

# Androgen receptor activation by polychlorinated biphenyls

## Epigenetic effects mediated by the histone demethylase Jarid1b

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**Keywords:** androgen receptor, polychlorinated biphenyls, Jarid1b, histone modification, environment, endocrine disruptors, epigenetic

**Abbreviations:** PCBs, polychlorinated biphenyls; DHT, dihydrotestosterone; AR, androgen receptor; polyQ, polyglutamine; EDC, endocrine disruptor compounds; AhR, aryl hydrocarbon receptor; ASD, autism spectrum disorder

The exposure to environmental endocrine disrupting compounds (EDC), as polychlorinated biphenyls (PCBs), widely diffused in the environment may produce epigenetic changes that affect the endocrine system. We found that PCBs activate AR transcriptional activity and that this effect is potentiated by the demethylase Jarid1b, a histone demethylase that catalyzes the removal of trimethylation of lysine 4 on histone H3 (H3K4me3), induced by PCB. The aim of the present study was to investigate the effect of the treatment of cultured cells (HEK293) with a mixture of the most diffused environmental PCBs and, also with dihydrotestosterone (DHT), on the functional interaction between AR and Jarid1b. Although the effect induced by DHT on the AR transactivation was considerably higher, the PCB mixture produced an AR-mediated transactivation in a dose-dependent manner. Cotransfection with plasmids expressing Jarid1b and various AR isoforms containing polyglutamine tracts (polyQ tracts) of different lengths showed that Jarid1b potentiates the AR transcriptional activity induced by PCBs but only with the shortest AR isoform. The potentiating effect of Jarid1b on the AR is mediated by a direct interaction of the enzyme with the AR promoter. In fact, utilizing constructs containing AR promoters with a different length and a luciferase reporter gene, we showed that the effect of PCBs, but not of DHT, needs the presence of Jarid1b and of at least two DNA binding sites for Jarid1b.

### Introduction

Epigenetic marks, including DNA methylation, histone modifications and small inhibitory RNAs, are heritable and provide critical layers of gene regulatory control; however, they are dynamic and can be influenced by environmental exposures. Studies primarily focused on cancer and late-onset diseases have revealed that a growing number of human diseases are linked to epigenetic disruptions, including cancer, osteoporosis, autoimmune diseases neurodevelopmental disorders, depression or autism.<sup>1</sup> Thus, it is of great interest to determine the epigenetic marks associated with these diseases, and the environmental factors that cause them to vary, which could dramatically advance our diagnostic ability and potentially lead to the development of new therapies.<sup>1</sup>

Evidences now suggest that epigenetic mechanisms link genetics and environment in shaping endocrine function.<sup>2,3</sup> Epigenetic mechanisms partition the genome into active and inactive domains based on endogenous and exogenous environmental

changes and developmental stages. This creates phenotype plasticity that can help to explain inter-individual and population endocrine variability.<sup>2</sup>

Several enzymes involved in epigenetic key process, such as histone modifying enzymes, are affected and modulate endocrine system. It is also known that nuclear receptors interact with histone-modifying enzymes to regulate gene transcription and chromatin remodeling.<sup>4</sup> Interestingly, histone demethylases, the enzymes that catalyze the removal of the methyl groups from histones, complex with steroid receptors (androgen receptor, AR) facilitating the transcription of their target genes.<sup>5</sup> For example, ligand-bound AR regulates target-gene transcription, interacting with different cofactors that include the enzymes responsible for histone methylation and acetylation.<sup>4,6</sup> AR also interacts with histone demethylases (such as Jarid1b, also named PLU1 or KDM5B, and LSD1)<sup>4,7</sup> and these enzymes regulate AR mediated transcription, especially during prostate cancer development, proliferation, and progression.<sup>8-10</sup>

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Notably, exposure to environmental pollutants, particularly to compounds with endocrine interfering activity (EDC), affect epigenome.<sup>11</sup> Some EDCs like polychlorinated biphenyls (PCBs) are widely present in the environment; PCBs are a group of 209 congeners with a broad spectrum of biological and toxic effects<sup>12</sup> and with different biochemical characteristics. PCBs are classified as dioxin like (or coplanar) (DL-PCBs), and non-dioxin compounds (NDL-PCBs). In the environment, animals and humans are exposed contemporary to both classes of PCBs and the global effect is cumulative. Many effects of PCBs, at least those of DL-PCBs, are mediated by the aryl hydrocarbon receptor (AhR), an ubiquitous nuclear transcription factor<sup>13</sup> capable to serve as a transcriptional co-regulator of AR.<sup>14</sup>

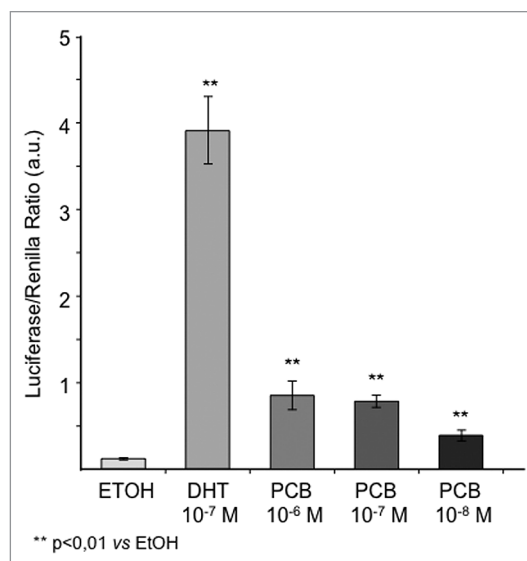
Moreover, PCBs affect the profile of testosterone metabolic enzymes (5- $\alpha$  reductase and aromatase) in the CNS<sup>15,16</sup> in a sex dimorphic way. PCBs also produce epigenetic changes including DNA methylation global content,<sup>17</sup> histone pattern of the Cyp11a1 promoter<sup>13</sup> and histone post-translational modifications in liver<sup>18</sup> and in brain (Casati et al., unpublished data).

