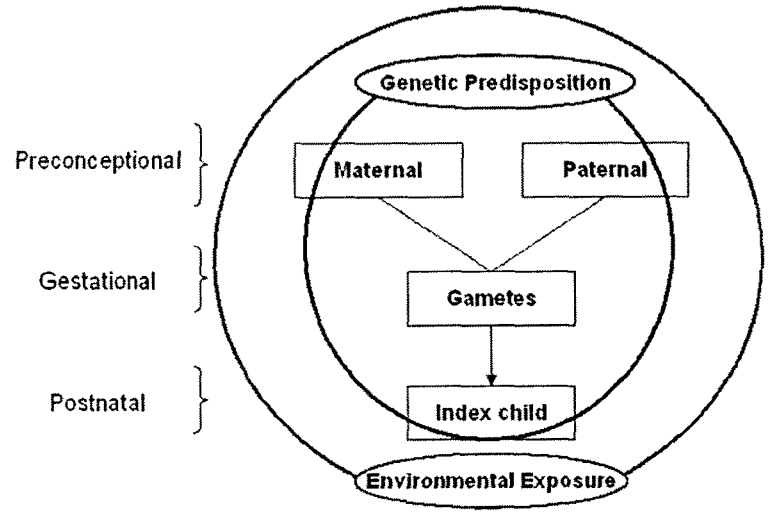
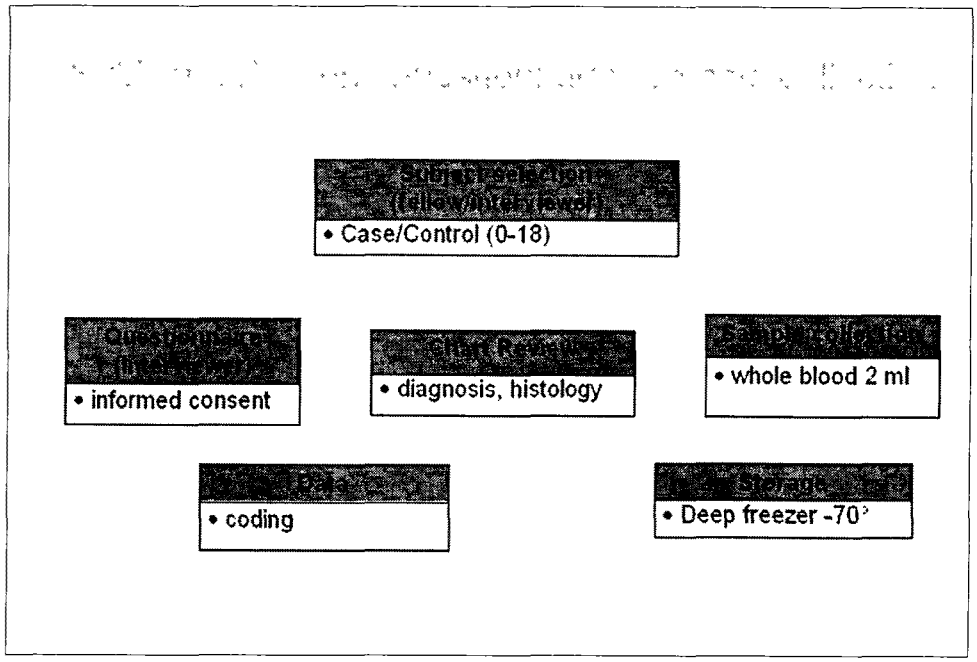


Figure 1. Etiology of childhood cancer: Environmental exposure and genetic predisposition

Objectives

- to evaluate the association between environmental factors and childhood cancer
- to evaluate the association between *CYP1A1* haplotype and childhood cancer
- to evaluate the interaction between parental smoking and *CYP1A1* haplotype on the development of childhood cancer



Subjects (Arp/2003~May/2005)

