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INVITED REVIEW

Reproductive Health

Gene-environment interactions in male reproductive health: special reference to the aryl hydrocarbon receptor signaling pathway

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Over the last few decades, there have been numerous reports of adverse effects on the reproductive health of wildlife and laboratory animals caused by exposure to endocrine disrupting chemicals (EDCs). The increasing trends in human male reproductive disorders and the mounting evidence for causative environmental factors have therefore sparked growing interest in the health threat posed to humans by EDCs, which are substances in our food, environment and consumer items that interfere with hormone action, biosynthesis or metabolism, resulting in disrupted tissue homeostasis or reproductive function. The mechanisms of EDCs involve a wide array of actions and pathways. Examples include the estrogenic, androgenic, thyroid and retinoid pathways, in which the EDCs may act directly as agonists or antagonists, or indirectly via other nuclear receptors. Dioxins and dioxin-like EDCs exert their biological and toxicological actions through activation of the aryl hydrocarbon-receptor, which besides inducing transcription of detoxifying enzymes also regulates transcriptional activity of other nuclear receptors. There is increasing evidence that genetic predispositions may modify the susceptibility to adverse effects of toxic chemicals. In this review, potential consequences of hereditary predisposition and EDCs are discussed, with a special focus on the currently available publications on interactions between dioxin and androgen signaling.

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INTRODUCTION

In 1992, a meta-analysis indicated falling sperm counts in otherwise normal men over a 50-year period.¹ At the same time, the incidence of testicular cancer had risen dramatically in some western countries.² There are also reports on increasing frequency of genital malformations such as cryptorchidism and hypospadias in newborn boys.³

Since these trends have emerged over a relatively short time span, they have been proposed to be caused by exposure to environmental antiandrogens or lifestyle factors with adverse effects on the male reproductive system. In support of this, epidemiological studies on testicular cancer have shown that first generation Nordic immigrants to Sweden had a prevalence of testicular cancer similar to the one found in their country of origin, whereas their offspring, who were born and raised in Sweden, had a frequency of testicular cancer similar to the prevalence found in Swedish men.⁴ Ethnic differences in the incidence of reproductive disorders, such as cryptorchidism,⁵ hypospadias,⁶ testicular cancer^{7,8} and prostate cancer,^{9,10} illustrate that genetic components that contribute to the susceptibility are also operating. This is also observed in animal models, where certain rodent strains differ in their response to xenobiotics.^{11,12}

It is increasingly recognized that the etiology of most common diseases involves not only discrete genetic and environmental factors, but also interactions between the two. Obvious gene-environment

interactions include the much stronger effect of sunlight exposure on skin cancer risk in fair-skinned humans compared to dark-skinned individuals. Other well-described examples where gene-environment interactions affect the susceptibility to common human diseases include diabetes¹³ and cancer.¹⁴

Gene-environment interactions can be exploited to improve human health. A well-known example of this relates to a rare autosomal recessive mutation in the gene encoding the hepatic enzyme phenylalanine hydroxylase, which leads to the metabolic disease phenylketonuria. Under normal dietary conditions, carriers of the phenylketonuria mutation suffer severe impairment in cognitive development, but a phenylalanine-deficient diet substantially improves their prognosis. Another example is illustrated by the influence of smoking and alcohol intake on the impact of polymorphisms in the genes encoding apoE and alcohol dehydrogenase, respectively, on coronary heart disease (reviewed by Talmud¹⁵).

Identifying and validating gene-environment interactions will further increase our understanding of how specific environmental exposures cause disease by pinpointing genes through which the environmental effects are conveyed. Since mechanisms through which most endocrine disrupting chemicals (EDCs) affect reproductive health are largely unknown, it is of interest to investigate whether adverse environmental effects on reproductive health are modified by genetic predisposition. Among all EDCs, the mechanism through which dioxins

and dioxin-like chemicals exert their biological and toxicological effects has been characterized best. Moreover, several crosstalk mechanisms between dioxin-induced signaling pathways and reproductive signaling pathways have been described. Therefore, this review aims to examine whether genetic variation in genes involved in dioxin-induced signaling contribute to an individual's susceptibility to the adverse effects of dioxins and dioxin-like EDCs on male reproductive health.

