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Data in Brief

Toxicogenomic analysis of placenta samples from mice exposed to different doses of BPA

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ABSTRACT

Bisphenol A (BPA), a widespread Endocrine Disrupter mainly used in food contact plastics, may induce adverse effects especially on susceptible lifestages, first of all pregnancy. The present study considered placental development as a potential target of BPA and investigated potential differences in the modes of action of two doses of BPA by a toxicogenomic approach. Pregnant CD-1 mice were administered with vehicle, 0.5 (BPA05) or 50 mg/kg (BPA50) body weight (bw)/die of BPA, from gestational day (GD) 1 to GD11. At GD12 dams were sacrificed and transcriptomic analysis was performed on placenta samples. Histological, histomorphometrical and immunohistochemical analyses were also performed to phenotypically anchor transcriptional changes associated with BPA exposure. The interpretation and description of the overall data are included in a manuscript under revision [1]. Here we describe the experimental design and the analysis performed on the gene expression data which are publicly available through the Gene Expression Omnibus (GEO) database with accession number GSE63852.

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Specifications	
Organism/cell line/tissue	CD-1 mice
Sex	Female
Sequencer or array type	Agilent 4 × 44K Whole Mouse Genome Microarray G4122F
Data format	Raw data; normalized data
Experimental factors	Bisphenol A treated vs control mice
Experimental features	Pregnant CD-1 mice were administered with 0 (vehicle = olive oil), 0.5 (BPA05) or 50 mg/kg (BPA50) body weight (bw)/die of BPA, from gestational day (GD) 1 to GD11. At GD12 dams were sacrificed and placenta samples were excised and flash frozen in liquid nitrogen. RNA was extracted and gene expression profiles were analyzed by Agilent microarrays.
Consent	The animal study was conducted according to the European Community Council Directive 2010/63/UE, the Italian Law 4 marzo 2014, n. 26 and the OECD Principles on Good Laboratory Practice.
Sample source location	CD1 female mice (30–35 g bw) were purchased from Harlan (Italy). Experiments were conducted in the Animal Facility at Istituto Superiore di Sanità

Direct link to deposited data

Raw and normalized data are available through the Gene Expression Omnibus. http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE63852.

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Experimental Design, materials and methods

Animal treatment

CD1 female mice (30–35 g bw) were purchased from Harlan (Italy) and kept under standard laboratory conditions (22 ± 0.5 °C, 50–60% relative humidity, 12 h dark–light alternation with 12–14 air changes/h) with water and food (4RF25 GLP "Top Certificate" diet purchased from Mucedola, Milan, Italy) available *ad libitum*. After 1 week of acclimatization, females were bred with CD sires of proven fertility. A sperm-positive vaginal plug served to define the Gestational day (GD) 0. Pregnant mice were treated daily p.o. by gavage with 0 (vehicle only = olive oil; Sigma-Aldrich, Milan, Italy), 0.5 or 50 mg/kg bw pro die of BPA (CAS no. 80-05-7, purity ≥99%, Sigma-Aldrich), named BPA05 and BPA50 respectively, dissolved in olive oil, from GD1 to GD11, a period covering implantation through early placentation phase. On GD 12 the dams were sacrificed by asphyxiation with CO_2, followed by cervical dislocation. Four placentae per litter/group were flash frozen in liquid nitrogen then stored at −80 °C. The remaining placentae were excised and fixed for histological, histomorphometrical and immunohistochemical analyses.

RNA extraction

Placenta samples were homogenized and total RNA was extracted by using the RNAeasy Mini Kit (QIAGEN), containing β-mercaptoethanol diluted 1:100. RNA quantity was assessed with NanoDrop (NanoDrop, Wilmington, DE, USA), purity was assessed by UV absorbance ratio

