“The Hokkaido Study of Environment and Children's Health” --- *Exploiting Gene-Environment Interaction to Detect Adverse Health Effects of Environmental Chemicals on the Next Generation*

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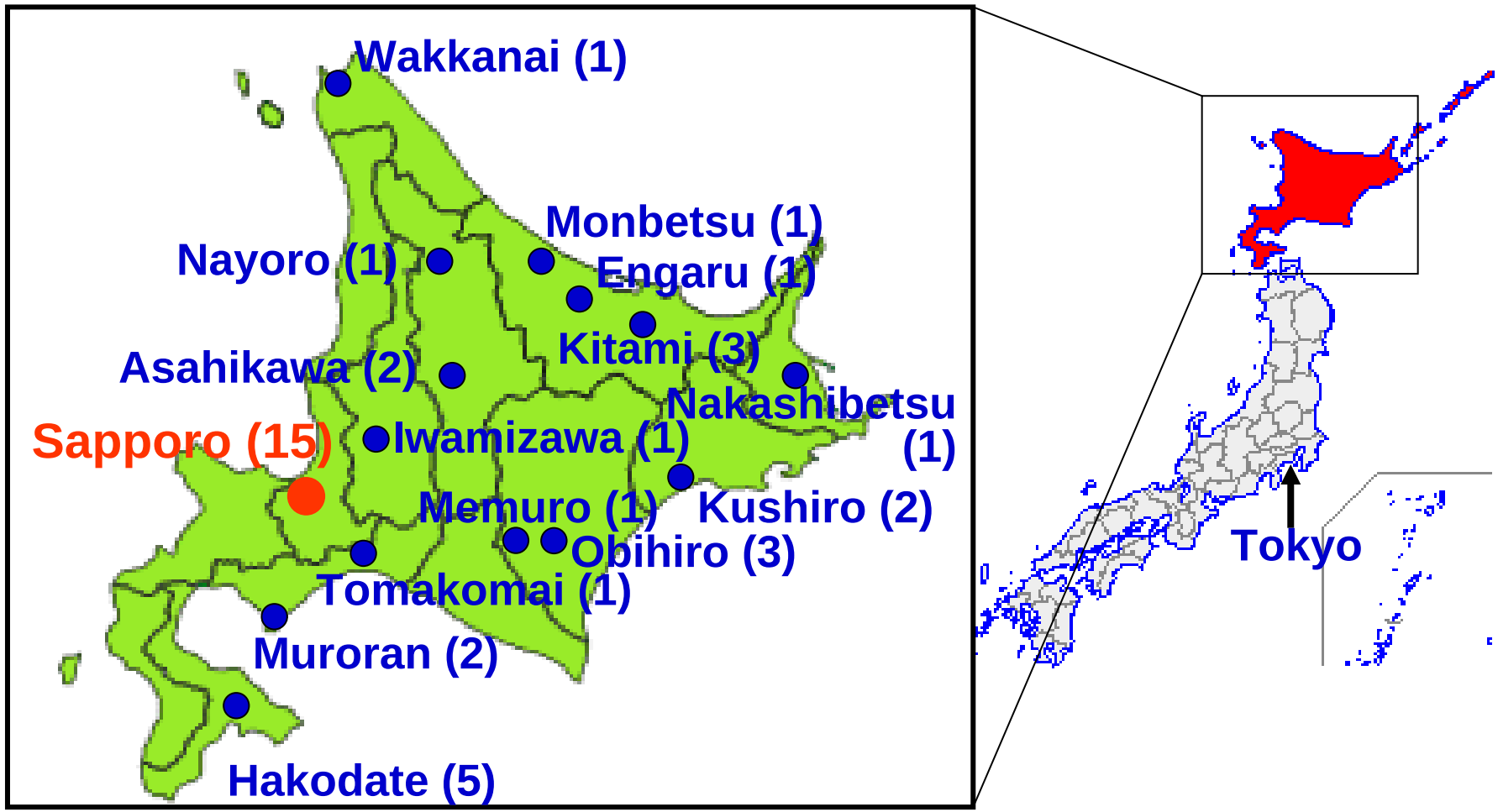
The Hokkaido Study on Environment and Children's Health

Populations: 5,6 millions: epidemiological studies



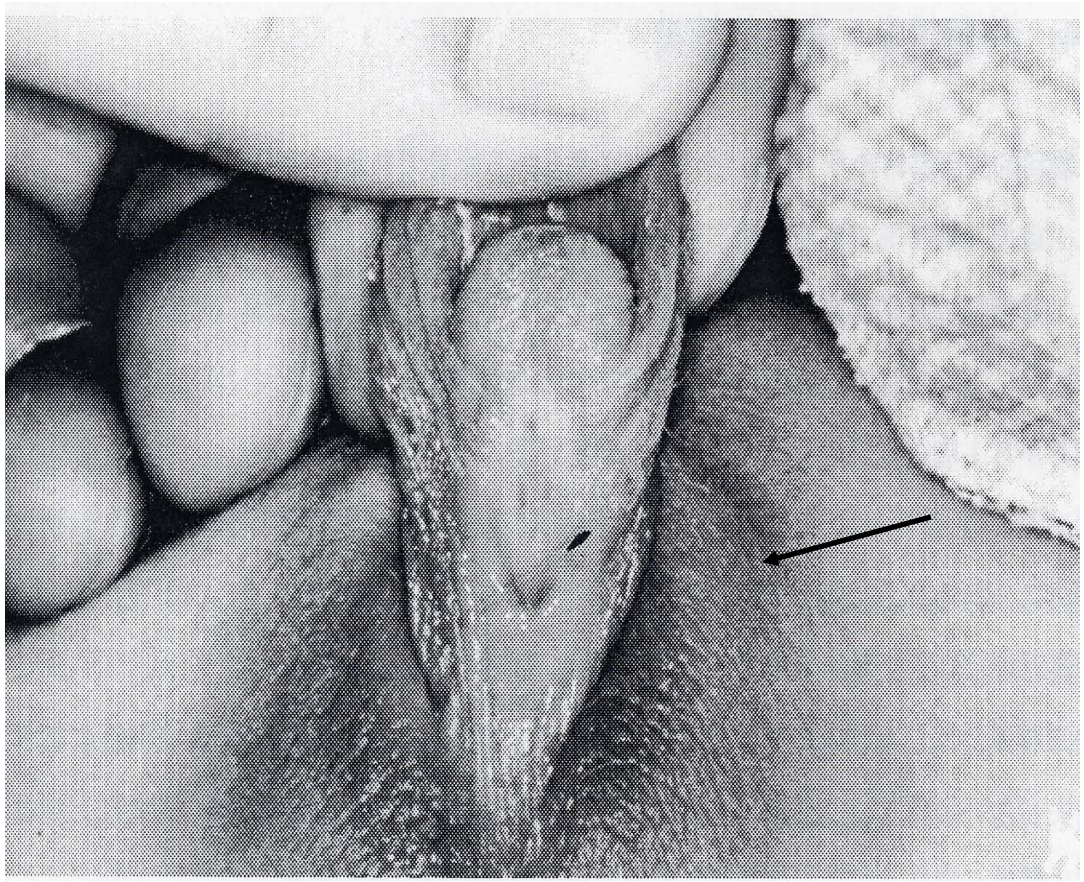
1. **Hypospadias and its genetic and environmental risk factors**

