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Cell cycle, apoptosis, cell differentiation, and lipid metabolism gene expression in endometriotic tissue and exposure to parabens and benzophenones

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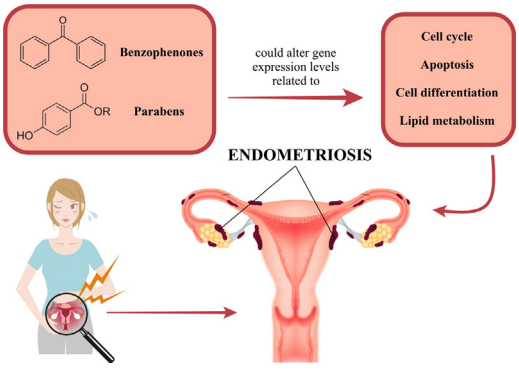
HIGHLIGHTS

- Endometriotic gene expression profiles and urinary PB/BP levels were studied.
- PB exposure was related to overexpression of cell cycle-related genes.
- Increased expression of cell differentiation genes was related to PB exposure.
- Upregulation of lipid metabolism-related genes was related to PB/BP exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Aim: To describe the expression profile in endometriotic tissue of genes involved in four signaling pathways related to the development and progression of endometriosis (cell cycle, apoptosis, cell differentiation and lipid metabolism) and to explore its relationship with the women exposure to chemicals with hormonal activity released from cosmetics and personal care products (PCPs).
Methods: This cross-sectional study, encompassed within the EndEA study, comprised a subsample of 33 women with endometriosis. Expression levels of 13 genes (*BMI1*, *CCNB1*, *CDK1*, *BAX*, *BCL2L1*, *FOXO3*, *SPP1*, *HOXA10*, *PDGFRA*, *SOX2*, *APOE*, *PLCG1* and *PLCG2*) in endometriotic tissue and urinary concentrations of 4 paraben (PB) and 3 benzophenone (BP) congeners were quantified. Bivariate linear and logistic regression analyses were performed to explore the associations between exposure and gene expression levels.

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Results: A total of 8 out 13 genes (61.5 %) were expressed in >75 % of the samples. Exposure to congeners of PBs and/or BPs was associated with the overexpression of *CDK1* gene (whose protein drives cells through G2 phase and mitosis), *HOXA10* and *PDGFRA* genes (whose proteins favor pluripotent cell differentiation to endometrial cells), and *APOE* (whose protein regulates the transport and metabolism of cholesterol, triglycerides and phospholipids in multiple tissues) and *PLCG2* genes (whose protein creates 1D-myo-inositol 1,4,5-trisphosphate and diacylglycerol, two important second messengers).
Conclusions: Our findings suggest that women exposure to cosmetic and PCP-released chemicals might be associated with the promotion of cell cycle and cell differentiation as well as with lipid metabolism disruption in endometriotic tissue, three crucial signaling pathways in the development and progression of endometriosis. However, further studies should be accomplished to confirm these preliminary data.

1. Introduction

Endometriosis is a common gynecological disease that affects 10–15 % of women of childbearing age (Zondervan et al., 2020). It is characterized by the presence of endometrial-like tissue outside the uterine cavity, commonly in the abdomino-pelvic cavity such as the pelvic peritoneum and the ovaries (Bulun, 2009; Giudice, 2010; Vercellini et al., 2014). Although the symptomatic burden varies among endometriosis patients, most of them often experience dysmenorrhea, dyspareunia, chronic pelvic pain, menstrual irregularities and infertility, leading to a significant decrease in their quality of life (Bulun, 2009).

To date, various theories, including retrograde menstruation, coelomic metaplasia, embryonic cell rest, induction, and lymphatic and vascular dissemination, have been proposed to explain the origin of this disease (Nezhat et al., 2008). However, its etiology and pathophysiology have not been fully elucidated. It has been reported aberrations in the regulation of the cell cycle and cell differentiation, such as *CDK1* and *HOXA10* genes that could be involved in the proliferation and malignant transformation, two processes linked to endometriosis (Charrasse et al., 2000; Ito et al., 2005; Tang et al., 2009; Taylor et al., 1998; Taylor et al., 1997; Van Langendonckt et al., 2010). Moreover, a reduced apoptotic index with overexpression of anti-apoptotic and downregulation of pro-apoptotic genes has been shown in endometriotic lesions (Dmowski et al., 2001; Gebel et al., 1998; Harada et al., 2004; Meresman et al., 2000; Nasu et al., 2011). In addition, an alteration of the lipid metabolism signaling pathway, which is closely linked to the inflammatory process, has been also reported in women with endometriosis (Liu et al., 2021; Lo Vasco et al., 2012).

Currently, it is suspected that the interaction of genetic and environmental factors could be crucial in the onset and development of the disease since there is a growing number of women with this estrogen-dependent disease. For that reason, women exposure to chemicals with hormonal activity (so called endocrine-disrupting chemicals, EDCs) has been postulated as potential risk factors for endometriosis (Smarr et al., 2016). In fact, we have previously showed that the magnitude of women exposure to various congeners of parabens (PBs) and benzophenones (BPs) was related to a higher consumption of cosmetics and personal care products (PCPs) and that they could be associated with an increased risk of endometriosis (Peinado et al., 2021). The family of PBs, which includes methyl- (MeP), ethyl- (EtP), propyl- (PrP), and butyl-paraben (BuP) congeners, are alkyl esters of p-hydroxybenzoic acid commonly used in a wide range of PCPs, pharmaceuticals, food, and beverages for their antimicrobial and preservative properties (Błędzka et al., 2014; Darbre and Harvey, 2008; Iribarne-Durán et al., 2020; Moos et al., 2015). The congeners of the BPs, which includes BP-1, BP-3 and 4-OHBP congeners, are frequently added as synthetic UV filters and used in a wide range of cosmetics and PCPs, food packaging materials, and textiles (Molins-Delgado et al., 2016). The main routes of human exposure to PBs and BPs are dermal absorption of PCPs and consumption of pharmaceuticals and foods (Benech-Kieffer et al., 2000; Díaz-Cruz et al., 2012; Janjua et al., 2008; Schlumpf et al., 2010). Both of them are considered EDCs because of their (anti-)estrogenic, (anti-)androgenic and/or (anti-)thyroid actions (Charles and Darbre, 2013; Chen et al., 2007; Darbre and Harvey, 2008; Kerdivel et al., 2013; Molina-Molina et al., 2008). However, the mechanisms of action of these EDCs in the pathophysiology of endometriosis are still unknown.