AROMATIC HYDROCARBONS

EDCs form a highly heterogeneous group of compounds, of which aromatic hydrocarbons, also known as arenes or aryl hydrocarbons, are among the most significant groups of persistent EDCs. Aromatic hydrocarbons can be monocyclic, e.g. benzene, furan and pyridine, or polycyclic, also called polyaromatic hydrocarbons (PAHs), with two to seven fused benzene rings, e.g. naphthalene and benzo[a]pyrene. Halogenated PAHs like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and dioxin-like compounds such as some polychlorinated biphenyls (PCBs) are released from household sources as well as from industrial and natural emissions. Some of them, such as benzo[a]pyrene, also occur as by-products of cigarette smoking.

The aryl hydrocarbon receptor

Both PAHs and halogenated PAHs exert their biological and toxic effects through activation of the aryl hydrocarbon receptor (AHR), also known as the dioxin receptor. Although the human AHR was discovered almost 4 decades ago,¹⁶ its physiological role and endogenous ligand initially remained unknown and it was therefore designated as an orphan receptor. Several candidate activators have since been presented, including the heme metabolites indirubin and bilirubin,^{17,18} the arachidonic acid metabolite lipoxin 4A¹⁹ and most recently the tryptophan metabolite kynurenine.^{20,21}

The AHR is evolutionary well-conserved and ubiquitously expressed in mammalian tissues.²² AHR knockout mice are characterized by liver fibrosis²³ as well as hampered embryonic development of a wide range of organs,^{24,25} reduced xenobiotic metabolism, immune system defects²⁶⁻²⁸ and regulation of hematopoiesis.²⁹ These animal studies also revealed that the AHR is essential in the reproductive system, for example for testosterone synthesis and sperm production³⁰ as well as for normal prostate and seminal vesicle development.³¹ Knocking out the AHR gene in a prostate cancer mouse model (TRAMP) inhibited prostatic carcinogenesis, while treating TRAMP mice with an AHR modulator inhibited metastasis.³²

Mice with constitutively active AHR on the other hand, had a reduced life span and spontaneously developed liver³³ and stomach tumors.³⁴ In three independent founder lines of the mice, heterozygous mice showed less severe stomach tumors than homozygous mice, indicating a gene-dosage effect. Furthermore, the severity of the gastric tumors increased with age, and males were affected more severely and died earlier than females further illustrating a sex difference in susceptibility to dioxin.

Molecular function of the AHR

The AHR is a ligand-activated transcription factor that belongs to a family of signal transduction proteins that contain a basic helix-loop-helix motif,³⁵ which is a conserved region in many transcription factors, and in addition a Per/AHR nuclear translocator (ARNT)/Sim (PAS) domain. Based on sequence similarity, these basic helix-loop-helix/PAS proteins can be divided into two phylogenetic groups; the ARNT group containing: ARNT, ARNT2, ARNT3 and Per, and the AHR group containing: AHR, Sim and hypoxia-inducible factor 1 α .³⁶⁻⁴⁰

The unliganded AHR resides in the cytosol associated with HSP90 and AHR-interacting protein (AIP), also known as hepatitis B virus X-associated protein (XAP2) or AHR activator 9 (ARA9). Upon ligand binding at the PAS domain, the receptor undergoes a conformational change and migrates to the nucleus, where it heterodimerizes with the ARNT protein⁴¹ and the complex subsequently interacts with consensus dioxin or xenobiotic responsive elements in enhancers/promoters of specific target genes including the genes for the enzymes CYP1A1 (cytochrome P450, subfamily I, polypeptide 1, also known as aryl hydrocarbon hydroxylase) and CYP1B1,⁴² resulting in the activation of several metabolic and detoxification pathways (Figure 1). Whereas the AHR-mediated transcription is modulated by nuclear coactivators and corepressors, AHR signaling is abrogated through a negative feedback mechanism, in which the AHR/ARNT heterodimer stimulates the expression of AHR repressor (AHRR) that competes with ARNT for binding to AHR.⁴³ Alternatively, AHR may undergo nuclear export, ubiquitination and subsequent degradation by the 26S proteasome.⁴⁴