2. **Prenatal exposure to PCBs･Dioxin, birth weight, & developments**

3. **Maternal smoking, polymorphisms & birth size**

1. Hypospadias and its genetic and environmental risk factors

Hypospadias



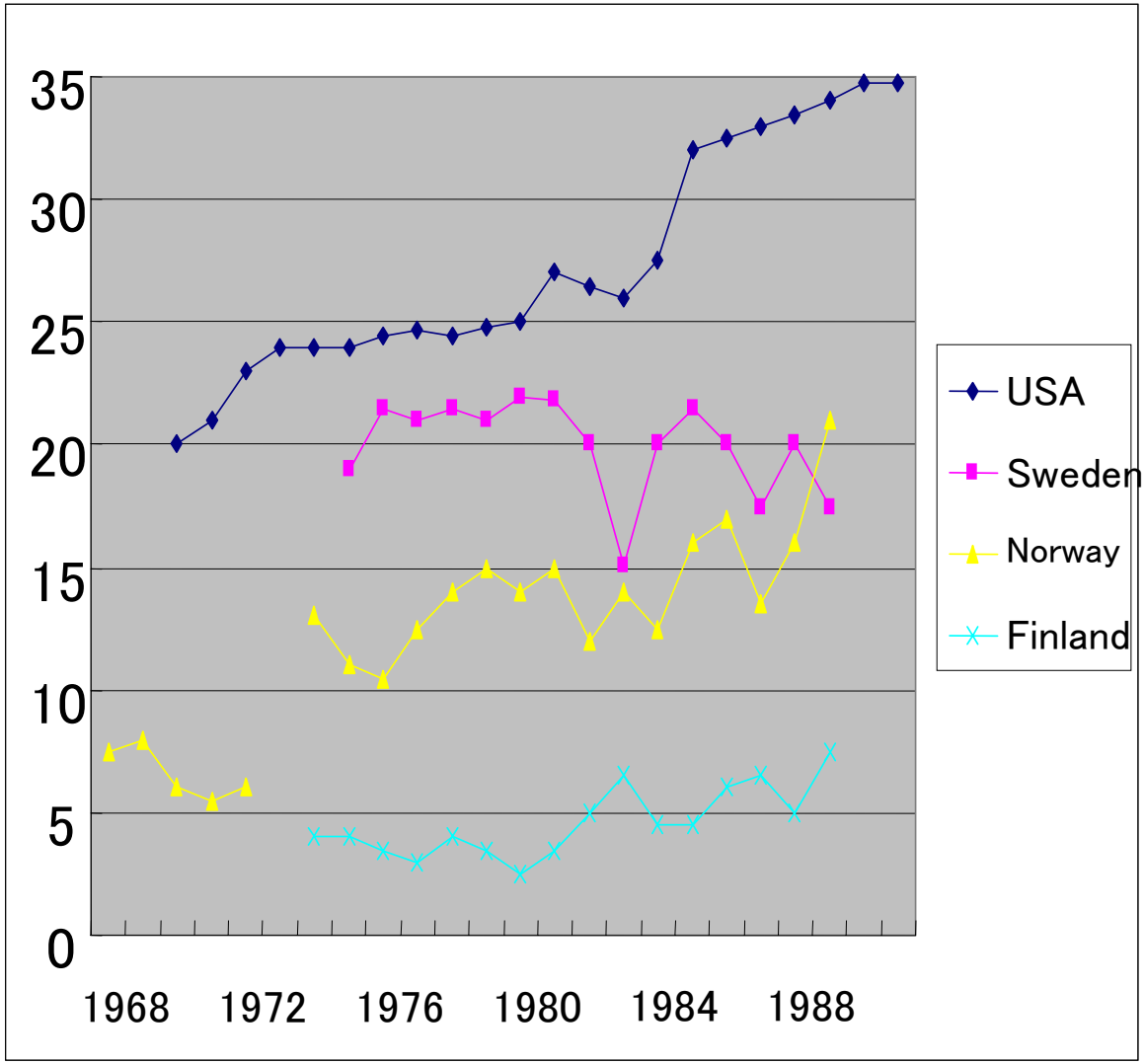
A common congenital anomaly,

the incomplete fusion of the urethral folds

The urethral opening is ventral surface of the penis, or on the scrotum, or the perineum

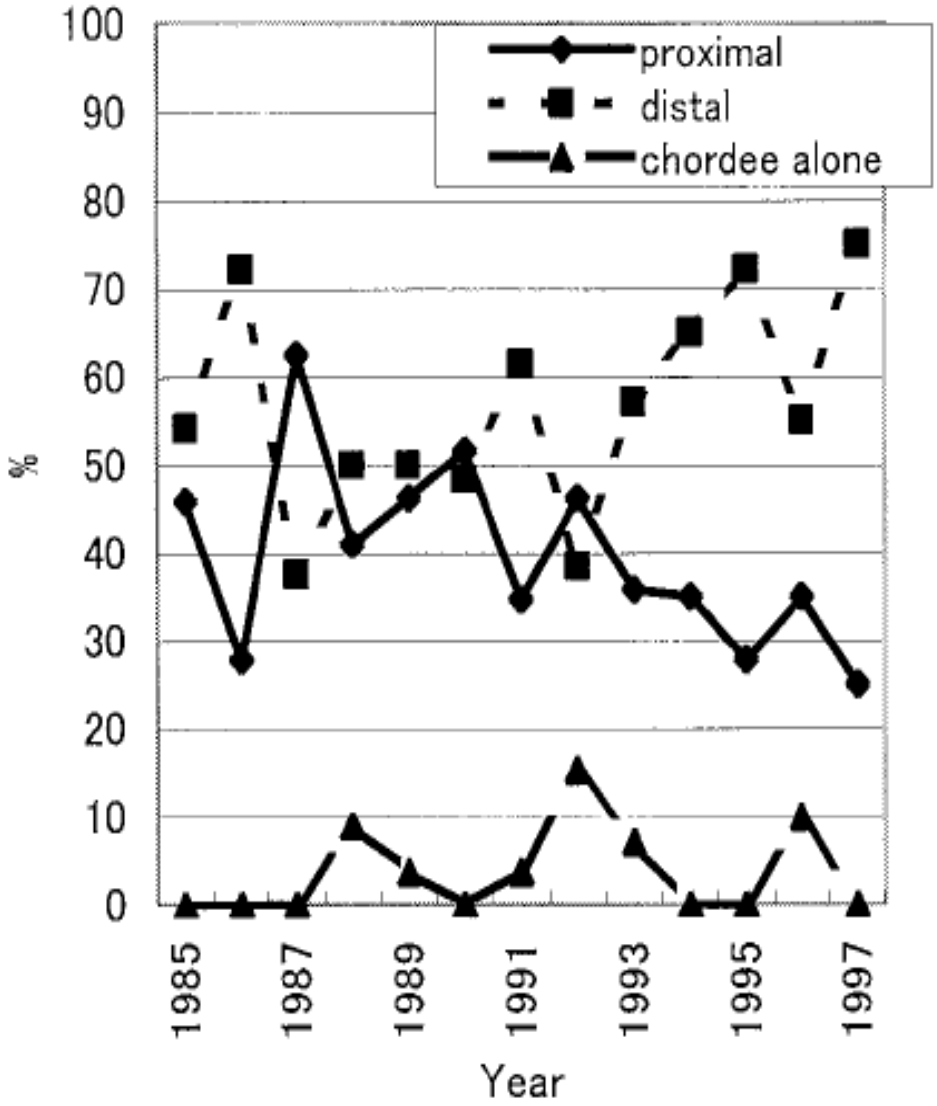
Photo by Department of Renal and Genitourinary Surgery, Hokkaido University Hospital, Sapporo