- Cases (n=284)
  - recruited from the Department of Pediatrics in SNUH, SMC and AMC
  - leukemia (63%, n=178), brain tumor (22%, n=62) and lymphoma (16%, n=44)
  - age: 0-18
- Controls (n=298)
  - non-cancer controls (acute gasteroentritis (11%), hernia (10%), peumonia (8%), LCP (5%), fracture, infectious diseases etc.)
  - recruited from SNUH, SMC, and Borame Hospital
  - age: 0-18
- Informed consent (reviewed by IRB of each hospital)

Table 1. Age distribution of subjects

Age	Leukemia	Brain tumor	Lymphoma	All cases	Controls
<1	12 (5)	2 (3)	2 (5)	13 (5)	39 (13)
1~4	59 (33)	20 (32)	10 (23)	89 (31)	125 (42)
5~9	55 (31)	19 (31)	15 (34)	89 (31)	78 (26)
10~14	48 (27)	17 (27)	15 (34)	80 (28)	50 (17)
15+	7 (4)	4 (6)	2 (5)	13 (5)	6 (2)
Total	178 (100)	62 (100)	44 (100)	284 (100)	298 (100)

Histology of childhood cancer

- Leukemia
  - acute lymphoblastic leukemia (ALL) (66%)
  - acute myeloid leukemia (AML) (21%)
  - acute biphenotypic leukemia (9%)
- Brain tumor
  - medulloblastoma (40%)
  - germ cell tumor (26%)
  - primitive neuroectodermal tumor (21%)
- Lymphoma
  - B-cell (including Brukitt's lymphoma) (65%), T-cell (23%), and Hodgikin's disease (12%)

Questionnaire

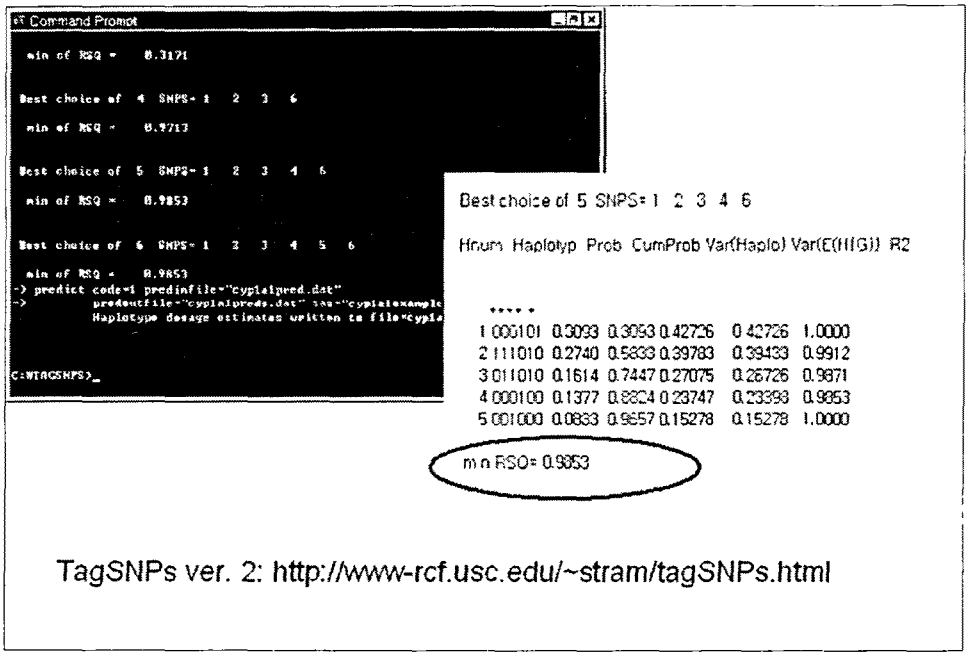
- interview with mother
- collected information
  - child: gender, birth order, birth weight, duration of breast feeding
  - farther & mother: education, smoking status & habit, alcohol consumption, occupation one year before the birth of the child
  - mother: medication of supplements during pregnancy, oral contraceptives
  - others: family history of cancer, family income, distance to transmission line, electric appliances at home (duration and frequency of the use), pesticide use, history of hospitalization of the child for 3 days or more