EFFECTS OF DIOXIN EXPOSURE ON MALE REPRODUCTIVE HEALTH

Data on the effects of exposure to dioxins and dioxin-like compounds on male reproductive health have mainly been obtained from a few large-scale accidental human exposures including the one in Seveso, Italy, in a herbicide production plant in 1976,⁴⁵ contaminated rice oil consumption in Japan (Yusho incident) in 1968⁴⁶ and in Taiwan region (Yucheng incident) in 1979⁴⁷ as well as US Air Force veterans who handled Agent Orange, a mixture of herbicides contaminated with TCDD, that was sprayed during operation Ranch Hand in Vietnam between 1962 and 1972.⁴⁸

Boys that were exposed to TCDD in Seveso during infancy or puberty exhibited permanently reduced estradiol and increased follicle-stimulating hormone (FSH) levels,⁴⁹ whereas boys born to

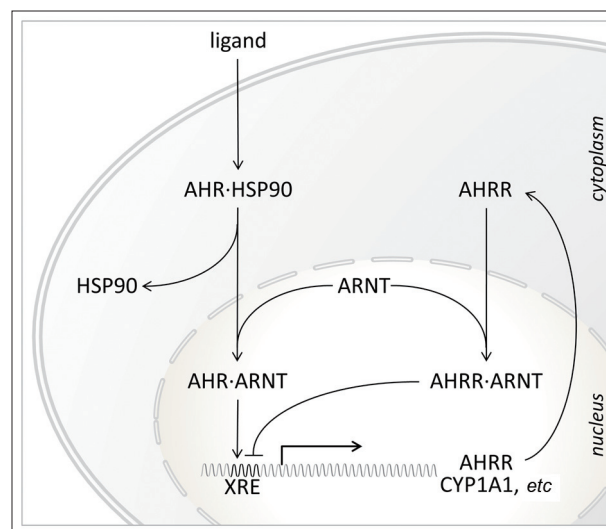


Figure 1: Schematic representation of ligand-activated AHR signaling. In the unliganded form, AHR resides in the cytosol as a complex with several chaperone proteins including heat shock protein 90 (HSP90). Ligand binding induces a conformational change in the AHR, revealing a nuclear localization signal that targets the AHR for nuclear translocation. In the nucleus, the AHR dimerizes with ARNT and interacts with the xenobiotic response element (XRE) in regulatory regions of specific target genes including AHRR and CYP1A1. In turn, AHRR acts as a repressor by competing for the dimerization with ARNT or directly at the XRE. AHR: aryl hydrocarbon receptor; AHRR: AHR repressor; ARNT, AHR nuclear translocator

Yucheng mothers showed decreased serum testosterone and increased serum estradiol and FSH at the age of puberty.⁵⁰ An inverse relationship between dioxins and testosterone was also observed in Ranch Hand veterans⁵¹ and in men from the general population in Belgium.⁵² Results from the latter study further suggested that decreased testosterone levels may interfere with the secretory function of the prostate and seminal vesicles without affecting spermatogenesis. Exposure data from general populations are sparse, but in a study on Greenlandic Inuit and three European populations, inconsistent relationships were seen with gonadotropin, sex hormone binding globulin, estradiol and testosterone levels, where positive associations of PCB 153 were seen with sex hormone binding globulin and luteinizing hormone in some but not all groups.⁵³

Widespread expression of AHR in the human testis may explain why dioxins and dioxin-like chemicals interfere with spermatogenesis and male fertility.⁵⁴ Studies on reproductive function in sons of the exposed mothers from Seveso have shown that *in utero* and lactational exposure to relatively low dioxin doses can permanently reduce sperm quality, manifested as reductions in sperm concentration, total count, progressive motility and total motile count.⁴⁵ Increased abnormal sperm morphology and decreased motility were also observed in a small cohort of young men born to Yucheng victims compared to controls, whereas semen volume and sperm count were not affected.⁵⁵ Negative correlations between dioxin-like compounds and sperm quality have been supported by several relatively small studies,⁵⁶⁻⁵⁸ although a larger study that included 798 European men that were exposed to much lower levels did not confirm these associations.⁵³ A recent study on 135 men from Seveso, who were exposed to TCDD during infancy, showed reduced sperm concentration and motility, whereas it had the opposite effect in men exposed during puberty and no effect in an older group with a mean age at exposure of 21.5 years.⁴⁹ This indicates that exposure during the fetal or early life period is the most sensitive window of exposure. Indeed, studies on Agent Orange veterans, who were exposed during adult life, have also not shown associations between serum TCDD concentrations and sperm parameters.⁵⁹ Reduced androgen levels during the period when Sertoli cells are most dependent on androgens could explain the permanently decreased sperm counts in adults who were exposed to TCDD before puberty.