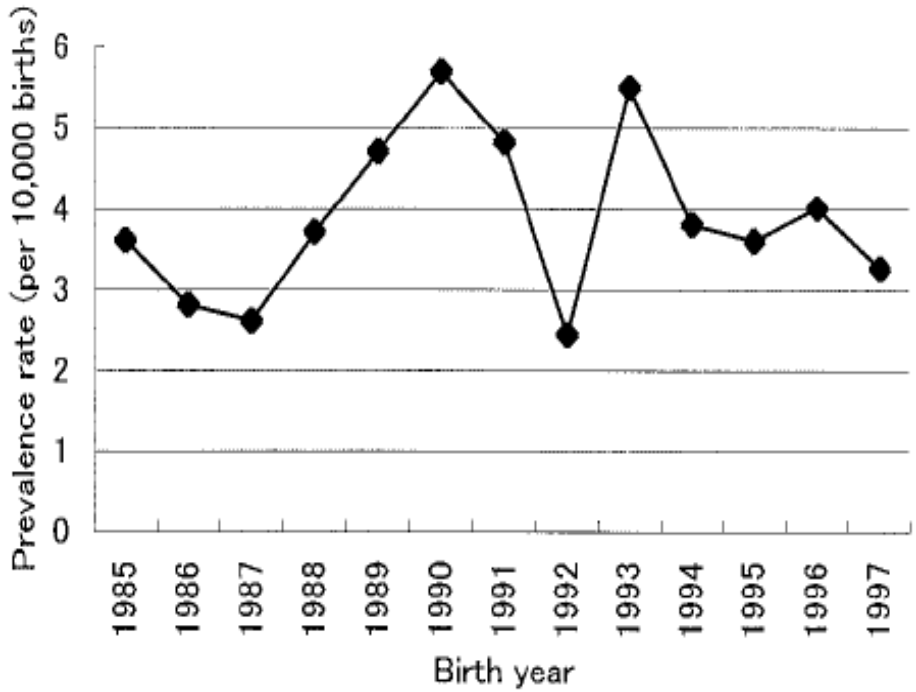
Transition of Incidence of Hypospadias



Toppari et al.（2001）

Prevalence of hypospadias from 1985 to 1997 in Hokkaido





Kurahashi et al., 2004

Environmental risk factors for hypospadias reported previously

drugs	progestins
	Diethylstilbestrol (DES)
	antiepileptic drugs
chemicals	pesticides
	residence near landfill sites
natural toxins	mycotoxins
phytoestrogens	vegetarian diet
Accidents or Disasters	Seveso accident
	Agent Orange exposure in the Vietnam War

Studies on the environmental exposure and risk of hypospadias

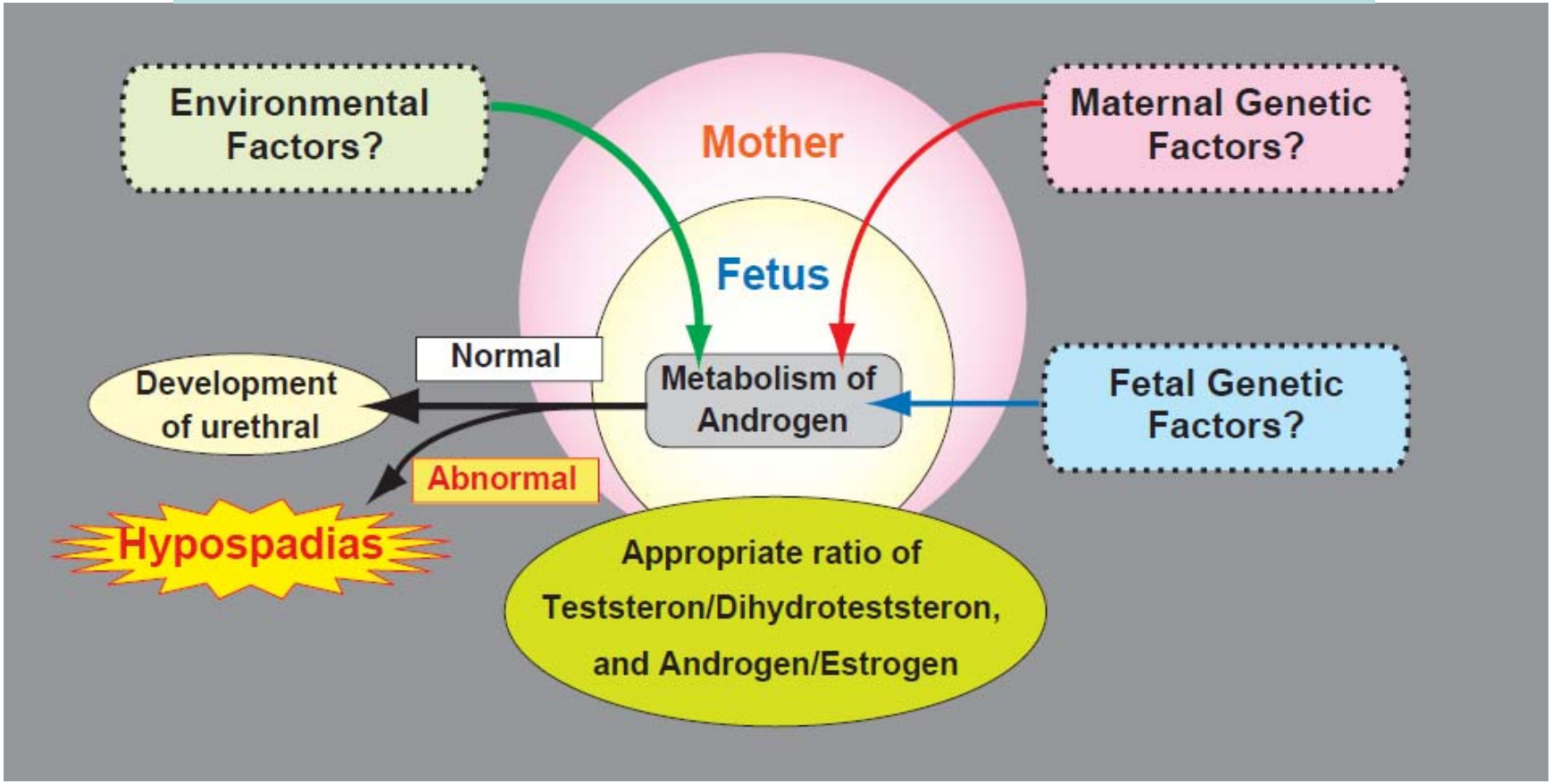
Study	Exposure	Statistical inference
Kristensen et al. (1997)	Farmers	No significant association
	Tractor spraying equipment and grain	OR = 1.51 (CI = 1.00-2.26)
Weidner IS *et al.* (1998)	Parents in farming or Gardening	No significant association
Longnecker MP *et al.* (2002)	Maternal serum DDE levels during pregnancy	No significant association
Pierik FK *et al.* (2004)	Paternal smoking	OR = 3.8 (CI = 1.1-13.4)
Carmichael SL *et al.* (2004)	Maternal smoking	No significant association
Meyer KJ *et al.* (2006)	Diclofop-methyl (per 0.05 lb applied)	OR = 1.08 (CI = 1.01-1.15)
	All study pesticides (per 0.5 lb applied)	OR = 0.82 (CI = 0.70-0.96)
	Applications of permethrin	OR = 0.37 (CI = 0.16-0.86)
Carbone P *et al.* (2006)	"Pesticide impact" on the basis 3 quantitative criteria on intensity of agricultural activities of population	Higher association of the birth prevalence of hypospadias with "Pesticide impact" (*P*=0.003)
Carbone P *et al.* (2007)	EDC at work, Work in agriculture, pesticides	No significant association