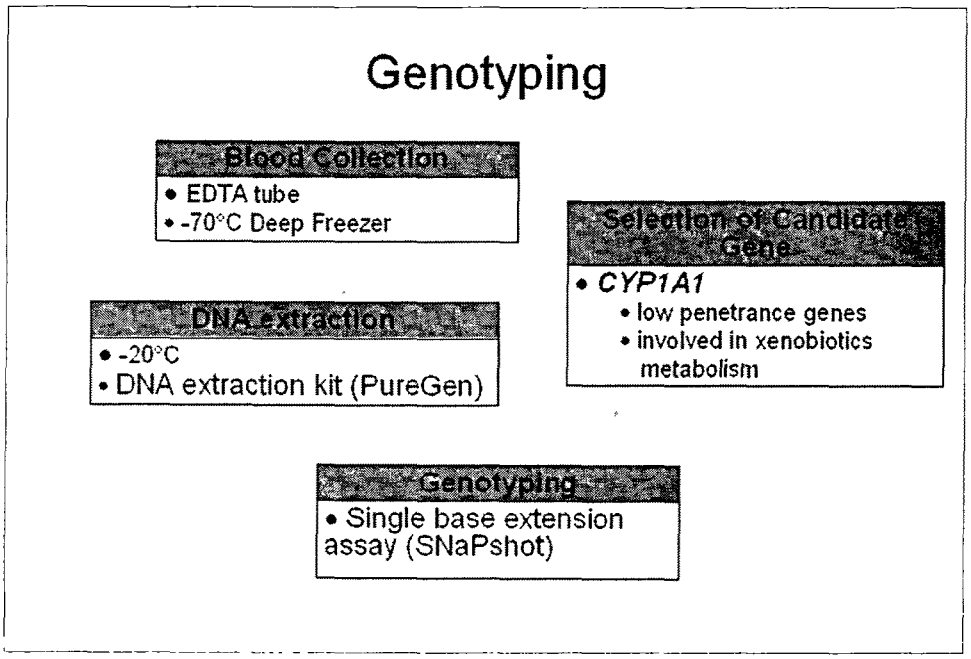
Selection of SNPs in *CYP1A1*

- CYP1A1
  - Phase I enzyme: activation of PAHs
- SNP searching
  - SNP500Cancer/JSNP/NCBI SNP database
  - minor allele frequency >10%
  - 10 locus
- 5 tagSNPs selected from 48 genotype data
  - min RSQ>0.98

Table 2. Selected haplotype tagging SNP of *CYP1A1* with minor allele frequency more than 10%

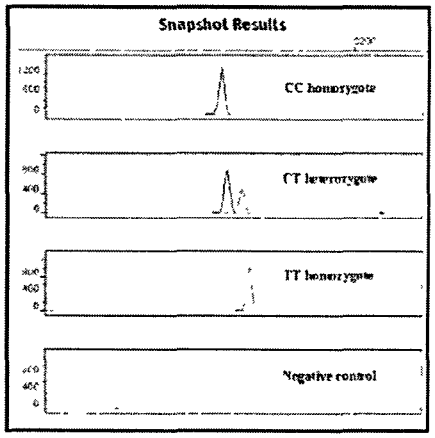
No.	rs#	Locus
1	rs2472299	5'-region
2	-	5'-region
3	rs1048943	exon7
4	rs4646903	3'-region
5	rs2198843	3'-region