The proportions of male births has slightly, but significantly, decreased in industrialized countries.^{60,61} Additionally, sharper changes in sex ratios have been observed in regions with demonstrated exposure to EDCs. For example, lower proportions of male offspring have been observed after paternal exposure to dioxin-like compounds in Seveso⁶² and Japan⁶³ as well as in Russian pesticide producers.⁶⁴ In the Yucheng cohort, birth sex ratio was not affected according to one study,⁶⁵ whereas in another study exposed men had a lower proportion of male offspring than unexposed men when exposed before 20 years of age.⁶⁶ Birth sex ratio was also not affected in US veterans who were exposed during adulthood.⁶⁷

Interestingly, Taylor *et al.*⁶⁸ reported that the proportion of male births was increased in women with higher estrogenic PCB levels, whereas it was decreased in women with higher levels of antiestrogenic PCBs.

In short, from these studies it is apparent that although exposure to dioxins or dioxin-like compounds may have a suppressive effect on testosterone levels throughout life, it only seems to affect male reproduction when exposed during reproductive development, i.e., either *in utero* or in the period up to puberty. Interestingly, increased PCB concentrations have been shown in mothers of men with testicular cancer but not in the men themselves.⁶⁹

Crosstalk mechanisms between aryl hydrocarbon receptor and AR signaling pathways

Dioxins and dioxin-like compounds exert their biological and toxicological effects by activation of the AHR signaling pathway. The importance of the AHR signaling pathway in dioxin toxicity was independently demonstrated by three groups who developed Ahr-null mice that were highly resistant to diverse manifestations of dioxin toxicity.⁷⁰⁻⁷² Similarly, mice that express low levels of ARNT from an engineered hypomorphic Arnt allele are highly resistant to hepatic toxicity of TCDD.⁷³ But actions of the direct target genes of AHR alone do not fully explain its toxicological and physiological effects and it has become clear that AHR exhibits additional regulatory functions by modulating the activity of other signaling pathways. Proposed crosstalk occurs via mechanisms including competition for cofactors, protein-protein interaction, competition for DNA binding and proteasomal degradation. Crosstalk with the AHR signaling pathway has been demonstrated for estrogen receptor (ER) α ,⁷⁴ ER β ,⁷⁵ hypoxia-inducible factor 1 α ,⁷⁶ thyroid hormone receptor/retinoblastoma-interacting protein 230,⁷⁷ nuclear factor erythroid 2-related factor 2,⁷⁸ specificity protein 1⁷⁹ and nuclear factor- κ B.⁸⁰

AHR signaling may interfere with the male reproductive system through several mechanisms, for example by directly affecting steroid hormone levels via induction of CYP1A1 and CYP1B1, which are representative phase I drug metabolizing enzymes that catalyze the conversion of steroid hormones.⁸¹

Activation of AHR may also alter the transcriptional activity of steroid hormone receptors. TCDD has been shown to inhibit testosterone-dependent transcriptional activity and testosterone-regulated prostate specific antigen expression⁸² and to block androgen-dependent proliferation of prostate cancer cells.⁸³ These *in vitro* findings are supported by a prospective cohort study of Vietnam veterans where an inverse relationship between TCDD body burden and risk of benign prostate hyperplasia was observed.⁵¹