The associations of hypospadias with maternal and fetal characteristics

Study	Design	Population and sample size	Mother/ Infant	Factor	Statistical inference
Hughes *et al.* (1997)	Case-control, US	51 cases, 7839 boys and 7393 girls in ALSPAC (1991-1992)	Infant	Birth weight ▼	$P < 0.0001$
				Birth length ▼	$P < 0.0001$
				Head circumference ▼	$P < 0.0001$
Hussain *et al.* (1998)	Retrospective cohort, US	112 cases of 6738 male infants in NICUs (1987-2000)	Infant	Birth weight ▼	$R^2 = 0.98$, $P < 0.0001$
				Birth length ▼	$R^2 = 0.93$, $P < 0.02$
				Head circumference ▼	$R^2 = 0.98$, $P = 0.0004$
			Mother	More than 2nd born	$P < 0.001$
Aschim *et al.* (2002)	Case-control, Norway	N = 961,396, 2382 cases, live Born in 1967-1998	Mother	Having health problems	OR = 1.25 (CI = 1.14-1.38)
				Parity (more than 1)	$P < 0.001$
			Infant	Retained placenta	OR = 1.67 (CI = 1.18-2.37)
				Gestational age ▼	$P = 0.002$
				Birth weight ▼	$P < 0.001$
				Other malformation	OR = 2.72 (CI = 2.30-3.20)
Picric *et al.* (2004)	Case-control, Netherlands	56 cases and 313 controls	Mother	Height ▼	$P < 0.05$
				Educational level ▼	$P < 0.05$
				General health ▼	$P < 0.05$
Boise et al. (2004)	Prospective cohort, with 3 yr follow-up, Denmark	n =1,072, with 74.4% completing In the study	Infant	Birth weight ▼	$P = 0.027$
				Birth length ▼	$P = 0.030$
				Head circumference ▼	$P = 0.030$
				Weight for gestational age ▼	$P = 0.011$
Chong *et al.* (2006)	Case-control, Singapore	27 cases and 6511 controls, 1999-2005	Mother	Preeclampsia	OR = 3.90 (1.50-10.14)
			Infant	Very low birth weight	OR = 14.12 (5.48-36.39)
				Small for gestational age	OR = 3,23 (1.25-8.37)

Fetal small birth physique and maternal health problem may be related with hypospadias.

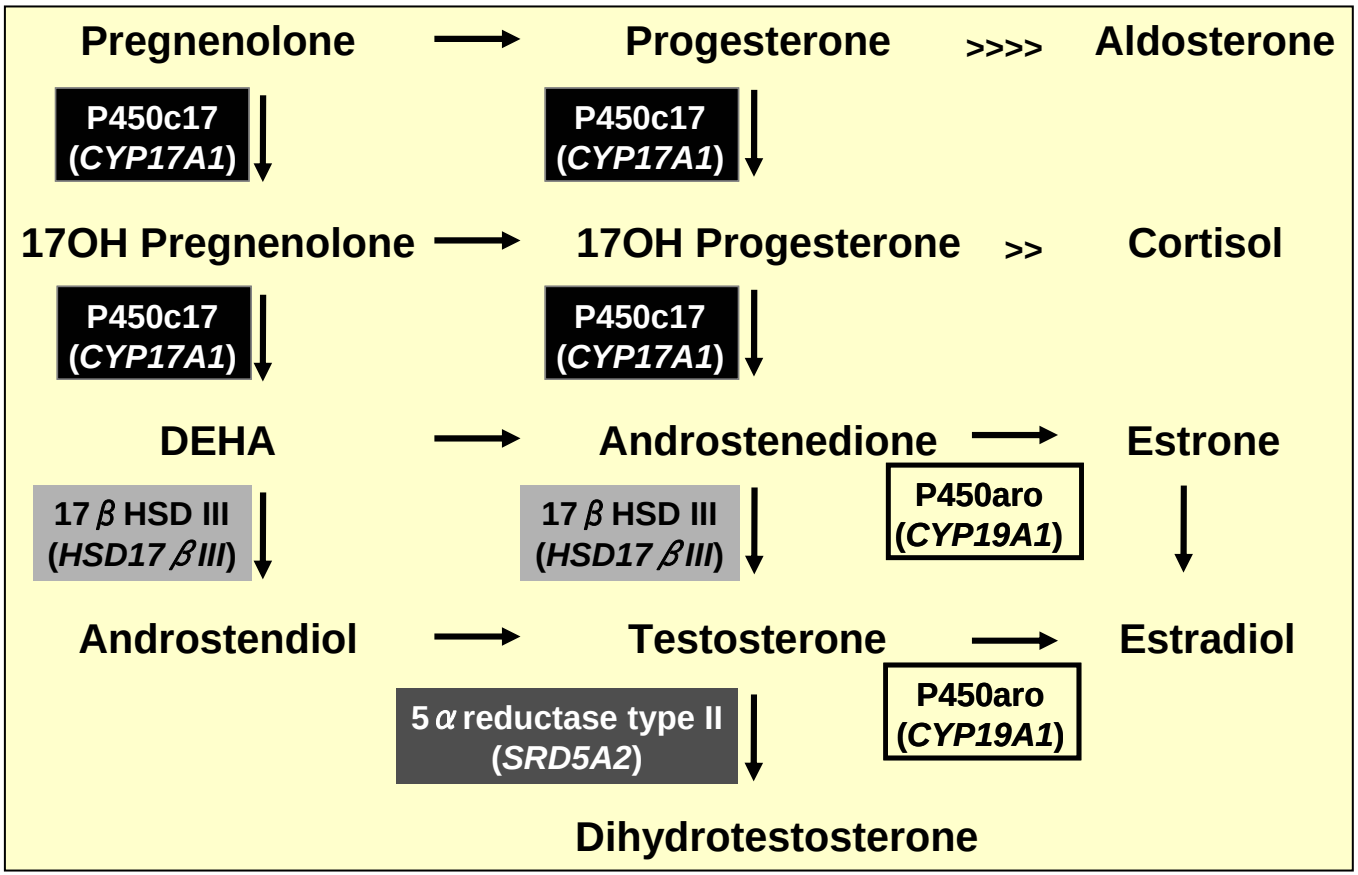
Etiology of hypospadias



The etiology of hypospadias is regarded as a complex disorder that has both maternal/fetal genetic and environmental factors that influence to androgen dependent development of the urethral and external genital system

CYP17A1, CYP19A1, ESR1 & ESR2, HSD17 and SRD5A2 influence estrogen and androgen activity. CYP17A1 catalyzes 2 sequential reactions in steroid metabolism. The gene *CYP17A1 m*ediates both 17 α hydroxylase and 17, 20 lyase activity in steroid biosynthesis, while *CYP19A1* mediates the step in the metabolites in both testosterone & androstenedione to estrogens. The gene ESR1 and ESR2 encode the estrogen receptor.

Steroid synthesis



Several studies showed the risk of hypospadias with gene polymorphism, which exert the androgen and estrogen activities.

Studies about the associations of hypospadias and fetal gene polymorphisms

Study	Design	Gene polymorphism	Statistical inference
Aschim *et al.* (2004)	Case-control (51 cases, 210 controls), Caucasian	*Androgen receptor gene* (CAG/GGN repeats)	●GGN numbers were significantly higher in subjects with penile hypospadias compared with controls (P = 0.003). ●The frequency of cases with GGN 24 or more/GGN = 23, differed significantly among subjects with penile hypospadias (69/31%) compared with controls (31/54%).
Thai *et al.* (2005)	Case-control (58 cases, 96 controls), Swedish, Turkish, and Middle Eastern	*SRD5A2* (V89L)	●A significant negative association for Val/Val genotype in hypospadias (OR = 0.24; CI = 0.14-0.41)
Beleza-Meireles *et al.* (2006)	Case-control (92 case, 94 controls), Swedish, Turkish, and Middle Eastern	*ESR2* (2681-4A>G, CA repeat)	●The CA repeat polymorphism is prolonged in hypospadias compared with controls ($P < 0.05$). ●The heterozygous genotype of 2681-4A>G polymorphism was a significantly higher frequency than in the controls ($P < 0.05$).
Radpour *et al.* (2007)	Case-control (92 cases, 190 controls), Iranian	*Androgen receptor gene* (CAG/GGN repeats)	●GGN numbers were significantly higher in subjects with penile hypospadias compared with controls (P = 0.001).

These polymorphisms decrease the androgen/estrogen activity as a result of being hypospadias.

Maternal genetic polymorphisms had not been studied.