Command Prompt
min of RSQ = 0.3171
Best choice of 4 SNPS= 1 2 3 6
min of RSQ = 0.9713
Best choice of 5 SNPS= 1 2 3 4 6
min of RSQ = 0.9853
Best choice of 6 SNPS= 1 2 3 4 5 6
min of RSQ = 0.9853
-> predict code=1 predinfile="cyp1a1pred.dat"
Haplotype dosage estimates written to file=cyp1a
C:\TAGSNPS>_
Best choice of 5 SNPS= 1 2 3 4 6
Hnum Haplotyp Prob CumProb Var(Haplo) Var(E(I|G)) R2
1 000101 0.3093 0.3093 0.42726 0.42726 1.0000
2 111010 0.2740 0.5833 0.39783 0.39433 0.9912
3 011010 0.1614 0.7447 0.27075 0.26726 0.9871
4 000100 0.1377 0.8824 0.23747 0.23393 0.9853
5 001000 0.0833 0.9657 0.15278 0.15278 1.0000
min RSQ= 0.9853
TagSNPs ver. 2: http://www-rcf.usc.edu/~stram/tagSNPs.html


Genotyping
Blood Collection
• EDTA tube
• -70°C Deep Freezer
DNA extraction
• -20°C
• DNA extraction kit (PureGen)
Selection of Candidate Gene
• CYP1A1
• low penetrance genes
• involved in xenobiotics metabolism
Genotyping
• Single base extension assay (SNaPshot)

Single base extenstion assay

- PCR
  - PTC-225 Thermal cycler (MJ Research, MA)
- Primer extension
  - ABI 3800 DNA analyzer (ABI, USA)
- Quality Control
  - 5*5 QC samples
  - 124/125=99% concordance rate



Statistical Analysis

- frequency matching for sex and age (SAS proc surveyselect)
  - age: 0, 1-2, 3-4, 5-6, 7-8, 9-10, 11-12, 13-14, _15
  - Leukemia: 166/166; Brain tumor: 62/62, Lymphoma: 44/44
- multiple logistic regression: OR (95% CI)
  - adjusted for age of child, father's education, and birth weight
- *CYP1A1* haplotype estimation
  - Bayesian method: PHASE ver. 2.0.2
- diplotype analysis: multiple logistic regression

Results

Table 3. Characteristic of childhood leukemia and controls

		Cases	Controls	P-value/ OR (95% CI)
sex, N (%)	male	103 (62.1)	103 (62.1)	1.00
	female	63 (37.9)	63 (37.9)	
age, mean (±SD)		7.00 (±4.32)	7.11 (±4.39)	0.82
birth weight (kg), mean (±SD)		3.38 (±0.46)	3.18 (±0.53)	<0.01
	<3.25	62 (37.4)	96 (57.8)	1.0 (ref.)
	3.25~3.7	55 (33.1)	46 (27.7)	1.9 (0.12-3.07)
	3.7≤	49 (29.5)	24 (14.5)	**3.2 (1.76-5.67)**
	p-for trend			***<0.01***

Table 4. Medicine during pregnancy and childhood leukemia

		Case	Controls	OR (95% CI)*
		N (%)	N (%)	
iron supplements	no	81 (50.3)	86 (52.4)	1.00 (ref.)
	yes	80 (47.6)	78 (47.6)	1.1 (0.70-1.74)
medicine during pregnancy	no	151 (93.8)	161 (98.2)	1.00 (ref.)
	yes	10 (6.2)	3 (1.8)	**4.6 (1.19-17.7)**

*adjusted for age of child, father's education, and birth weight

Table 5. Paternal smoking and childhood leukemia

		Cases N (%)	Controls N (%)	OR (95% CI)*
Paternal smoking status				
	400≤cigarettes	34 (10.5)	42 (25.5)	
	>400 cigarettes	132 (89.5)	123 (74.5)	1.3 (0.73-2.26)
Paternal smoking at home				
	no	89 (53.9)	106 (65.8)	
	yes	76 (46.1)	55 (34.2)	**1.7 (1.04-2.66)**
Paternal smoking at home at the presence of child				
	no	127 (77.0)	135 (83.9)	
	yes	38 (23.0)	26 (16.2)	1.5 (0.83-2.69)

*adjusted for age of child, father's education, and birth weight

Table 6. *CYP1A1* haplotype distributions in childhood brain tumor cases and control subjects

