A GENOME INFORMATICS AND EPIDEMIOLOGICAL STUDY IDENTIFIES ALLELES IN ARNT2 ASSOCIATED WITH RISK OF HYPOSPADIAS AND MICROPENIS

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Introduction

At the early developmental stages (embryonic, foetal and infant), humans are highly vulnerable to environmental hazards. Several epidemiological studies have suggested the association of perinatal exposure to diethylstilbestrol, allylestrenol, and environmental chemicals, such as organochlorine pesticides and PCBs, with male genital organ abnormalities[1-4]. These chemicals exert their effects via binding to nuclear receptors and activating various genes, including steroid- or drug-metabolizing enzymes. The expression of some of these effects may be promoted by predisposing genetic traits. Ogata et al. recently identified the association of male genital abnormalities with homozygosity for the specific ESR1 (estrogen receptor α) "AGATA" haplotype[5]. In the present study, we systematically selected SNPs in the nuclear receptor genes interacting with ESR1 using informatics and examined the association of certain SNPs with male genital organ abnormalities.

Materials and Methods

Subjects

A total of 234 genome DNA samples were obtained from Dr. Ogata (National Research Institute for Child Health and Development, Tokyo, Japan); they consisted of 134 samples from patients aged 1-13 yrs (62 cryptorchidism (CO), 30 hypospadias (HS), 42 micropenis (MP)) and 100 control samples from 34 boys and 66 adult men.

This study was approved by the Institutional Review Board Committees at the National Institute for Environmental Studies and National Center for Child Health and Development, and informed consent was obtained from each subject and parent(s).

Target gene selection by informatics

As homozygosity for the specific ESR1 (estrogen receptor α) "AGATA" haplotype associated with male genital organ abnormalities, we searched genes, proteins and low-molecular-weight chemicals which interact with ESR1 using KeyMolnet, a comprehensive bioinformatics platform (IMMD, Tokyo, Japan). Metabolism maps containing responsive genes to nuclear receptors that interact with ESR1 were obtained from KEGG (Kyoto Encyclopedia of Genes and Genomes: http://www.genome.jp/kegg/). We then searched for genes that are responsive to environmental chemicals via nuclear receptors that are involved in the biosynthesis pathway from cholesterol to steroid hormones.

Genotyping

SNP genotyping was performed on 221 samples with the Golden Gate Assay (Illumina, CA). The genotyping success rate was 95.2%, and the locus success rate was 98.43%.

SNP selection

The precise human allele information of locus genes selected using informatics was obtained from HUGO (The Human Genome Organization (http://www.hugo-international.org/)). Information on SNPs and tagSNPs was obtained from the dbSNP database (National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/)) and the HapMap Project (http://www.hapmap.prg). The selected SNPs were 20kb upstream to 10kb downstream from the coding region of each gene and more than 60kb from each other; tagSNPs were given priority. Table 1 shows the list of selected genes and the number of SNPs examined in this study.

Statistical analysis

The tests of Hardy-Weinberg equilibrium and correlation between the ESR1 and ARNT2 haplotype block were carried out with GeneSpring GT (ver2.0). Case-control incidences were compared by the χ^2 test. Analysis of the haplotype block was carried out using Haploview (http://www.broad.mit.edu/mpg/haploview/). Other statistical analysis was performed using SAS (SAS Analysis Pro, SAS Institute, Inc., Cary, NC).

Results and Discussion

A total of 378 SNPs of 210 samples (91 controls and 119 cases: 62 cryptorchidism; 23 hypospadias; 34 micropenis) were genotyped. In the case-control analysis, 7 SNPs from three genes were significantly different from the control for all phenotypes combined. Twenty-five SNPs from 5 genes, i.e., CYP17A1, ARNT2, CYP1A2, NR1I2, and AHR, were significantly different from the control in any single phenotype. For these 25 SNPs, the odds ratio for the most frequent allele of each SNP was calculated. Alleles from CYP17A1, ARNT2, and CYP1A2 showed odds ratios of more than 2.0. Five SNPs from CYP17A1 and CYP1A2 showed odds ratios of more than 3.0 (95% CI > 1.0). As many SNPs from CYP17A1, ARNT2, and CYP1A2, such as rs206951 and rs4778607, had no variant, the odds ratio could not be calculated. Instead, trend analysis was performed. Table 2 shows SNPs from CYP17A1, ARNT2, and CYP1A2 with significantly different frequency of allele variants by trend analysis.

As Ogata et al. had shown 4 SNPs in an ESR1 haplotype block related to male genital organ abnormality, the correlation between the 4 SNPs and 31 SNPs which revealed significant differences by case-control analysis (χ^2 test) from 5 genes (AHR, ARNT2, CYP17A1, CYP1A2, NR1I2 (PXR)) was tested. ESRI and ARNT2 were highly correlated. The correlation coefficient for rs3020371 (ESR1) and rs4778607 (ARNT2) was 1.0.

Case-control analysis revealed differences in 5 genes (AHR, ARNT2, CYP17A1, CYP1A2, NR1I2 (PXR)). As these genes are related to dioxin binding (AHR, ARNT2), dioxin induction (CYP1A2), estrogen synthesis (CYP17A1), and bisphenol A induction (NR1I2 (PXR)), the relevance of genetic susceptibility to a dioxin and estrogenic endocrine disruptor in the development of male genital organ abnormality was suggested.