1. We, (Kurahashi et. al. 2005) studied 31 case mothers and 64 control mothers. The frequency of low birth weight was significantly higher in hypospadias.

2. The heterozygous CYP1A1 and hetero and homo-zygous CYP1A1 were less frequent in case mothers. We found no effect of maternal smoking on the hypospadias risks among the gene polymorphisms.

3. CYP1A1 metabolizes not only environmental chemicals but also metabolizes endogenous estrogens. A maternal genetic factor related to bio-transformation enzymes to estrogen metabolism, CYP1A1 may affect the risk of hypospadias.

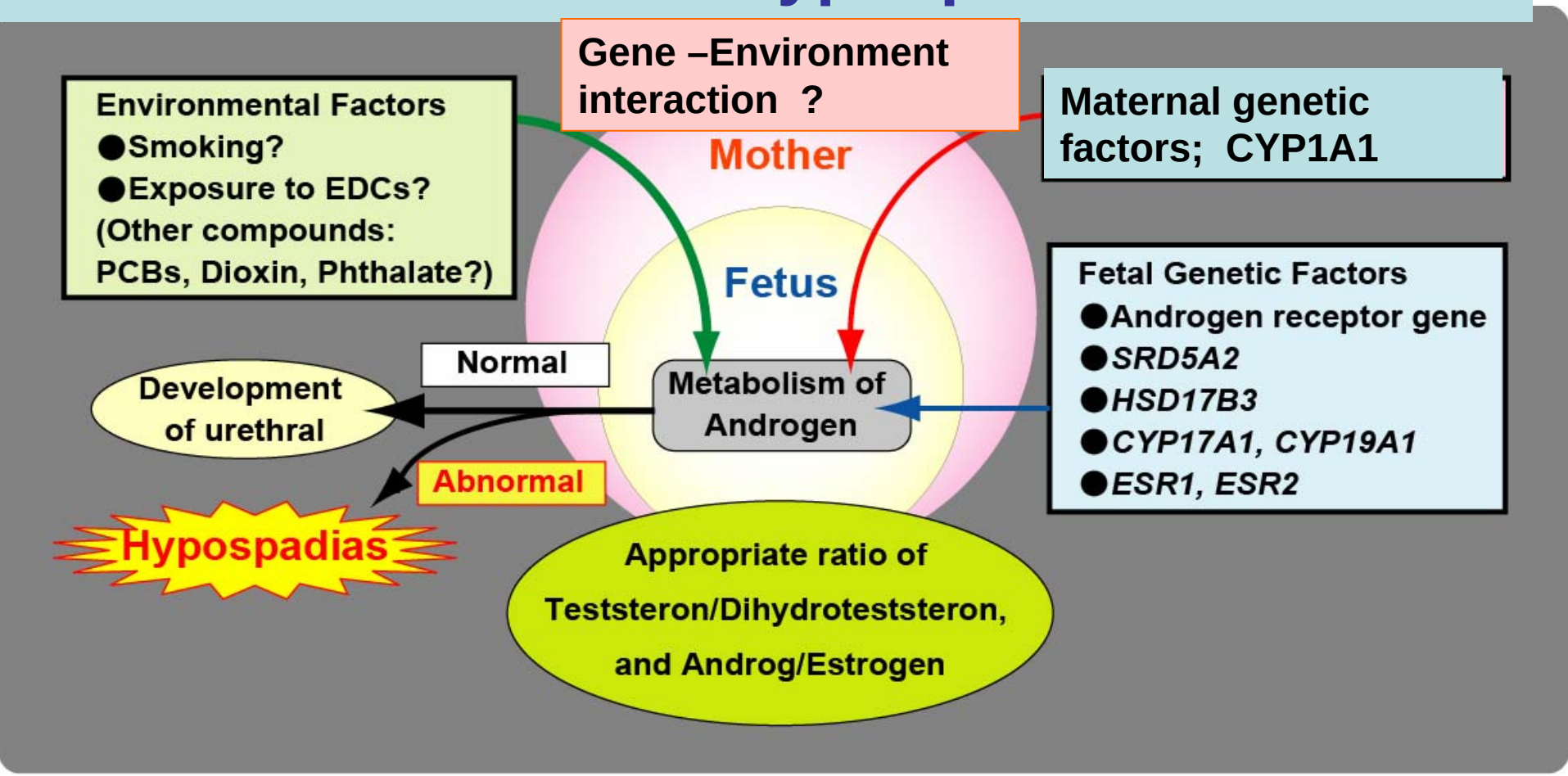
Kurahashi et. al. Molecular Human Reproduction (2005)

Association of the risk of hypospadias with maternal *CYP1A1* polymorphism

Genotype	Cases (n = 31) %	Controls (n = 64) %	OR	95% CI	*P*
GSTM 1					
Present	41.9	49.2	1.00		
Null	58.1	50.8	1.11	0.34-3.64	0.87
GSTT1					
Present	61.3	54.2	1.00		
Null	38.7	45.8	1.14	0.34-3.79	0.83
CYP1A1 Msp I					
m1/m1	48.4	28.1	1.00		
m1/m2	29.0	54.7	0.17	0.04-0.74	0.02*
m2/m2	22.6	17.2	0.73	0.14-3.90	0.71
m1/m2* or *m2/m2	51.6	71.9	0.28	0.08-0.97	0.04*

Kurahashi et. al. (2005)

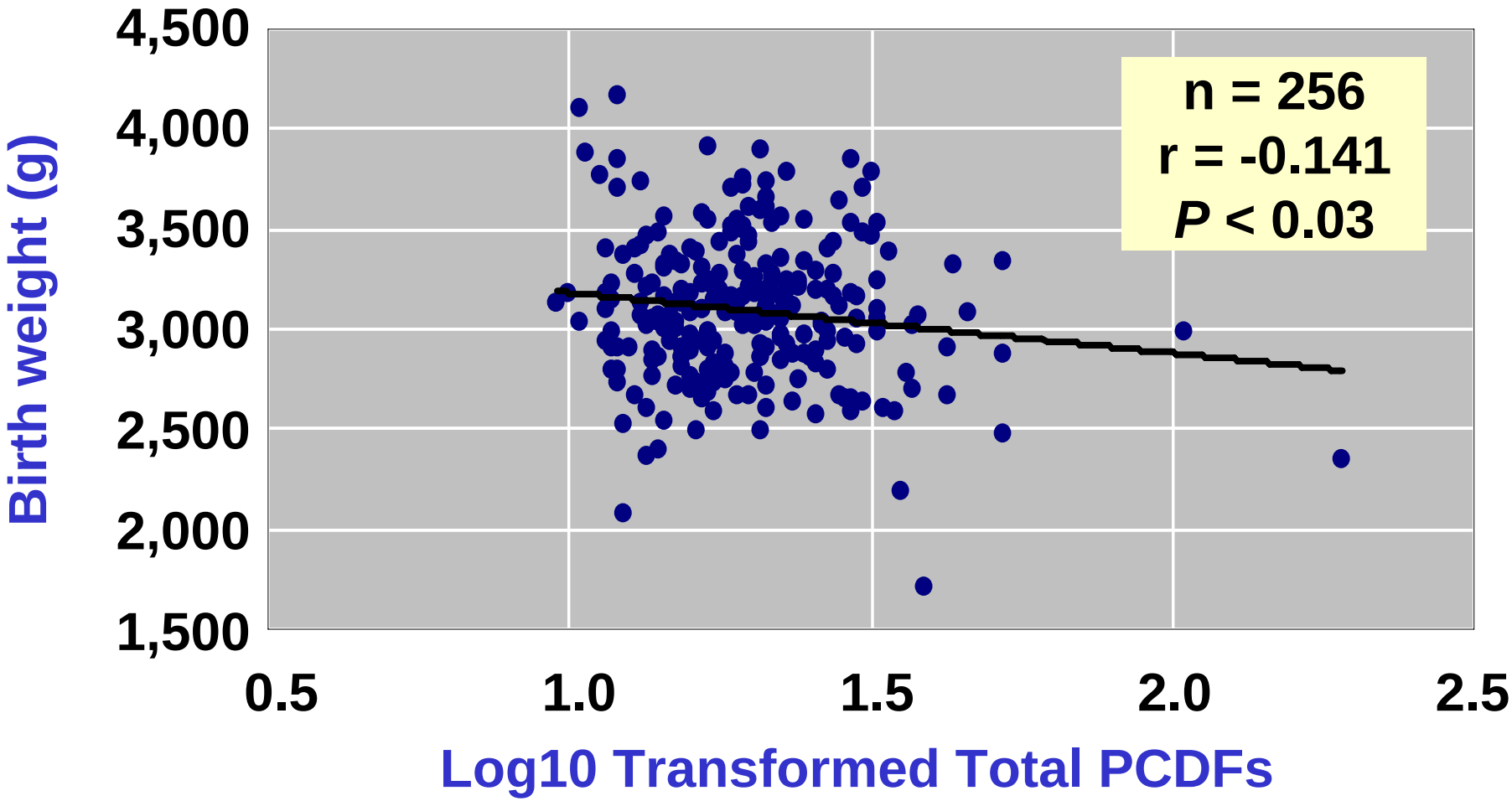
Summary of environmental and genetic factors for hypospadias



