Use of a proteomics chip to discover mechanisms of actions for different polychlorinated biphenyls in humans

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Introduction

The dioxin-like Polychlorinated biphenyls (PCBs) (coplanar) have since long been known to activate the Ah-receptor (AHR). AHR is a ligand-activated basic helix-loop-helix transcription factor, and a member of the PER-ARNT-SIM (PAS) superfamily of transcription factors [1]. Binding of PCB to AhR induces AhR dimerization with ARNT (AhR nuclear translocator) and translocation to the nucleus, leading to the transcription of several genes, including hepatic enzymes, such as CYP1A1 And CYP1B1 [2]. The transcriptional activity of the complex needs co-activators such as thyroid hormone receptor/retinoblastoma protein-interacting protein 230 (TRIP230), and co-repressors such as the estrogen receptor alpha isoform (ERα) [3].

Although the binding of PCBs to receptors have been widely studied, the downstream effects of such activation/inhibition are not well characterized. One way to study the effects on the protein level is by transcriptomics and it has recently been shown that children exposed to high levels of PCB showed a very different expression profile in whole blood compared to less exposed controls [4]. Another way to study the effects of PCBs on the protein level is by the use of proteomics. Recently a novel technology enabled us to measure 86 different proteins by use of a proteomics chip in a human sample in which we previously have measured different PCBs, the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study. In this sample, we studied how different PCBs were related to the plasma levels of 92 proteins with special emphasis on dioxin-like activity.

Material and methods

The population-based Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study (n=1016, 50% women, all aged 70 years) was used for analyses.

PCB levels were measured in stored plasma samples collected at baseline using a Micromass Autospec Ultima (Waters, Milford, MA, USA) high-resolution gas chromatography coupled to a high resolution mass spectrometry (HRGC/ HRMS) system based on the method by Sandau and co-workers with some modifications. A more detailed description of the analysis in this sample has previously been presented [5]. The levels were normalized for plasma lipids.

In the present study we only used data for six of the 16 PCBs evaluated (PCB 118, 126, 153, 169, 170, 209), since we found that these six PCBs represents the major variation in all the 16 PCBs measured (6) and this reduction will reduce the multiple comparison problem.

The Olink Proseek Multiplex Cardiovascular 96X96 kit was used to measure proteins in plasma by real-time PCR using the Fluidigm BioMark HD real-time PCR platform. Of the wells, one is a negative control while 3 are positive controls (spiked in IL-6, IL-8 and VEGF-A) resulting in 92 measured proteins. Each sample includes two incubations, one extension, and one detection control used to determine the lower detection limit and to normalize the measurements. The resulting relative values obtained are log2-transformed for subsequent analysis. Further details regarding LOD, reproducibility and validations are given at Olink´s webpage (http://www.olink.com/products/proseek-multiplex/downloads/data-packages). In this sample, six of the 92 proteins showed levels below LOD in >25% of the population, and was therefore not used in the analyses.

As a first step, the overall hypothesis was tested that the relationships between the proteins and the PCBs were different for the 8 PCBs. For that purpose, multivariate regression (mvreg) that fits a multivariate regression model for several dependent variables (all proteins) with the same independent variables (all PCBs).
As the second step, linear regression models were used to evaluate the relationships between the plasma levels of the six PCBs and 86 different proteins as independent variables in separate models for each protein. Two levels of adjustments were used. First, adjustment for sex and storage time in freezer (age same in all subjects) was applied. Second, further adjustment for multiple potential life-style confounders (education level, exercise habits, daily energy intake, alcohol intake, and smoking) was applied.
The p-value for significance was adjusted both by the number of proteins, as well as for the six PCBs evaluated (0.05/86/6) to 0.0000969 according to Bonferroni.

Results and discussion
The primary overall analysis showed that the relationships between all proteins and the PCBs were different amongst the PCBs. This is graphically illustrated in the heatmap-plot in figure 1, where the p-values for the relationships between each protein vs. each of the 6 PCBs are shown.

While no proteins were significantly related to PCB126, four proteins were related to PCB118 following adjustment for sex only (age same in all subjects), one protein was related to PCB153, two were associated with PCB169, six proteins were related to PCB170 and eight proteins were associated with PCB209. All of these relationships were highly significantly also following adjustment for multiple life-style factors.

Two of the proteins were related to four of the six evaluated PCBs, Leptin (LEP) and Fatty acid-binding protein 4 (FABP4). It should however be noted that these two proteins were positively related to PCB118 and inversely related to PCB169, PCB170 and PCB209.