The AHR has been shown to directly bind to a large number of coactivators and other nuclear proteins, including p300, cyclic AMP response element-binding (CREB)-binding protein, steroid receptor coactivators 1/2 and receptor-interacting protein 140.^{84,85} Many of these also interact with other nuclear receptors such as ER and the androgen receptor (AR), and as a result AHR and AR may compete with each other to recruit shared cofactors such as steroid receptor coactivators 1 and p300.^{86,87} Similar mechanisms have indeed been shown to account for the interaction between AHR and ER.^{88,89} It has been suggested that competition for the shared coactivator nuclear receptor coactivator 4, also known as AR associated protein 70, provides the basis for the bilateral transcriptional interference and that AHR modulation of AR activity is differentially altered by the level of four and a half LIM domain 2 protein and the amount of AHR present in the cell.⁹⁰ Furthermore, PAHs have been shown to stimulate c-jun and c-fos expression in prostate cancer LNCaP cells.⁹¹ Since activator protein-1, a heterodimer of c-jun and c-fos, is known to inhibit binding of AR to androgen responsive elements by protein-protein interaction with AR, this suggests the involvement of AHR-induced activator protein-1 in the antiandrogenic effects of PAHs. Recently, Bjork and Giwercman reported that the suppressive effect of TCDD on AR activity depends on the polymorphic glutamine repeat in the transactivating domain of AR,⁹² lending further support that crosstalk between AR and AHR signaling is mediated at the level of cofactor binding.

Conversely, testosterone has been reported to repress TCDD-induced transcription of AHR-regulated CYP1A1 gene and CYP1A1 enzymatic

activity in LNCaP cells.^{82,93} Furthermore, 5 α -dihydrotestosterone was shown to suppress transcription of AHR-regulated genes by facilitating complex formation between AR and AHR, which results in reduced transcriptional activity.⁹⁴

Crosstalk between AHR and AR signaling pathways could also occur by direct competition for DNA binding sites in the promoter of androgen-responsive genes, as has been shown for ER-regulated genes.⁹⁵ TCDD treatment blocked ER binding to estrogen responsive elements and, conversely, estradiol blocked TCDD mediated CYP1A1 enzymatic activity by decreasing AHR binding to dioxin responsive elements in breast cancer cells.

Finally, AR signaling can be abrogated by the ability of the AHR to assemble an ubiquitin ligase complex, which subsequently promotes proteasomal degradation of the AR protein.^{96,97}

Genetic polymorphisms

Polymorphisms in the AHR gene, enzymes that are transcriptionally regulated by AHR, or other genes involved in the AHR signaling pathway, may not only cause variations in the individual susceptibility to dioxin-like compounds, but may also affect the cross talk between AHR signaling and other signaling pathways as described above. As such, these polymorphisms may determine to what extent dioxins and dioxin-like compounds disrupt for example androgen signaling. Details of the polymorphisms discussed in this paper are provided in **Table 1**.

Genetic variations in the rodent AHR have been shown to dramatically alter ligand binding and transactivation by the receptor. For example, a single nucleotide change at codon 375 in the ligand binding domain of the murine AHR reduces the binding affinity for TCDD approximately 10-fold in the resistant DBA/2 strain as compared to the sensitive C57BL/6J strain.^{11,98} Correspondingly, the latter shows higher CYP1A1 induction and a greater sensitivity to TCDD.⁹⁹ Due to a deletion in the transactivation domain of the rat AHR, the Han/Wistar rat strain is a 1000-fold more resistant to TCDD than the sensitive Long-Evans rat strain.^{12,100} The most dioxin-sensitive species is the Guinea pig, while the hamster, which has a modified transactivation domain similar to the resistant Han/Wistar rat, tolerates a 1000-fold higher dose.^{101,102} Interestingly, although humans are considered to be relatively insensitive to dioxins, the human AHR is highly homologous to the guinea pig AHR.¹⁰³ Besides important implications for testing of pollutants in

animal models, these inter- and intraspecies differences indicate that genetic polymorphisms in the AHR structure can have profound effects on the individual sensitivity to polycyclic and halogenated aromatic hydrocarbons.

In humans, genetic polymorphisms have been identified in the coding regions of the genes encoding AHR, ARNT and AHRR. The first identified and most widely studied single-nucleotide polymorphism (SNP) in the human AHR gene is a G > A substitution in exon 10, which causes an arginine to lysine change at codon 554 (Arg554Lys) in the transactivating domain of the receptor.¹⁰⁴ Its functional significance is currently unclear as both upregulation¹⁰⁵ as well as loss¹⁰⁶ of transactivational activity have been reported for the lysine variant. Conflicting associations of this SNP with human cancer risk exist, but in a recent systematic meta-analysis Luo *et al.*¹⁰⁷ concluded that this SNP does not contribute to the development of cancer.