Until now, hypospadias has been studied from either the environmental or the genetic side. Further studies are necessary to examine gene –environment interaction.

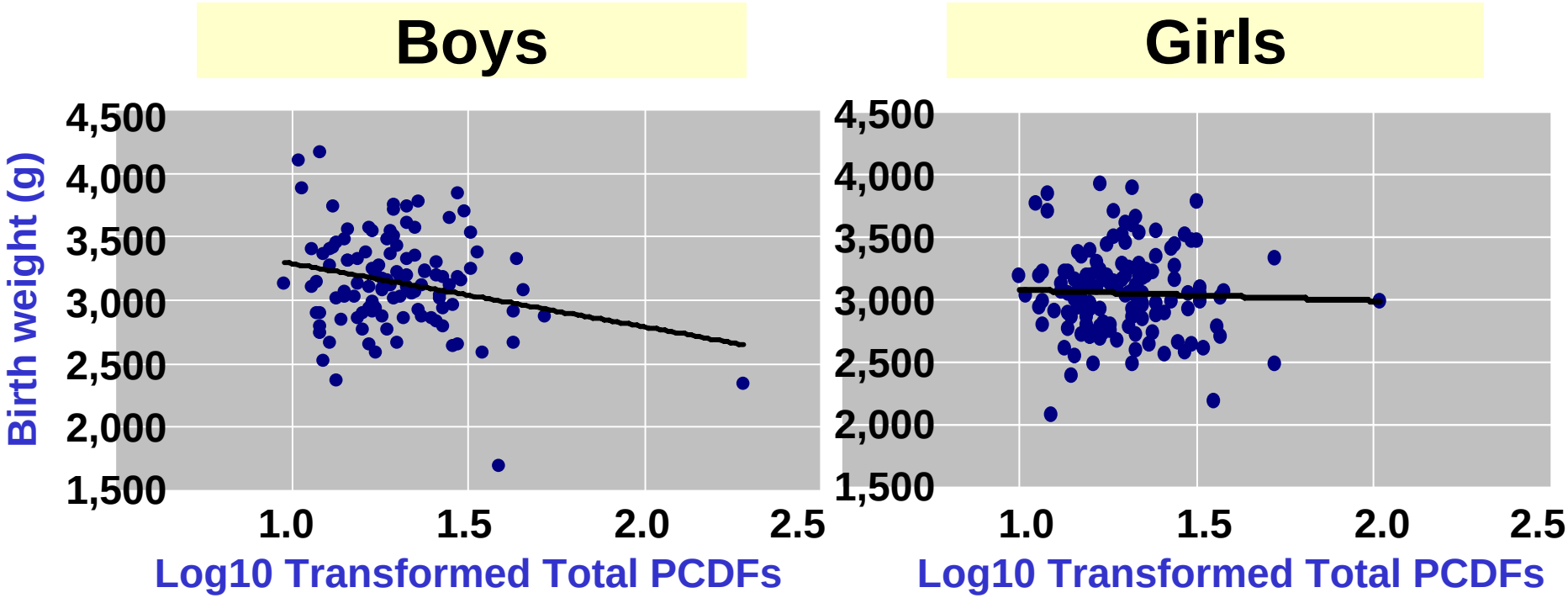
3. Birth weight, developments & prenatal exposure to PCBs･Dioxin

Correlation of birth weight with Log10 transformed Total PCDFs levels of maternal blood - Among all children -



Konishi et. al. (Dioxin 2007)

Correlation of birth weight with Log10 transformed Total PCDFs levels of maternal blood
- Among boys and girls -



r = -0.241
***P* < 0.03**

r = -0.04
***P* = 0.65**

Konishi et. al. (Dioxin 2007)

MDI and PDI Scores for Infants in Relation to the Level of Isomers of PCBs and Dioxins in Maternal Blood①

	MDI				PDI			
	β	*t*	*p*		*β*	*t*	*p*	
＜PCDD＞								
2,3,7,8-TCDD	-0.150	-1.714	0.089		-0.105	-1.235	0.219	
1,2,3,7,8-PeCDD	0.067	0.771	0.442		-0.036	-0.423	0.673	
1,2,3,4,7,8-HxCDD	-0.035	-0.394	0.694		-0.124	-1.462	0.146	
1,2,3,6,7,8-HxCDD	0.023	0.259	0.796		-0.045	-0.520	0.604	
1,2,3,7,8,9-HxCDD	0.002	0.026	0.979		-0.189	-2.284	0.024	*
1,2,3,4,6,7,8-HpCDD	-0.219	-2.395	0.018	*	-0.240	-2.749	0.007	**
OCDD	-0.173	-1.864	0.065		-0.172	-1.927	0.056	
＜PCDF＞								
2,3,7,8-TCDF	-0.050	-0.584	0.560		-0.178	-2.175	0.031	*
1,2,3,7,8-PeCDF	0.014	0.158	0.875		-0.196	-2.412	0.017	*
2,3,4,7,8-PeCDF	0.022	0.252	0.801		-0.046	-0.544	0.588	
1,2,3,4,7,8-HxCDF	-0.107	-1.199	0.233		-0.137	-1.615	0.109	
1,2,3,6,7,8-HxCDF	-0.099	-1.117	0.266		-0.167	-1.990	0.049	*
2,3,4,6,7,8-HxCDF	0.026	0.302	0.763		-0.167	-2.012	0.046	*
1,2,3,7,8,9-HxCDF	ND	ND	ND		ND	ND	ND	
1,2,3,4,6,7,8-HpCDF	-0.042	-0.482	0.631		-0.064	-0.763	0.447	
1,2,3,4,7,8,9-HpCDF	ND	ND	ND		ND	ND	ND	
OCDF	-0.057	-0.656	0.513		-0.032	-0.390	0.697	

Adjusted for gestational age, smoking during pregnancy, and blood sampling time. * $p < 0.05$; ** $p < 0.01$

Nakajima et al., Env. Health Perspectives, 2006

MDI and PDI Scores for Infants in Relation to the Level of Isomers of PCBs and Dioxins in Maternal Blood②