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References

1. Gill, W. B., Schumacher, G. F., Bibbo, M., Straus, F. H., 2nd & Schoenberg, H. W. (1979) *J Urol* **122,** 36-9.
2. Czeizel, A. & Huiskes, N. (1988) *Clin Ther* **10,** 725-39.
3. Longnecker, M. P., Klebanoff, M. A., Brock, J. W., Zhou, H., Gray, K. A., Needham, L. L. & Wilcox, A. J. (2002) *Am J Epidemiol* **155,** 313-22.
4. Fujita, H., Kosaki, R., Yoshihashi, H., Ogata, T., Tomita, M., Hasegawa, T., Takahashi, T., Matsuo, N. & Kosaki, K. (2002) *Teratology* **65,** 10-8.
5. Watanabe, M., Yoshida, R., Ueoka, K., Aoki, K., Sasagawa, I., Hasegawa, T., Sueoka, K., Kamatani, N., Yoshimura, Y. & Ogata, T. (2007) *Hum Reprod* **22,** 1279-84.

Table 2. *p* values for trend analysis for *CYP17A1, CYP1A2* and *ARNT2* SNPs

	Analysis Type	All diseases	Cryptorchidism	Hypospadias	Micropenis
CYP17A1					
rs10786712	Fisher's exact	0.3970	0.2471	0.2101	0.6837
	Cochran-Armitage	0.2477	0.1667	**0.0712**	0.5260
Number		119	62	23	34
rs743572	Fisher's exact	0.4215	0.2711	0.2188	0.5436
	Cochran-Armitage	0.3329	0.2151	**0.0864**	0.4615
Number		119	62	23	34
CYP1A2					
rs2069521	Fisher's exact	**0.0634**	**0.0102**	0.1389	0.2573
	Cochran-Armitage	**0.0888**	**0.0551**	0.7488	0.1237
Number		119	62	23	34
rs2069522	Fisher's exact	0.1038	**0.0287**	0.1803	0.2111
	Cochran-Armitage	**0.0803**	0.1862	0.2858	0.1174
Number		119	62	23	34
rs2069526	Fisher's exact	0.1273	**0.0287**	0.1803	0.1717
	Cochran-Armitage	0.1496	0.1862	0.2858	0.1765
Number		119	62	23	34
ARNT2					
rs4778607	Fisher's exact	0.1644	0.1038	1.0000	0.1795
	Cochran-Armitage	**0.0333**	**0.0392**	1.0000	**0.0536**
Number		119	62	23	34
rs8024819	Fisher's exact	0.9561	0.2505	0.7440	0.3782
	Cochran-Armitage	1.0000	**0.0994**	0.8034	0.1030
Number		119	62	23	34

Number of control samples is 91. Bold figure indicates statistically significant at $p < 0.01$.

Table 1. Selected genes and number of SNPs examined in this study.

HUGO approved gene symbol	Approved Gene Name	Location	Sequence	Previous symbol	Aliases	SNP[a]	tgSNP[b]
AHR	aryl hydrocarbon receptor	7p15	L19872, NM_001621			17	10
AHRR	aryl-hydrocarbon receptor repressor	5p15.33	AB033060, NM_020731	AHH, AHHR	KIAA1234	29	14
ARNT	aryl hydrocarbon receptor nuclear translocator	1q21	AF001307		HIF-1beta	31	6
ARNT2	aryl-hydrocarbon receptor nuclear translocator 2	15q24	AB002305		KIAA0307	69	32
CYP17A1	cytochrome P450, family 17, subfamily A, polypeptide 1	10q24.3	M19489, NM_000102	CYP17	P450C17, CPT7, S17AH	18	4
CYP19A1	cytochrome P450, family 19, subfamily A, polypeptide 1	15q21	D14473	CYP19	ARO, P-450AROM, CPV1, ARO1, CYAR	40	21
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	15q24.1	BC023019, NM_000499	CYP1	P450DX, P1-450, P450-C, CP11	9	2
CYP1A2	cytochrome P450, family 1, subfamily A, polypeptide 2	15q24.1	AF182274, NM_000761		P3-450, CP12	10	2
CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1	2p22.2	U56438, NM_000104	GLC3A	CP1B	20	6
CYP2B6	cytochrome P450, family 2, subfamily B, polypeptide 6	19q13.2	AF182277, NM_000767		CPB6, CYPIIB6, CYP2B	13	9
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4	7q21.1	AF280107	CYP3A3		27	1
NR1I2	nuclear receptor subfamily 1, group I, member 2	3q12-q13.3	AF061056		ONR1, PXR, BXR, SXR, PAR2	21	14
RXRA	retinoid X receptor, alpha	9q34	X52773		NR2B1	46	19
RXRB	retinoid X receptor, beta	6p21.3	M84820		NR2B2, H-2RIIBP, RCoR-1	0	0
RXRG	retinoid X receptor, gamma	1q22-q23	U38480, NM_006917		NR2B3	34	19