The Arg554Lys SNP is in linkage disequilibrium with two other non-synonymous SNPs in exon 10 (Pro517Ser and Val570Ile), which are very rare except in African ancestry.^{105,106,108} Combinations of Lys554/Ile570 or Lys554/Ile570/Ser517 variant alleles are unable to drive the CYP1A1 gene expression *in vitro*.¹⁰⁶ This could be beneficial to individuals who are carriers of these nonresponsive genotypes and who are exposed to AHR inducing chemicals. This is supported by the nearly total resistance to tumor formation in benzo[a]pyrene-exposed AHR knockout mice, also displaying loss of the ability to induce CYP1A1.¹⁰⁹ A recent study confirmed that lower AHR, ARNT and CYP1B1 mRNA expression was associated with the homozygous variant Lys554 genotype of the AHR,¹¹⁰ but it remains to be seen whether humans who carry the variant codons at 570 and 517 will have a lower cancer risk as well.

Four additional human AHR variants (Lys17Thr, Lys401Arg, Asn487Asp and Ile514Thr) have been described.¹¹¹ Reduced transcriptional activity was reported in the Lys401Arg and Asn487Asp variants, but so far the phenotypic consequences of these variants remain unknown.

The AHRR gene harbors a missense mutation leading to a Pro185Ala amino acid change in exon 6.^{112,113} Although the functional properties of this variant are unclear, it has been linked with endometriosis in women¹¹⁴ and infertility in men,¹¹⁵⁻¹¹⁷ possibly through a reduced negative feedback on dioxin-induced AHR signaling.¹¹⁸ Since this SNP influences CYP1A2 activation *in vivo*, with carriers of the Ala-genotype being the most inducible,¹¹⁹ the 185Ala is suggested to have a lower repressor activity towards the AHR. Recently, in a candidate association

Table 1: Details of polymorphisms discussed in the text according to dbSNP build 137

| rsID | Gene | Chromosome | Location | Chromosomal position | Nucleotide change | Amino acid change | MAF ^a |
|------------|-------|------------|-------------|----------------------|-------------------|-------------------|------------------|
| NA | AHR | 7 | Exon 10 | 17338938 | 50A>C | Lys17Thr | NA |
| rs2158041 | AHR | 7 | Intron | 17368420 | 450+948T>C | | T=0.192 |
| NA | AHR | 7 | Exon 10 | 17378651 | 1202A>G | Lys401Arg | NA |
| rs75519181 | AHR | 7 | Exon 10 | 17378908 | 1459A>G | Asn487Asp | G=0.003 |
| NA | AHR | 7 | Exon 10 | 17378990 | 1541T>C | Ile514Thr | NA |
| rs72552768 | AHR | 7 | Exon 10 | 17378998 | 1549C>T | Pro517Ser | T=0.001 |
| rs2066853 | AHR | 7 | Exon 10 | 17379110 | 1661G>A | Arg554Lys | A=0.274 |
| rs4986826 | AHR | 7 | Exon 10 | 17379157 | 1708G>A | Val570Ile | A=0.022 |
| rs2292596 | AHRR | 5 | Exon 6 | 422955 | 565C>G | Pro185Ala | G=0.308 |
| rs10305741 | ARNT | 1 | Exon 16 | 150789864 | 1551T>G | Asp517Glu | C=0.009 |
| rs1805133 | ARNT | 1 | Exon 16 | 150789884 | 1486G>A | Asp511Asn | T=0.003 |
| rs2228099 | ARNT | 1 | Exon 7 | 150808889 | 522G>C | Val189= | G=0.428 |
| rs2278705 | ARNT2 | 15 | near 5'-UTR | 80694630 | C>T | | T=0.077 |
| rs5000770 | ARNT2 | 15 | intron | 80716483 | 31+19595G>A | | A=0.322 |