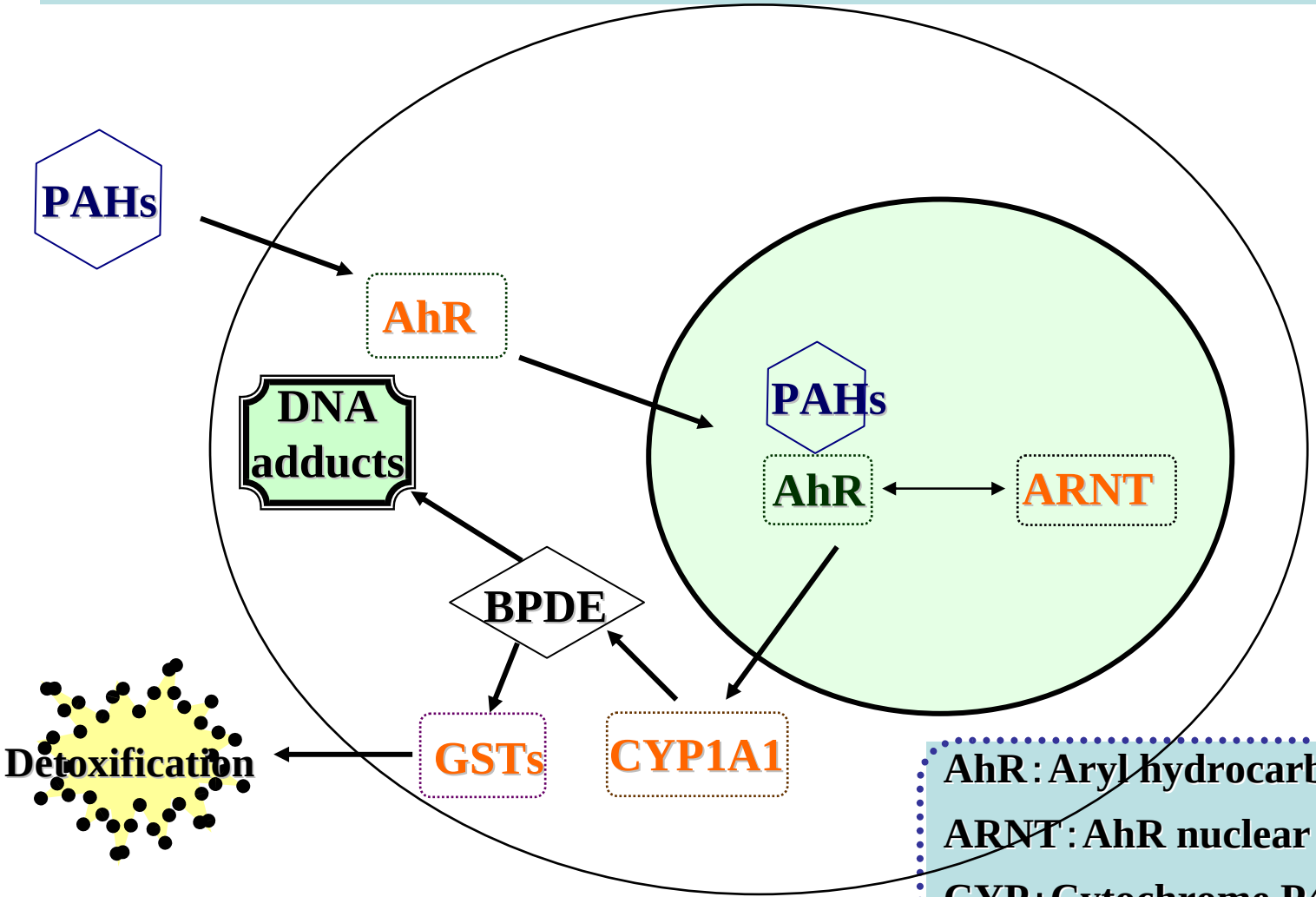
	MDI			PDI		
	β	*t*	*p*	*β*	*t*	*p*
＜non-ortho PCB＞						
33'4'4'-TCB(#77)	0.035	0.405	0.686	-0.007	-0.082	0.935
344'5-TCB(#81)	ND	ND	ND	ND	ND	ND
33'44'5-PenCB(#126)	-0.005	-0.056	0.956	-0.106	-1.277	0.204
33'44'55'-HxCB(#169)	0.008	0.091	0.928	-0.075	-0.898	0.371
＜mono-ortho PCB＞						
233'44'-PenCB(#105)	-0.007	-0.082	0.935	-0.090	-1.083	0.281
2344'5-PenCB(#114)	-0.030	-0.348	0.729	-0.110	-1.325	0.187
23'44'5-PenCB(#118)	-0.020	-0.232	0.817	-0.111	-1.334	0.185
2'344'5-PenCB(#123)	0.032	0.367	0.714	-0.081	-0.970	0.334
233'44'5-HexCB(#156)	-0.007	-0.077	0.939	-0.077	-0.932	0.353
233'44'5'-HexCB(#157)	-0.045	-0.519	0.604	-0.126	-1.521	0.131
23'44'55'-HexCB(#167)	-0.018	-0.211	0.833	-0.112	-1.353	0.178
233'44'55'-HpCB(#189)	-0.106	-1.232	0.220	-0.117	-1.420	0.158
＜di-ortho PCB＞						
22'33'44'5-HpCB(#170)	-0.033	-0.386	0.700	-0.110	-1.327	0.187
22'344'55'-HpCB(#180)	-0.030	-0.349	0.728	-0.074	-0.891	0.374

Adjusted for gestational age, smoking during pregnancy, and blood sampling time. * p < 0.05; ** p < 0.01

Nakajima et al., Env Health Perspectives, 2006

3. Maternal smoking,
Birth size (weight, height, head circumstance),
&
Polymorphisms

Metabolic pathways of polycyclic aromatic hydrocarbons (PAHs)



AhR : Aryl hydrocarbon receptor

ARNT : AhR nuclear translocator

CYP : Cytochrome P450

GSTs : Glutathione-S-transferases

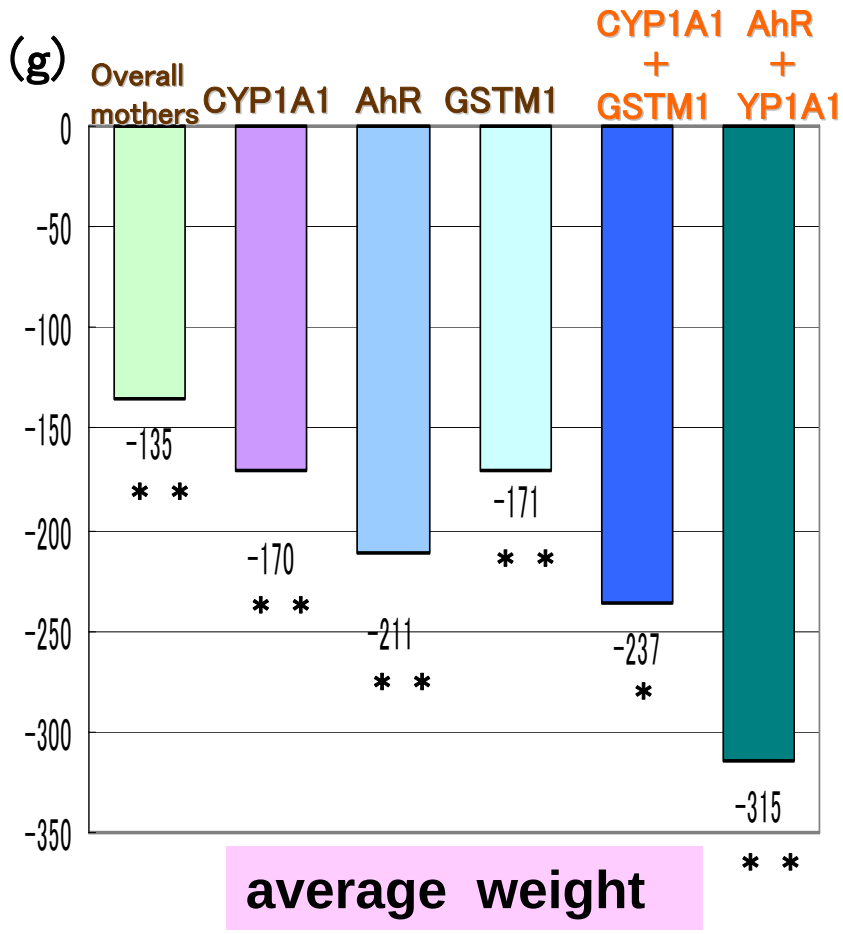
BPDE : B[a]p-7,8-dihydrodiol-9,10-epoxide