The aim of our studies is to evaluate the role of a mixture of the most diffused environmental PCBs on the AR-Jarid1b interaction. In particular, we have analyzed: (1) the AR transcriptional activity and nuclear localization induced by PCB; (2) the role of Jarid1b in the AR activation and interaction with the AR promoter and (3) the role of PCBs and Jarid1b in the transactivation of different AR poly Q variants (isoforms with different transcriptional activities; see the discussion for more details).

## Results

In order to assess the effect of different concentrations of the PCB mixture on the AR transcriptional activity, we employed a genetic reporter assay on the HEK293 cell line, derived from human embryonic kidney. HEK293 cells were cotransfected with a plasmid expressing the human androgen receptor (pCMV-hAR), a plasmid containing two AR response elements inserted in front of the coding region for firefly luciferase (GRE2E1bLuc), and the plasmid pGL4hRLuc expressing constitutively *Renilla* luciferase, which served as internal vector control for normalization of transfection efficiencies. As positive control for the transcription activity of AR, the effect of the natural androgen dihydrotestosterone (DHT,  $10^{-7}$  M) on the cotransfected cells was also determined. As shown in **Figure 1**, the mixture of PCBs activated the AR-mediated transcription in a dose-dependent manner, although this activation was quantitatively much lower than that induced by the natural androgen DHT.

To analyze the role of Jarid1b in AR transcriptional activity independently of cell phenotype, we cotransfected Jarid1b (pcDNA 3.1-hJarid1b-myc-his) and AR (same plasmids utilized above) in epithelial (HEK293) and neuronal (NSC34 and GN11) cells. Jarid1b potentiates (**Fig. 2**) the AR transactivation induced both by DHT and PCBs in HEK293 cells, but also in neuronal derived NSC34 and in GN11 cells. The effect of Jarid1b is strongest in the presence of DHT, while is absent without an AR ligand and appears to be comparable in the three types of cells utilized.



**Figure 1.** Dose-response effect of PCBs on AR transcriptional activity in HEK293 cells transiently cotransfected with pCMV-hAR, GRE2E1bLuc, and pGL4hR and treated with DHT  $10^{-7}$  M, or with the PCB mixture at  $10^{-6}$ ,  $10^{-7}$  and  $10^{-8}$  M, or with ethanol (EtOH) as negative control. EtOH, ethanol; PCB, polychlorinated biphenyls; AR, androgen receptor.

We ascertained that the overexpressed protein Jarid1b was functionally active evaluating its demethylating capacity on trimethylated lysine 4 of histone H3 (H3K4me4) into transiently transfected cells. Levels of H3K4me3 were determined by western blotting assay using a specific antibody. Cells transfected with plasmid expressing Jarid1b (pcDNA 3.1-hJarid1b-myc-his) contained decreased amounts of global H3K4me3 (**Fig. 3**), in comparison with control cells transfected with the empty vector (pcDNA 3.1). The reduction in levels of H3K4 trimethylation resulted to be independent of the treatment with AR ligands (**Fig. 3**) and it did not affect cell viability, since transfection with Jarid1b has not changed cell proliferation and apoptosis rate in HEK293 cells (data not shown). Since H3K4me3 is found in a constant balance with Polycomb-mediated repressive H3K27me3,<sup>19</sup> we have evaluated by western blotting analysis the level of H3K27me3 in HEK293 cells. The results indicate that neither the overexpression of Jarid1b nor the treatment with DHT or PCB modified the global level of this repressive histone mark (data not shown).

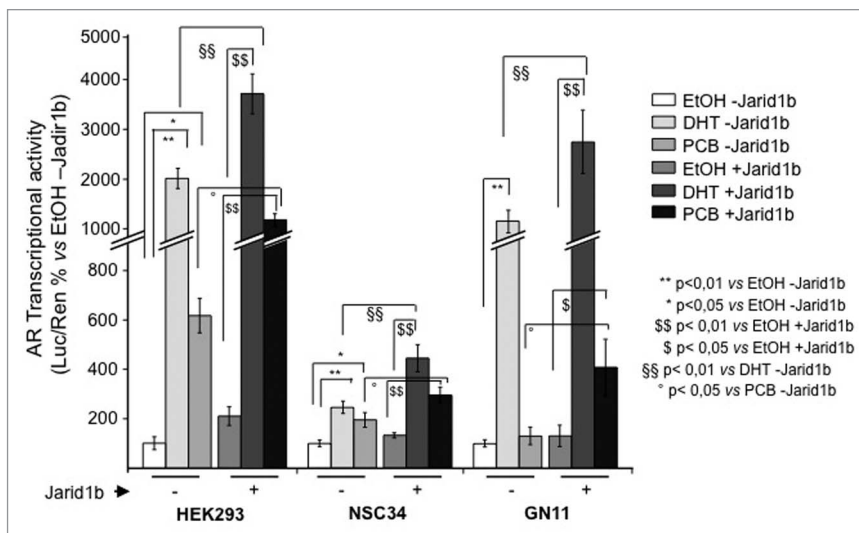
Next, we studied whether the nuclear translocation of AR could be promoted by Jarid1b. To do this, we analyzed by immunofluorescence microscopy the nuclear localization of AR in the presence or the absence of Jarid1b following activation by the natural ligand DHT or the PCB mixture. As it can be seen in **Figure 4**, exposure of transfected HEK293 (pCMV-hAR) cells to DHT or to the mixture of PCBs resulted in nuclear translocation of AR, as evidenced by the concentrated green label into the nucleus. Transfected Jarid1b (pcDNA 3.1-hJarid1b-myc-his) (labeled in red) appears to be present together with AR in the nuclei after ligand exposure. Even if a quantitative evaluation of the amount of nuclear translocation has not been done, the transfection of Jarid1b appears to potentiate this effect induced by both ligands.