AHR: aryl hydrocarbon receptor; AHRR: AHR repressor; ARNT: AHR nuclear translocator; NA: not available; UTR: untranslated region; ^aMAF: minor allele frequency according to 1000 genome phase 1 genotype data from 1094 worldwide individuals, released in the May 2011 dataset

study on testicular cancer in a combined Swedish and Danish cohort, it was shown that the risk of disseminated TGCC was associated with four different SNPs that tag two haplotypes in the AHRR, whereas no association was found with SNPs in the AHR.¹²⁰

In the ARNT gene (also known as ARNT1 and HIF1 β), a silent mutation at codon 189 in exon 7 has been identified.¹²¹ The functional significance of this SNP is unknown; in smokers it did not affect CYP1A1 activity.¹²² Two other variants, Asp511Asn and Asp517Glu, have also been identified in the ARNT gene, but since both are located in exon 16, which does not contain a known functional domain, the significance of these SNPs has not been determined to date. Taken together, known variations in ARNT do probably not explain susceptibility to dioxins.¹²³

In ARNT2 on the other hand, which is a close structural homologue of ARNT³⁸ and expressed in parallel with ARNT in many tissues,¹²⁴ a significant association was observed between two SNPs (rs2278705 and rs5000770) and having either cryptorchidism, hypospadias or both in Japanese boys, whereas, rs5000770 was linked to at least one genital malformation in Italian men.¹²⁵ Studies in populations that have been exposed to significant dioxin levels may be needed to further confirm the possible role of ARNT or ANRT2 variants in male genital development.

One could argue that the human AHR is not likely to be polymorphic with respect to susceptibility for two reasons: (i) the human AHR already harbors the mutation that in the DBA/2-mouse reduces its affinity or CYP1A1 inducibility, which may not be overcome by additional mutations and (ii) the critical importance of the AHR during development may not allow additional deleterious mutations in this gene.¹²⁶ However, a recent genetic association study in a Chinese cohort of 580 idiopathic infertile men and 580 fertile controls observed that men homozygous for the AHR rs2158041 AA genotype had lower sperm counts than carriers of the GG genotype.¹²⁷ Interestingly, the same polymorphism also associated with risk of lung cancer in a similarly-sized Chinese study.¹²⁸ The molecular mechanism underlying these associations is currently not clear since the SNP is intronic and therefore not expected to have a functional consequence. However, intronic SNPs can affect transcription,¹²⁹ produce alternative splice sites¹³⁰ or be in linkage disequilibrium with other, causal genes.

Gene-environment interactions

The concept of gene-environment interaction means that some people carry genetic factors that confer susceptibility or resistance to a certain disorder in a particular environment. Unfortunately, only few studies have been performed aiming at identifying gene-environment interactions with respect to male reproductive health. Animal studies have shown that daily sperm production is affected less by TCDD exposure *in utero*¹³¹ as well as during adulthood¹³² in resistant Han/Wistar rats who carry a mutated transactivation domain of the AHR as compared to Long-Evans rats carrying the wildtype allele.

In humans, studies on gene-environment interactions related to environmental exposure and male reproductive health are still limited, and have recently been reviewed by Axelsson *et al.*¹³³ For example, a polymorphism in CYP1A1 and hydroxysteroid 17 β -dehydrogenase 4 modifies the association between exposure levels to different PCB congeners and the risk of testicular cancer.¹³⁴ Men with a shorter polymorphic trinucleotide CAG repeat length in AR have been observed to be more sensitive to the deleterious effects of exposure to PCB and *p*, *p*'-1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (DDE, a metabolite of the pesticide dichlorodiphenyltrichloroethane) on sperm DNA fragmentation and total sperm counts, respectively.¹³⁵ Another study reported increased sperm DNA fragmentation in men with a variant of

the detoxifying enzyme glutathione-S-transferase M1 when exposed to polycyclic aromatic hydrocarbon metabolites found in air pollution.¹³⁶

Is there a case to be made for gene-environment interactions with respect to exposure to dioxins and dioxin-like compounds? On the one hand, exposure to these chemicals clearly has antiandrogenic effects on male reproductive health, especially when the exposure occurs during sensitive periods before adulthood, affecting reproductive hormone levels and ultimately sperm quality. On the other hand, polymorphisms in genes involved in AHR signaling pathway have been identified in humans, and in a limited number of studies these have been associated with male reproductive functions. Whether the antiandrogenic effects of dioxin-induced AHR signaling are mediated via cross talk with the AR, remains to be studied in more detail.