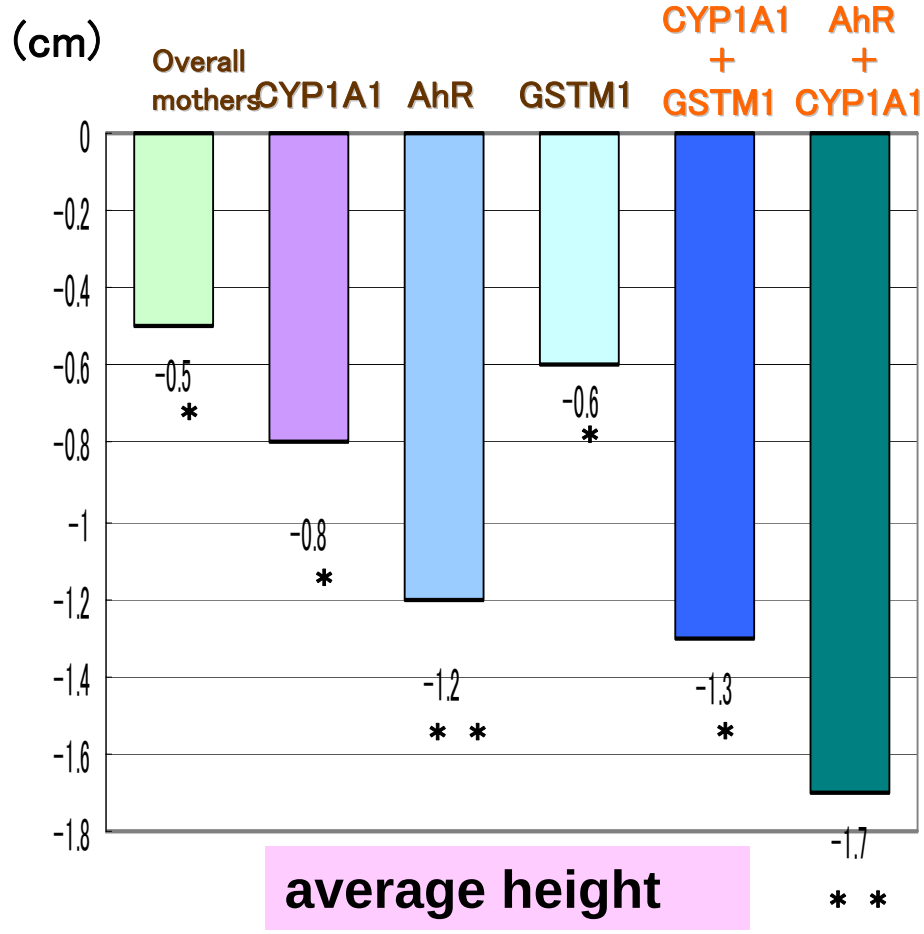
Reference : Baccarelli et al. (2004), Rybicki et al. (2005)

Epidemiological studies on the associations of birth size with gene polymorphisms by smoking

Author	Design	Gene	Outcome
Wang *et al.* (2002, U.S.A)	Nested case-control	*CYP1A1*	Maternal genetic polymorphisms in the PAH-metabolizing enzymes, i.e., CYP1A1 and GSTT1 genotypes modified the association between cigarette smoking and infant birth weight. The greatest reduction in birth weight was found among smoking mothers with the CYP1A1 Aa/aa and GSTT1 absent genotype.
		GSTT1	
Sasaki *et al.* (2005, Japan)	Cohort	*AHR*	Birth weight and length were significantly lower for infants of continuously smoking mother in the AhR wild type + CYP1A1 variant group and in the CYP1A1 variant + *GSTM1* null group.
		CYP1A1	
		GST M1	
Sram *et al.* (2006,Czech)	Cohort	*CYP1A1*	The risk of low birth weight and prematuring was significantly increased by genotypes of GSTM1 and CYP1A1*2C and the combination.
		GST M!	
Sasaki *et al.* (in press, Japan)	Cohort	*CYP2E1*	The adverse effects of maternal smoking on infant birth size were modified by the maternal genetic polymorphisms in the N-nitrosamines-metabolizing enzymes NQO1 wild genotype and CYP2E1 genotype.
		NQO1	

combined effects between maternal genetic polymorphisms of *AHR*, *CYP1A1* and *GSTM1* and smoking during pregnancy **(Sasaki et al, 2005)**





Adjusted for maternal age, height, weight before pregnancy, alcohol consumption during pregnancy, history of delivery, newborn sex, gestational weeks, house income * p<0.05 * * p<0.01

Birth size and smoking, by maternal polymorphism of *NQO1* (N-nitrosamine metabolizing enzymes)

Genotypes	status during pregnancy	Birth weight, (g)		Birth length, (cm)		Birth head circumference, (cm)	
		β	P	β	P	β	P
NQO1							
Pro/Ser+ Ser/Ser	Nonsmoking	Ref.		Ref.		Ref.	
	Quitting	-21	.664	0.2	.469	0.05	.808
	smoking	-58	.302	-0.1	.661	-0.3	.260
Pro/Pro	Nonsmoking	37	.372	0.1	.526	-0.02	.921
	Quitting	-8	.903	-0.2	.647	0.1	.656
	smoking	-207	.001	-0.8	.015	-0.7	.004
Interaction		-202	.001	-0.8	.06	-0.7	.003

Sasaki et. al, (in press, 2007)

Infant birth size by maternal *CYP2E1* *N*-nitrosamine-metabolizing enzymes genotypes

Genotypes	status during pregnancy	Birth weight, (g)		Birth length, (cm)		Birth head circumference, (cm)	
		β	*P*	β	*P*	β	*P*
CYP2E1							
c1/c2+ c2/c2	Nonsmoking	Ref.		Ref.		Ref.	
	Quitting	18	.776	0.5	.102	0.4	.144
	smoking	-158	.019	-1.0	.004	-0.6	.027
c1/c1	Nonsmoking	-64	.120	-0.4	.049	-0.1	.403
	Quitting	-119	.028	-0.7	.013	-0.2	.284
	Smoking	-185	.002	-0.5	.079	-0.5	.040
Interaction		-137	.008	-0.3	.316	-0.4	.063

Sasaki et. al. (2007, in press)

Conclusions and further directions

1. Prenatal environmental factors might have possible adverse effects on low birth weight , developments and congenital anomalies.
2. There may be a high risk group by genetic susceptibility factors.
3. Should be included
   - Genetic factors to chemical metabolites
   - Genetic factors susceptible to diseases
4. Consider about additive effects of various chemicals which surround our daily lives (e.g. smoking, PCBs, Dioxins, PFOS, mercury, pesticide, etc.).
5. Follow up the children for a long period

Collaborating Institutions

Hokkaido University Graduate School of Medicine
Department of Obstetrics and Gynecology
Department of Renal and Genitourinary surgery

Sapporo Medical University
Department of Obstetrics and Gynecology

Asahikawa Medical College
Department of Obstetrics and Gynecology

Sapporo City Institute of Public Health

Hokkaido University Graduate School of Veterinary Medicine
Department of Environmental Veterinary Sciences

Fukuoka Institute of Health and Environmental Sciences

Hoshi University School of Pharmacy and Pharmaceutical Sciences, Department of Analytical Chemistry

Hokkaido Association of Obstetricians and Gynecologists (40 institutes)

Sapporo Toho Hospital