Since the CAG repeat length present in the first coding exon of the AR gene ranges normally from 6 to 30 repeats and shorter length correlates with a greater transactivational activity, we have evaluated the effect of DHT and of the PCB mixture on AR transcriptional activity in HEK 293 cells cotransfected with plasmids (pCMV3.AR/Q12, pCMV3.AR/Q23, pCMV3.AR/Q46) coding for a AR with different CAG repeat length (12 PolyQ, 23 PolyQ, or 46 PolyQ) and Jarid1b (pcDNA 3.1-hJarid1b-myc-his) (or an empty vector, pcDNA 3.1). **Figure 5** shows that DHT is always very effective in inducing the AR transcriptional activity independently of CAG repeat length, while Jarid1b is able to significantly potentiate the AR transcriptional activity only in the presence of the two shorter AR isoforms. In particular the demethylase potentiate the effect of PCBs 2,2 times in the case of 12 PolyQ and 1,7 times in the case of 23 PolyQ).

Since the interaction between AR and Jarid1b may take place at the level of the AR promoter, we performed an *in silico* analysis on the binding sites present on AR promoter by the informatics platform MatInspector available on [www.genomatix.de/online](http://www.genomatix.de/online). We have considered 5000 nucleotides before start transcription site (TSS) and 2800 base pair after TSS. Among many putative hormone receptor binding sites, such as those responsive elements to androgens (ARE), glucocorticoids (GRE), estrogens (ERE), aryl hydrocarbon (AhRE; which receptor, precisely, binds coplanar PCBs), we found several potential interaction sites for Jarid1b (PLU 1) (**Fig. 6**). They are distributed both in the proximal and distal part of AR promoter always before the TSS. It is possible to localize Jarid1b binding sites at -2959 bp, at -2615 bp, at -1206 bp and -548 bp. On the contrary, the AhRE are mainly present after the TSS (**Fig. 6**) at +122 bp, +579 bp, +948 bp and +959 bp; the two AhRE present before the TSS are localized at -4436 bp and -2731 bp. The presence of binding sites for Jarid1b and AhR, along with ARE, on the AR promoter, is indicative of the possible direct effect of the demethylase in modulating the AR transactivation induced either by DHT or PCBs also in AR negative feedback.

To analyze the effects of the interaction between androgens PCB and Jarid1b, we have cotransfected HEK293 cells with plasmids coding for Jarid1b or with an empty vector (pcDNA 3.1) and the AR promoter with different regions upstream to the TSS plus 5'UTR (pgL7.0, pgL3.5, and pgL1.8) (see **Fig. 7**) driving the luciferase gene expression. The effect tested is the inhibition of AR promoter activation exerted by androgens (which is the basic mechanism by which AR establishes a negative control on its own functions<sup>20</sup>).

The data indicate that in presence of the longest promoter region (7.0), DHT significantly inhibits the promoter activation, but the effect is not potentiated by Jarid1b (**Fig. 7A**). Interestingly



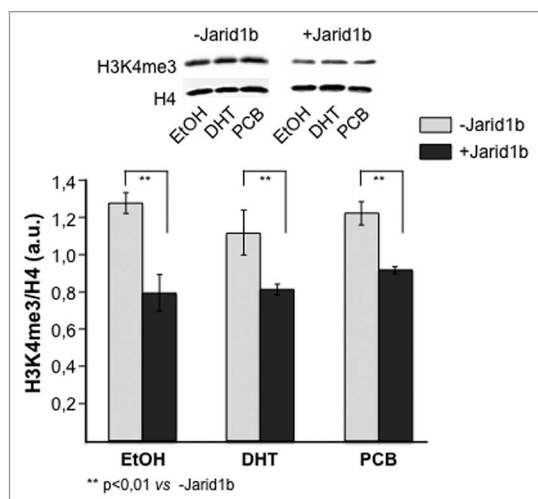
**Figure 2.** Enhancement of transcriptional activity of AR by Jarid1b in HEK293, GN11, and NSC34 cells. Cells cotransfected with pCMV-hAR, pcDNA 3.1-hJarid1b-myc-his (or the empty vector, as control), GRE2E1bLuc, and pGL4hRLuc were treated with DHT  $10^{-7}$  M; the PCB mixture  $10^{-7}$  M; or ethanol (EtOH). EtOH, ethanol; PCB, polychlorinated biphenyls; DHT, dihydrotestosterone; AR, androgen receptor.