Although animals show large differences in sensitivity to dioxins due to these polymorphisms, the functional effects are less obvious in humans. One of the reasons for this discrepancy is that most genetic polymorphisms in AHR, ARNT or AHRR have first been identified by massive high-throughput screening, without known phenotypes, whereas it was the opposite in animal models, i.e. genotypic variation was studied as a consequence of differences in sensitivity in the different strains. Genetic analyses of these genes in populations that are exposed to wide range of exposure levels may identify yet unknown polymorphisms that to a larger extent explain variation in susceptibility to EDCs.

CONCLUDING REMARKS

Virtually all human diseases result from the interaction between genetic susceptibility factors and modifiable environmental factors. Many environmental pollutants exert their biological effects through activation of the AHR signaling cascade and polymorphisms in the genes involved in this pathway may affect an individual's response to these pollutants. It may not be likely that polymorphisms in the human AHR gene affect receptor function, but there is evidence to suggest that polymorphisms in AHRR may indeed affect an individual's susceptibility to dioxin-related reproductive health effects. Whether these anti-androgenic effects are indeed mediated through cross talk between AHR and AR signaling, remains to be scrutinized. Whereas the mechanisms underlying the cross talk between ER and AHR signaling have been well-described, only a few studies have addressed similar mechanisms between AR and AHR.

When studying such complex mechanisms as gene-environment interactions, even relatively large-scale multicenter studies have a relatively low statistical power. Therefore, efforts should be made to establish international consortia merging different cohorts from which biological material for genetic as well as exposure analyses is available. To facilitate such collaboration, international standards regarding type of biological samples and questionnaire information to be collected as well should be developed. Furthermore, taking into consideration the limited availability of sufficiently sized study cohorts, efforts should be made to develop more efficient study designs and statistical models for studying gene-environment interactions.^{137,138}

QUESTIONS FROM THE PANEL

Q1: What can we learn from animal studies?

A1: Genetic polymorphisms in animals have convincingly been shown to affect susceptibility to dioxin-like compounds. However, given the fact that AHR receptor signaling is crucially important during development, the human AHR which already harbors a polymorphism that in the mouse reduces its activity may not allow further compromising genetic modifications.



Q2: Include gene-environment interaction studies with lifestyle factors as the “environment”.

A2: Smoking, which globally is an abundant lifestyle factor, has in a laboratory study been shown to affect men and women differently, so that women experienced greater abstinence induced anger than men, which could be an important factor for understanding and treating nicotine addiction in women.¹³⁹ In a study on more than 15 000 adolescents, these were shown to smoke more cigarettes and consume more alcohol when attending schools with elevated rates of tobacco and alcohol use. More important, an individual's susceptibility to school-level patterns of smoking or drinking is conditional on the number of short alleles he or she has in 5HTTLPR (serotonin-transporter-linked polymorphic region), which is a polymorphic region in SLC6A4, the gene that codes for the serotonin transporter. Overall, the findings demonstrate the utility of the differential susceptibility framework by suggesting that health behaviors reflect interactions between genetic factors and the prevalence of these behaviors in a person's context.¹⁴⁰

Q3: What is known about the genetic background in the susceptibility to certain EDCs in animals and humans? Are there indications of more or less susceptible subpopulations?

A3: Genetic variations in AHR in different rodent strains, as well as between different species, have indeed been shown to dramatically alter ligand binding and transactivation by the receptor, as detailed in the text. For example, the DBA/2 mouse strain is more resistant than the sensitive C57BL/6J strain,^{11,98,99} and the Han/Wistar rat strain is a 1000-fold more resistant to TCDD than the sensitive Long-Evans rat strain.^{12,100} The most dioxin-sensitive species is the guinea pig, while the hamster tolerates a 1000-fold higher dose.^{101,102}

Studies in humans have shown that serum level of CB-153, a marker for PCB exposure, is positively associated with DNA fragmentation. Interestingly, this was only seen in Caucasian but not in Inuit men.^{141,142} Whether this difference is solely due to difference in genetic background, or also related to other environmental exposures or lifestyle factors, remains to be seen.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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