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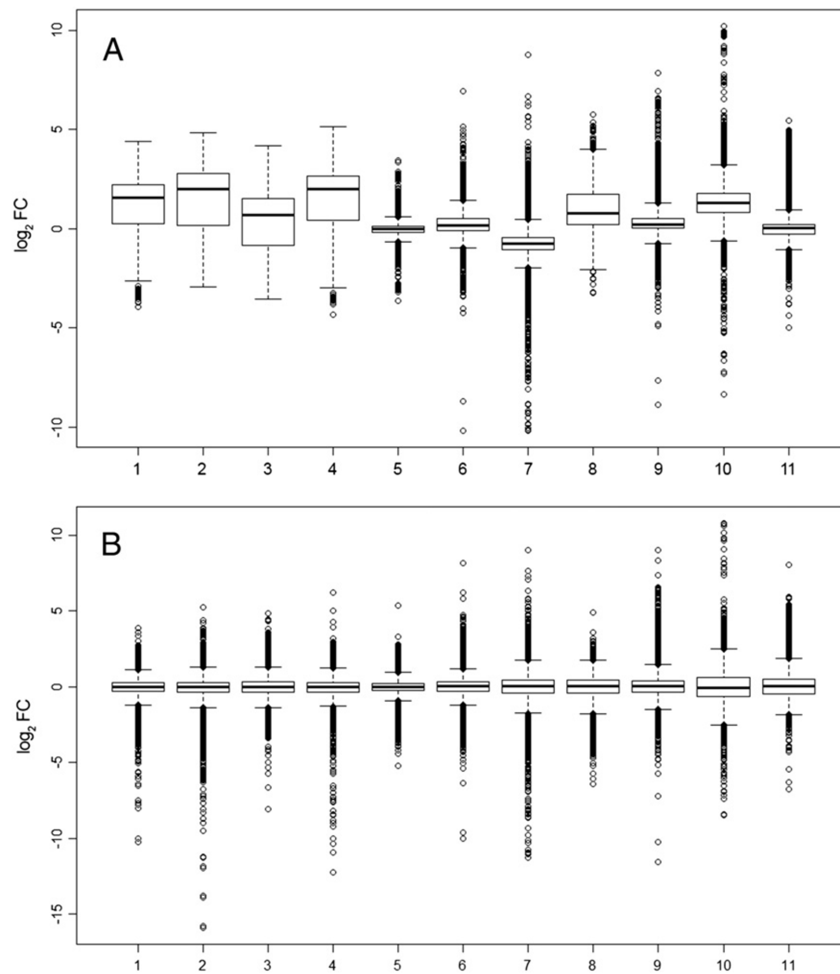

Fig. 1. Boxplots of signal intensities by array before (A) and after (B) normalization within (Loess) and between (quantile) arrays.

measurement at 260 nm and 280 nm whereas integrity was evaluated by 1% agarose gel electrophoresis.

Microarrays

cDNA and cRNA synthesis, amplification and labeling were performed using the Quick Amp Labeling Kit, Two Colors (Agilent, Santa Clara, CA, USA) with spike-in internal controls (Agilent) following manufacturer's protocol. Labeled samples and controls were co-hybridized following a dye swap experimental protocol (control and treatment samples alternatively labeled with Cy5 and Cy3) on Agilent 4 × 44K Whole Mouse Genome Microarray G4122F at 65 °C for 17 h, with a 10 rpm rotation in an Agilent Hybridization Oven.

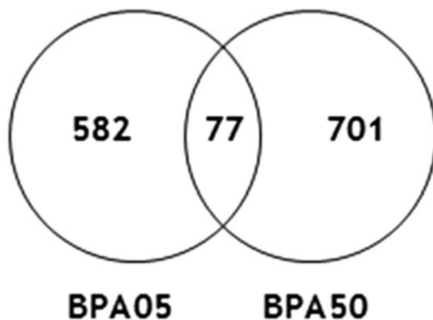

Fig. 2. Venn Diagram of differentially expressed genes in BPA05 and BPA50 mice placenta with respect to control placentas.

After washing with Gene expression wash buffers (Agilent), slides were scanned by the Agilent G2505B Microarray Scanner System, extracting data through the Agilent Feature Extraction 9.5 software. All arrays met the minimum Agilent QA/QC standards.

Data analysis

Normalization and differential expression analysis were performed using the R Bioconductor package *limma* [2–4]. Data were $\log_2$ transformed (Fig. 1A) and background subtracted, then a global Loess was performed to normalize each array. A quantile between-array normalization was performed to obtain the same distribution of probe signal intensities across arrays (Fig. 1B). Genes with $\log_2$ fluorescence intensity values (A) <6 were excluded. Arrays quality was also evaluated [5] and obtained array weights were applied to the statistical model, a simple linear model fit and moderated t-statistic, thus obtaining two lists of significant differentially expressed genes in the BPA-treated placentas at the two doses in comparison to controls. A total of 1220 genes were found differently expressed with a p-value < 0.01; in particular 582 were modulated by BPA05 and 701 by BPA50; only 77 genes were shared by the two groups (Fig. 2).

Hierarchical clustering with Euclidean distance and average linkage was performed to discriminate between the two gene expression profiles. Functional analysis of enriched GO terms and KEGG pathways was performed to determine the biological significance of the differentially expressed genes. Transcription factor binding analysis was performed using five different tools to minimize false positives to identify potential gene expression regulators. Interaction networks

with most significant modulated genes and transcription factors were calculated.

Real-time PCR was performed on critical differentially expressed genes to validate microarray data [1].

Discussion

We describe here a data set relative to gene expression profiles of mice placenta samples upon exposure to two different doses of BPA, namely 0.5 or 50 mg/kg bw day, during early gestation (GD1–GD11). The two doses altered two quite distinct patterns of genes which were associated with different effects on placental morphology and development, as supported by histological, histomorphometrical and immunohistochemical analyses as reported in detail in [1].

Acknowledgments

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