PCBs are ineffective in absence of Jarid1b, but gain a significant inhibitory effect in cotransfected cells. It is possible to observe that in 7.0 promoter there are 5 Jarid1b binding sites (at -2959 bp, at -2615 bp, at -1206 bp and -548 bp) and there are 4 AhRE (at -4436 bp, -2731 bp, at +122 bp, +579 bp, +948 bp, and +959 bp). The shorter 3.5 promoter region (which has lost 3 distal Jarid1b and 4 AhRE binding sites, but still possesses 2 proximal Jarid1b sites and most androgen binding sites) shows a similar behavior (**Fig. 7B**). The inhibitory effect of DHT, PCB and the potentiating effect of Jarid1b are completely lost in the shortest 1.8 promoter region, which possesses very few androgen responsive elements, only one Jarid1b binding site, at -548 bp and 4 AhRE at +122 bp, +579 bp, +948 bp, and +959 bp (**Fig. 7C**). These results have indicated the requirement of Jarid1b to observe the feedback inhibition of the promoter activation following the treatment with PCBs, but not with DHT. Furthermore, employing promoter versions of different length we have shown that this effect requires the presence at least two of the Jarid1b potential binding sites.

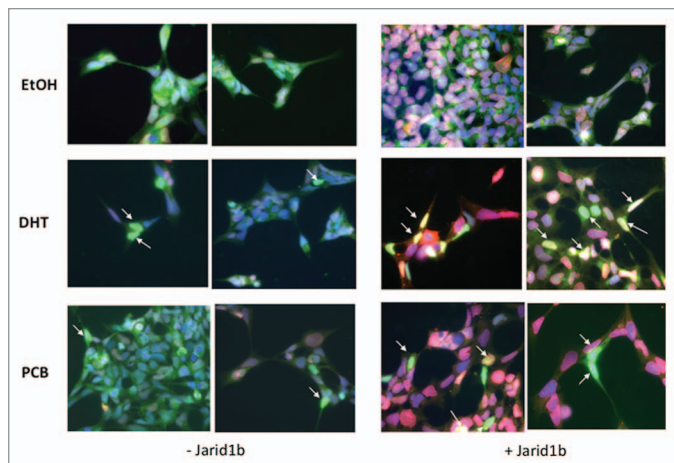
## Discussion

The aim of this study was to evaluate the role of Jarid1b in modulating the AR transcriptional activity triggered by its physiological ligand, DHT, or by a PCB mixture of the most diffused environmental components of this family of pollutants.

From the dose response studies performed in HEK293 cells transfected with AR it clearly appears that the PCB mixture produces a dose dependent AR transactivation, which is much lower than the effect induced by the endogenous ligand DHT. DHT represents the main “active” 5 $\alpha$ -reduced testosterone metabolite possessing a much higher affinity than the parent compound for the AR. In the androgen-dependent tissues (e.g.,



**Figure 3.** Western blot analysis of H3K4me3 levels in HEK293 cells transfected with the plasmid pcDNA 3.1-hJarid1b-myc-his (or the empty vector, as control) overexpressing histone demethylating enzyme Jarid1b. The relative amount of H3K4me3 was normalized by the signal obtained for total H4 with an anti-H4 antibody. EtOH, ethanol; PCB, polychlorinated biphenils; DHT, Dihydrotestosterone; J, Jarid1b.



**Figure 4.** Immunofluorescence analysis of AR nuclear translocation in HEK293 cells transiently cotransfected with the plasmid 3.1-hJarid1b-myc-his following DHT or PCB treatment. Primary antibodies against AR and pcDNA 3.1-hJarid1b-myc-his were used to detect them. Secondary conjugated antibodies were used to detect AR (green channel) or Jarid1b (red channel). Images were obtained at either 20 $\times$  or 32 $\times$  magnification.

the prostate) the local DHT formation is essential for producing a “normal” androgenization. Furthermore, the cotransfection of AR with the H3K4me demethylase Jarid1b, an enzyme that we have previously shown to potentiate the androgenic signal in rat liver,<sup>12,18</sup> resulted in a further increase of the AR transcriptional activity induced by DHT or by PCBs in three different cell types: HEK293, and two neuronal cell lines, NSC34 and GN11. It is worth of note that this demethylase appears widely diffuse, since the presence of Jarid1b activity/expression has been

shown in several cell lines from HeLa cells to different prostatic cell lines.<sup>7,10</sup>

The transfected Jarid1b plasmid codes for a full functional enzyme which is able to reduce global H3k4me3 levels (but not global H3K27me3) in HEK293 cells independently of the treatment, and is also able to potentiate AR nuclear translocation when the receptor is activated by DHT or PCBs. Utilizing plasmids that express AR with a different CAG repeat length we have shown that Jarid1b potentiates the AR transcriptional activity induced by PCBs only in the presence of the shorter AR isoforms.

The mechanism by which Jarid1b potentiates the AR transcriptional activity is still unclear. It is known that the enhancement of the AR transcriptional activity needs the enzymatic activity because deletion of the JmjC domain, the catalytic center of the demethylase, abolished the stimulation.<sup>7,21</sup> The enzymatic activity of Jarid1b requires not only the JmjC domain but also the Arid domain and zinc-finger-like domain and three plant homeodomains (PHD), involved in histone-tail recognition.