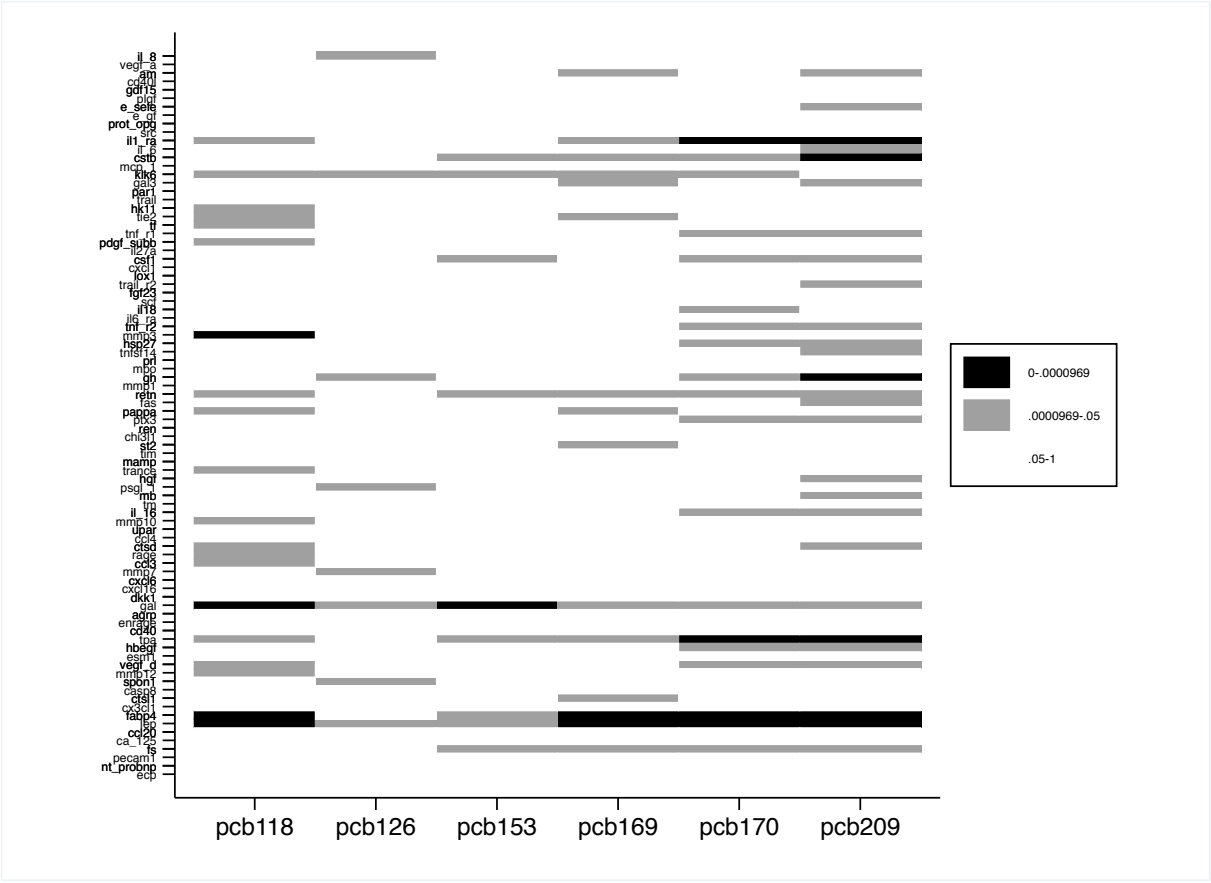

Figure 1. “Heat-map” of the p-values for the relationships between 6 PCBs and the 86 proteins. Relationships are adjusted for sex (age same in all subjects). The p-values are coded in grey-scale in three categories, >0.05 (white), 0.05- 0.0000969 (Bonferroni-adjusted significance level, grey), and < 0.0000969 (black).

As expected from the human study by Dutta et al [4] using transcriptomics as the outcome, the degree of PCB exposure was related with protein levels in humans. As also expected from the experimental data by Wens et al [6], the relationships between the PCBs and the proteins differed between the PCBs. In the present study we selected 6 PCBs that we previously have been found to represent the major variation in a larger set of PCB measurements [7]. Three of those were DL PCBs, including the potent AHR activator PCB126. We were however not able to demonstrate any obvious difference between the DL and non-DL PCBs regarding the links to the different proteins. Regarding leptin and FABP-4 for example, these two proteins were related to two DL and two non-DL PCBs.

The strength of the present study is measurement of a large number of proteins in a fairly large population in which also measurements of PCBs have been performed.

A limitation is that the study was performed in elderly Swedish subjects from a defined geographical area, so the results have to be replicated in other samples with different characteristics in order to be regarded as being general. Since we are not aware of any studies suitable for replication, we did chose a very strict adjustment for multiple comparisons (adjusting for 86 times 6 tests) in order to restrict the reporting of false positive findings.

In conclusion, proteomics disclosed that leptin and FABP4 levels were related to several PCBs suggesting one common mechanism of action tor these PCBs. In addition, some proteins were associated with only one of the PCBs suggesting more specific mechanistic links.

References

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