Haplotype*	Brain tumor		Controls		P-value
	N	%	N	%	(χ^2-test)
TGATG	37	30.8	36	30.0	0.20
CAGCC	26	21.7	34	28.3	
CGATG	21	17.5	16	13.3	
CGACC	12	10.0	20	16.7	
CGATC	19	15.8	9	7.5	
Others	5	3.3	5	4.2	
Total	120	100	120	100	

*estimated by Bayesian method using PHASE ver. 2.0.2: composed of four polymorphic sites: rs2472299 (C>T), rs4646903 (T>C), rs2198843 (C>G), and -9893G>A

Table 7. Interactive effect between paternal smoking and *CYP1A1* diplotypes and in childhood leukemia cases and control subjects

		CGACC containing diplotypes	Other diplotypes*	OR (95% CI)†
All		34/47‡	123/116	**1.5** (0.68-2.52)
Paternal smoking status				
	400≤cigarettes	8/8	24/32	0.7 (0.17-2.68)
	>400 cigarettes	26/39	99/83	**1.9** (1.03-3.39)
	p-for interaction			*0.11*
Paternal smoking at home				
	no	20/24	65/80	1.1 (0.53-2.19)
	yes	14/21	57/33	**2.3 (1.03-5.32)**
	p-for interaction			*0.12*
Paternal smoking at home at the presence of child				
	no	26/36	94/96	1.5 (0.81-2.72)
	yes	8/9	28/17	1.4 (0.43-4.81)
	p-for interaction			*0.99*

*other than CGACC combination; †adjusted for age of child, father's education, and birth weight; ‡no. of cases/no. of controls

Table 8. Interactive effect between paternal smoking and *CYP1A1* diplotypes in childhood brain tumor cases and control subjects

		Other diplotypes*	CGATC Containing diplotypes	OR (95% CI)†
All		43/52‡	17/8	**2.8** (1 06-7 41)
Paternal smoking status				
	400≤cigarettes	10/4	4/5	0 5 (0 07-3 68)
	>400 cigarettes	33/48	13/3	**6.1** (1 56-24 0)
	p-for interaction			*0 01*
Paternal smoking at home				
	no	21/26	6/7	1 5 (0 37-5 70)
	yes	22/26	11/1	**13.3 (1.49-119)**
	p-for interaction			*0 07*
Paternal smoking at home at the presence of child				
	no	35/36	11/7	2 2 (0 72-7 07)
	yes	8/16	6/1	**14.6** (0 88-241)
	p-for interaction			*0 24*

'other than CGATC combination; †adjusted for age of child, father's education, and birth weight; ‡no. of cases/no. of controls

Table 9. Interactive effect between paternal smoking and *CYP1A1* diplotypes in childhood lymphoma cases and control subjects

		CGACC containing diplotypes	Other diplotypes*	OR (95% CI)†
All		8/12‡	34/31	**1.5** (0 48-4 63)
Paternal smoking status				
	400≤cigarettes	3/1	4/9	0 1 (0 01-6 10)
	>400 cigarettes	5/11	30/22	**2.7** (0 68-10 8)
	p-for interaction			*0 03*
Paternal smoking at home				
	no	4/6	14/21	0 9 (0 18-4 21)
	yes	4/6	20/8	**3.4** (0 40-28 0)
	p-for interaction			*0 28*
Paternal smoking at home at the presence of child				
	no	6/7	26/25	1 2 (0 31-4 24)
	yes	2/5	8/4	**5.5** (0 29-105)
	p-for interaction			*0 35*

'other than CGACC combination; †adjusted for age of child, father's education, and birth weight; ‡no. of cases/no. of controls

Summary

- birth weight & childhood leukemia (p-for trend<0.01)
- medication during conception & childhood leukemia (OR=4.6)
- Paternal smoking at home & childhood leukemia (OR=1.7)
- Effect of genetic polymorphisms of *CYP1A1* was modified by the paternal smoking status & habit in a dose-response manner