The presence of binding sites for Jarid1b (PLU1) and AhR (XRE), along with some androgen responsive elements (ARE), on the AR promoter, is indicative of the possible direct effect of the demethylase in modulating the AR transactivation induced either by DHT and by PCBs also in AR negative feedback.<sup>20</sup>

Our studies with constructs containing AR promoters with a different length (long, intermediate, and short) and a luciferase reporter gene, show that the effect of PCBs, but not of DHT, needs the presence of Jarid1b and of at least 2 PLU1 binding sites. This result appears particularly important because it indicates that Jarid1b might have a specific role in potentiating the AR transactivation in presence of PCB pollution.<sup>16,18,20</sup> Moreover, it is noteworthy that PCB exposure stimulates the expression of the Jarid1b “in vivo” at least in the rat liver.<sup>18</sup> Due to the concomitant presence of XRE, ARE, and PLU1 on the AR promoter it is possible that the recruitment of Jarid1b is responsible of the complex AhR-AR interactions occurring after PCB exposure, in particular in presence of coplanar congeners, as PCB 126 contained in our mixture, which are able to bind AhR.<sup>22-24</sup> The association between AR and AhR is intricate and not fully understood.<sup>22,25,26</sup> Besides, ligand association studies have shown a direct and specific binding of several PCB congeners to the ligand-binding domain of the AR protein.<sup>27</sup>

It is unknown now which compound in the mixture is mainly responsible of the AR activation. It is noteworthy that if the receptor is activated by DHT, the concomitant exposure to the PCB mixture produces a weak antagonist effect, even if the Jarid1b potentiation of AR activity is still present (data not shown). Literature data available on AR agonist/antagonist activity of the individual PCB congeners present in the mixture has been already discussed in details.<sup>18</sup>

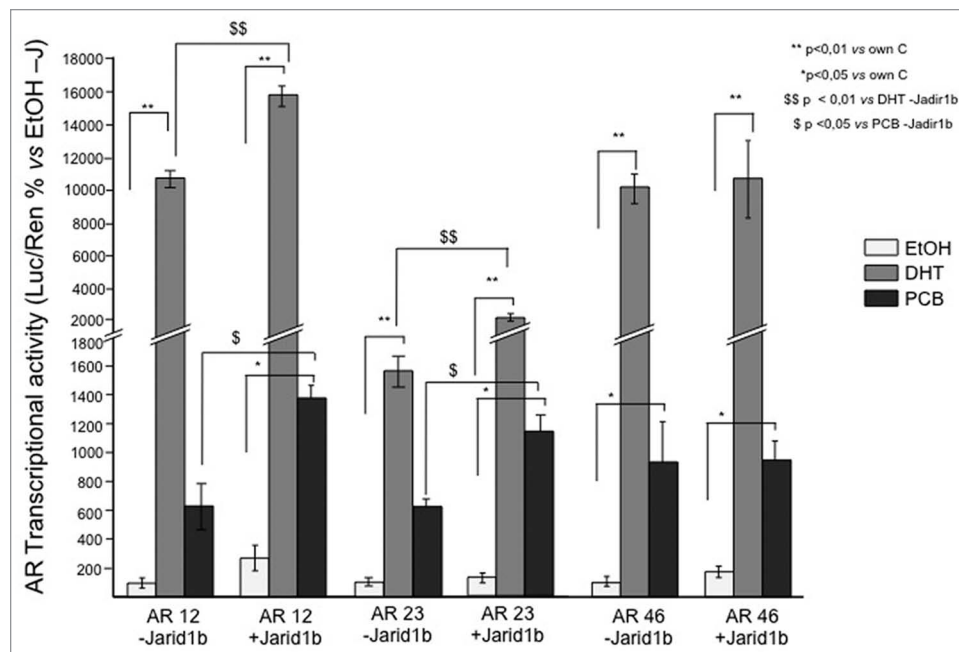
The interaction between PCB and AR is also affected by differences in the structure of the AR gene present among individuals, since its transcriptional activity depends on the polyglutamine tract (polyQ, coded by a CAG repeat) length located in the trans-activating region of the AR. In the AR gene the CAG repeat number vary both within and between human populations between 8 and 30 units<sup>28,29</sup> and thus the coded polyQ also is

polymorphic in length. An inverse relationship exists between the AR transcriptional activity and the poly Q repeat length (AR CAG<sup>n</sup><sup>30-32</sup>), and at least 64 different diseases and phenotypes have been investigated in relation to AR CAGn. Moreover, two recent reports by Bjork and coworkers<sup>14,33</sup> indicate that PCBs have a CAG/PolyQ length dependent effect on AR “in vitro”<sup>33</sup> and in some human prostatic cells.<sup>14</sup> In substantial agreement with the data presented in this paper, a single exposure to PCB 153, one of the component of our mixture, has the most pronounced effect on the activity of the shortest 16 repeat AR “in vitro.” The exposure of PANT1A prostatic cells, transfected with ARs containing a 16, 22, and 28 CAG/PolyQ repeat tract, to 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD), one of the most toxic coplanar PCB congeners, potentiates the DHT induced AR activity only in the cells with the shortest CAG/PolyQ replicate.

On the basis of the data of the present study, we propose that the higher activation of the short AR is mediated by a better interaction of this receptor with Jarid1b. This observation might be important in the pathogenesis of autism spectrum disorder (ASD) in which the genetic as well as important environmental effects might be involved. Indeed, ASD onset might be related to an excessive androgen prenatal exposure both in animals<sup>34</sup> and in humans.<sup>35</sup> The hyperandrogenization of the brain might be caused by a higher fetal androgen exposure, increased activity of testosterone (T) activating enzymes or enhanced AR activity, as might occur in presence of the short polyQ variant of AR and/or by the interaction of environmental induced epigenetic changes. A hyperactivation of AR induced by PCBs in presence of Jarid1b in some susceptible individuals could be an important etiological mechanism in this complex disease.

Indeed, a higher prevalence of short CAG alleles in ASD female subjects has been reported,<sup>36</sup> and mutations within the genes encoding the H3K4-specific histone demethylase, JARID1C/SMCX, have been linked to autism.<sup>37</sup> All these data suggest that an early-life exposure to PCB might be involved in modulating AR effects through AhR and by epigenetic mechanisms.<sup>13,18</sup> The AR CAG repeat genotype might be one of many genetic component interacting with environment through epigenome to contribute at ASD susceptibility.

In conclusion PCBs are able to modulate AR transcriptional activity by AhR-AR-Jarid1b interaction and chromatin remodeling. These results might be important in the explaining some etiological mechanisms of a complex, environmental sensitive, disorder like autism.



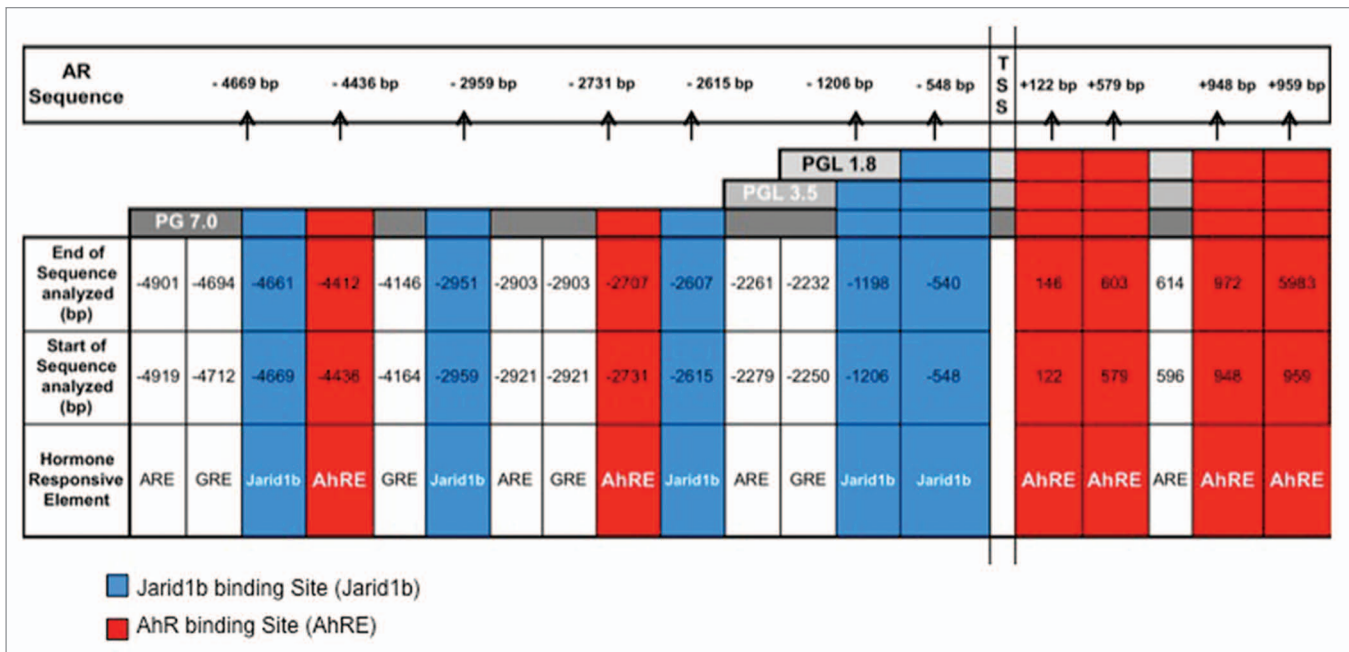
**Figure 5.** Transcriptional activity of different Poly Q variant of AR in HEK 293 cells cotransfected with plasmids (pCMV3.AR/Q12 or pCMV3.AR/Q23 or pCMV3.AR/46) coding for a AR with different CAG repeat length (12 PolyQ, 23 PolyQ or 46 PolyQ) and pcDNA 3.1-hJarid1b-myc-his (or an empty vector). Cells were treated with ETOH, DHT  $10^{-7}$  M or the PCB mixture  $10^{-7}$  M. EtOH, ethanol; PCB, polychlorinated biphenyls; DHT, dihydrotestosterone.

## Materials and Methods

**Cell cultures and treatments.** HEK293 and GN11 cells were grown in Dulbecco’s Modified Eagle Media (DMEM) containing phenol red and supplemented with 10% fetal bovine serum (FBS). NSC34 cells were grown in DMEM with phenol red and 5% FBS.

The polychlorinated biphenyls mixture contained PCB 138, PCB 153, PCB 180, and PCB 126 (in relative proportions, 1:1:1:1/10 000) dissolved in ethanol. The final and total concentration of PCBs applied to cells was  $10^{-7}$  M. For the dose-response experiments, PCBs were used at final concentrations between  $10^{-6}$  M and  $10^{-8}$  M. Dihydrotestosterone (DHT) was employed at concentration of  $10^{-7}$  M.

**Transfection.** Transfections were performed by using Lipofectamine 2000 (Life Technologies) in 96-well plates in steroid-free medium without phenol red, according to the manufacturer’s protocol. The following constructs were transfected: pCMV-hAR, for the expression of human androgen receptor; pcDNA 3.1-hJarid1b-myc-his, a plasmid expressing human Jarid1b, or the empty vector pcDNA 3.1 used as control; GRE2E1bLuc, the reporter plasmid expressing firefly luciferase under the control of AR response elements, to evaluate transcriptional activity of AR; pGL4hRLuc, expressing *Renilla* luciferase, as internal control vector. To analyze AR negative feedback, plasmids pgL7.0, pgL3.5, and pgL1.8 encoding different length of AR promoter driving the firefly luciferase gene, (see outline at Fig. 7D, for additional details) were transfected. Plasmids pCMV-hAR, GRE2E1bLuc, and also those expressing AR variants with



**Figure 6.** In silico analysis on the binding sites for hormone receptors and for Jarid1b present on AR promoter. The computer platform MatInspector (available on [www.genomatix.de/online](http://www.genomatix.de/online)) was used. A stretch of the AR gene sequence spanning from about 5000 bp before the transcription start site (TSS) to about 2800 bp after TSS was examined. ARE, Androgen Responsive Element; GRE, Glucocorticoid Responsive Element; ERE, Estrogen Responsive Element; AhRE Element responsive to AhR (the aryl hydrocarbon receptor) and Jarid1b (also known as PLU1) binding sites. Start and End rows represent the relative position of the potential binding site relative to the sequence analyzed. At the right side of the figure the plasmids used in figure above are aligned for the sequences. The position of binding site for Jarid 1b and AhRE are exactly localized. Bp, base pair; TSS, transcription start sites; AhRE, element responsive to AhR; ARE, androgen responsive element; GRE, glucocorticoid responsive element; ERE, estrogen responsive element; AR, androgen receptor.

distinct polyQ tracts, pCMV3.AR/Q46, pCMV3.AR/Q23 and pCMV3.AR/Q12 were kindly provided by Dr Angelo Poletti (Department of Endocrinology, Pathophysiology and Applied Biology of University of Milan, Italy). Plasmid pcDNA 3.1-hJarid1b-myc-his was kindly provided by Dr Charlie Degui Chen (Institute of Biochemistry and Cell Biology, Shanghai, China). Efficiency of transfection was evaluated by fluorescent microscopy transfecting pEGFP.

**MTT assay.** To evaluate cell proliferation we have performed a MTT assay. Briefly, cells were plated in 24 well plates at a density of  $5 \times 10^{-4}$  cells per well. The cells were cultured overnight and the day after they were transfected with pcDNA 3.1-hJarid1b-myc-his or the empty vector pcDNA 3.1.

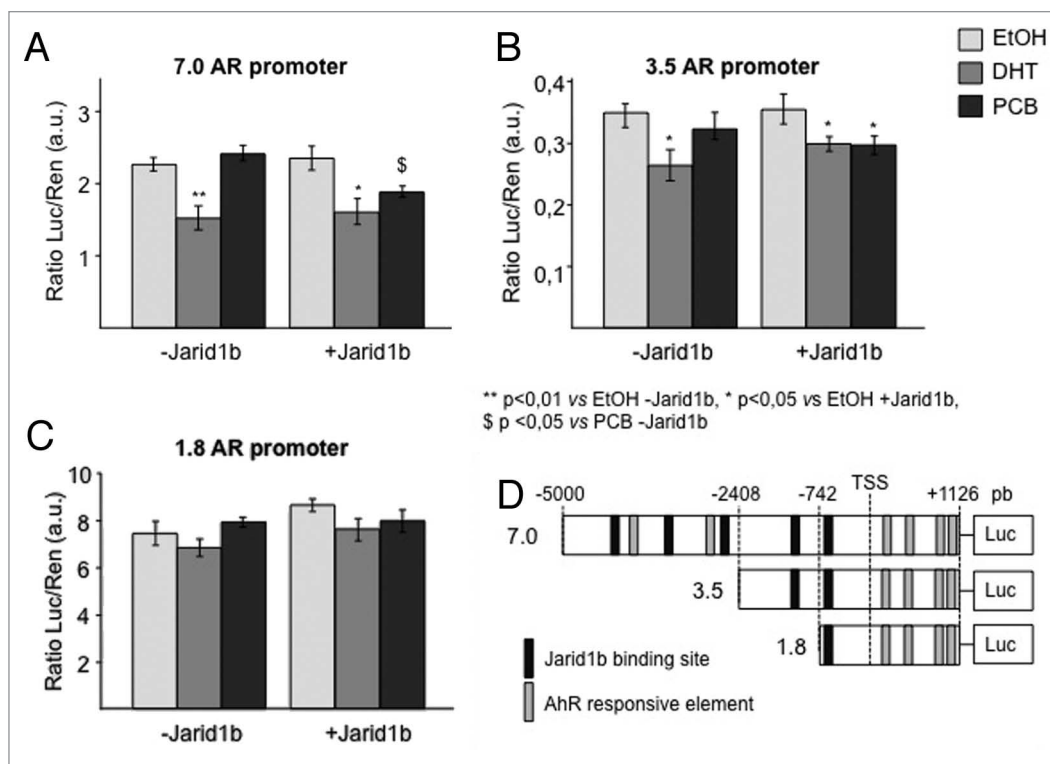
After 22 h, 300  $\mu$ l of MTT (Sigma-Aldrich) dissolved in DMEM at 1.5 mg/ml was added directly to the well and incubated at 37 °C for 1 h. The formazan crystals were dissolved in 500  $\mu$ l of isopropanol after careful removal of supernatant. The absorbance (490 nm) was read at microplate reader (VICTOR, Perkin Elmer).

**Detection of morphological apoptosis with Hoechst 33258 Staining.** Cells were plated at a density of  $5 \times 10^{-4}$  cells per well on 14-mm glass coverslips in 24 well plates. After the transfection with pcDNA 3.1-hJarid1b-myc-his or the empty vector pcDNA 3.1, cells were fixed with a solution of paraformaldehyde 3% and sucrose 2% for 10 min at 37 °C. After the fixation, cells were washed once with PBS with calcium and magnesium

and permeabilized with Triton X-100, 0.2% in PBS. The fixed cells were stained with 10  $\mu$ g/ml Hoechst 33258 for 10 min at 37 °C. Images were obtained at either 20 $\times$  magnification with a Zeiss Axiovert microscope equipped for fluorescence analysis with the corresponding sets of filters to detect Hoechst emission. Apoptotic cells were identified by condensation and fragmentation of nuclei (bright blue chromatin).<sup>38</sup>

**AR transcriptional activity.** Transcriptional activity was measured using the Dual-Glo Luciferase System (Promega) according to the manufacturer's protocols. Briefly, transfected HEK293 cells were cultured in 96-well plates and treated with PCB  $10^{-7}$  M, dihydrotestosterone (DHT)  $10^{-7}$  M, or ethanol for 22 h. Each sample was replicated ten times. Cell medium was removed and 75  $\mu$ l of fresh medium was added to each well. Seventy-five microliters of Dual-Glo Luciferase reagent have been added to each sample and firefly luminescence read after 10 min. At the end of this step, 75  $\mu$ l of Dual-Glo<sup>®</sup> Stop and Glo<sup>®</sup> Reagent were added in each well and *Renilla* luciferase luminescence read after 10 min. Data are expressed as the mean of the ratio  $\pm$  SE between luminescence of the experimental reporter (firefly luciferase) to that of the control reporter (*Renilla* luciferase). Each experiment was repeated three times.

**Immunoassays.** For western blotting of histones, samples were prepared as follow: cells were collected in PBS-EDTA and cell pellets were homogenized in Laemmli buffer 1 $\times$  (62.5 mM TRIS-HCl pH 6.8, 7.5% glycerol, 2% SDS, 0.125 M dithiothreitol),



**Figure 7.** Transcriptional activity of AR by different length AR promoters on HEK 293. The cells were cotransfected with pcDNA 3.1-hJarid1bmyc-his (or the same empty vector), pCMV human androgen receptor, pgL7.0, pgL3.5, and pgL1.8 (coding for AR promoters of differential length). Cells were treated with ETOH, DHT  $10^{-7}$  M or the PCB mixture  $10^{-7}$  M. EtOH, ethanol; PCB, polychlorinated biphenils; DHT, dihydrotestosterone; bp, base pair; AhRE, element responsive to AhR; AR, androgen receptor; J: Jarid.

subjected to ultrasonic bath for 10 min prior to heat samples at 95 °C for 5 min. The resulting whole cell extracts were then centrifuged at 13 200 rpm for 10 min and supernatants were saved and used directly for immunoblotting. Protein extracts were resolved by SDS/15%PAGE. Protein gels were electrotransferred to 0.2  $\mu$ m pore nitrocellulose membranes by standard wet procedures. After transfer, membranes were stained with Ponceau S to verify the correct loading and transfer, and then blocked and probed overnight at 4 °C with the primary specific antibodies: anti-H3K4me3 (ref. Ab8580, Abcam), anti-H3K27me3 (ref. 07-449, Millipore) and anti-H4 (ref. 10158, Abcam). Membranes were processed with WesternDot™ 625 Western Blot Kits (Life Technologies) as described by the manufacturer. Fluorescent signals were quantified using ChemiDoc system (Bio-Rad). Data are expressed as mean  $\pm$  SE of the relative amounts of H3K4me3/H4 (arbitrary units).

For immunofluorescence microscopy, HEK293 cells transiently transfected with expression vectors for AR and Jarid1bmyc-his were processed for immunolabeling as follow. Cells were fixed with a solution of paraformaldehyde 3% and sucrose 2%. After incubation, cells were washed once with PBS with calcium and magnesium and permeabilized with Triton X-100, 0.2% in PBS containing BSA 0.25% for 10 min. Samples were then incubated overnight at room temperature with a dilution 1:100 of the primary antibodies against AR (Ab3509, Abcam) and poly-His (for Jarid1b myc-his detection) (Ab5000, Abcam). After

washing with PBS, cells were incubated with secondary antibodies for one hour. AlexaFluor 488 (Life Technologies) conjugated goat anti-rabbit secondary antibody was used to detect AR (green channel), and AlexaFluor 594 conjugated goat anti-mouse secondary antibody (Life Technologies) was used for Jarid1bmyc-his (red channel). DNA was counterstained with DAPI. Images were obtained at either 20 $\times$  or 32 $\times$  magnification with a Zeiss Axiovert microscope equipped for fluorescence analysis with the corresponding sets of filters to detect triple fluorescent emission.

**In silico analysis.** In silico analysis of the binding sites present on AR promoter was performed using the informatics platform MatInspector available on [www.genomatix.de/online](http://www.genomatix.de/online). It was considered 5000 nucleotides before start transcription site (TSS) and 2800 base pair after TSS of the nucleotide sequence of human androgen receptor.

**Statistical analysis.** Data were analyzed by unpaired student's t-test and two-way ANOVA and expressed as mean  $\pm$  SE (SPSS 20, PRISM 6).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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