Therefore, the aim of this study was to describe the expression levels in endometriotic tissue of 13 genes involved in four signaling pathways related with the development and progression of endometriosis (cell cycle, apoptosis, cell differentiation and lipid metabolism), and to explore their potential associations with women exposure to PBs and BPs. Despite the variety of chemicals released from cosmetics and PCPs (including bisphenols, phthalates, camphenes, dimeticones and oxycinnamates, among others), the selection of these two families of chemicals was based on (i) the large evidence on the relationship between cosmetic and PCP consumption - PB/BP exposure - health adverse effects in women, (ii) the previous evidence supporting a relationship between PB/BP exposure and endometriosis risk (Kunisue et al., 2012; Peinado et al., 2021) and (iii) the lack of knowledge on the adverse outcome pathways between PB/BP exposure and endometriosis.

2. Material and methods

2.1. Study population and sample collection

This study is enclosed in a wider research project (EndEA study, **End**ometriosis y **E**xposición **A**mbiental) focused on the elucidation of the contribution of EDCs to endometriosis and their potential mechanisms of action, which has been described elsewhere (Peinado et al., 2020; Peinado et al., 2021). This cross-sectional study was conducted in a subsample of 33 women with confirmed endometriosis by laparotomy or laparoscopic surgery and further histological confirmation, recruited between January 2018 and July 2019 in two public hospitals ('San Cecilio' and 'Virgen de las Nieves') in Granada, Southern Spain. Inclusion criteria were: premenopausal woman aged between 20 and 54 years, receipt of abdominal surgery, and body mass index (BMI) below 35 kg/m^2. Exclusion criteria were: history of cancer (except non-melanoma skin cancer), pregnancy at study enrolment, and inability to read and sign the informed consent document. Cases were categorized in stages I/II and III/IV according to Revised American Fertility Society's classification (Canis et al., 1997). Informed consent was obtained from each participant, and the study was approved by the Research Ethics Committee of Granada.

Before surgery, all participants were clinically examined and anthropometric characteristics were collected, recording their height and weight for calculation of their body mass index (BMI). In addition, epidemiological and clinical questionnaires were completed by the women to collect sociodemographic, lifestyle and clinical information, and surgical questionnaires were completed by the surgeons, gathering data on: age (years), residence (rural or urban/sub-urban), educational level (university degree or less), working outside the home (yes or no), current smoker (yes or no), parity (nulliparous or primiparous/multiparous), average intensity of menstrual bleeding (spotting/light or moderate/heavy), and endometrioma location (deep infiltrating endometriosis or ovarian/peritoneal endometriosis). First morning urine samples were collected the same day immediately before surgery and divided into 1 mL aliquots. Endometriotic tissue samples (3 g) were collected intraoperatively by the surgeon, avoiding any potential risk to the patient's health. Both urine and endometriotic tissue samples

were placed in PB- and BP-free glass tubes, anonymously coded, and immediately stored at −80 °C at the Biobank of the Public Andalusian Healthcare System until further laboratory analyses. Banking of biological samples and completion of questionnaires followed the standardized procedures of the EHPect project (http://endometriosisfoundation.org/ephect/).

2.2. Chemical extraction and analysis

A total of four PB [methyl- (MeP), ethyl- (EtP), propyl- (PrP) and butyl-paraben (BuP)] and 3 BP congeners [benzophenone-1 (BP-1), benzophenone-3 (BP-3), and 4-hydroxybenzophenone (4-OHBP)] were analyzed in urine samples. Chemical extraction of PBs and BPs were carried out through dispersive liquid-liquid microextraction (DLLME), while chemical quantification was performed with a ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS), as previously described (Vela-Soria et al., 2014).

Briefly, samples were centrifuged (2600 x*g* for 10 min) and 1.0 mL sample was used. Then, samples were enzymatically treated with β-glucuronidase/sulfatase, previously prepared by dissolving 10 mg of β-glucuronidase/sulfatase ($3{\cdot}10^6$ U g $solid^{-1}$) in 15 mL of 1 M ammonium acetate/acetic acid buffer solution (pH 5.0), in order to determine the total amount (free and conjugated) of the aforementioned chemicals. After incubation (at 37 °C for 24 h), 20 μL of labeled standards solution (5 mg·L^{-1} of EtP-$^{13}C_6$, 2 mg·L^{-1} of BPA—D_{16}, and 2 mg·L^{-1} of BP-D_{10}) were added and the samples were diluted with 10 mL of 10 % aqueous NaCl solution (pH 2.0, adjusted with 0.5 M HCl). Then, a mixture of 1 mL of acetone (dispersing solvent) and 0.5 mL of trichloromethane (extraction solvent) was added, and samples were shaken manually for 30 s and centrifuged at 4000 x*g* for 10 min. Next, the organic phase was carefully collected from the bottom of the glass tube using a 1 mL pipette and placed in 2 mL glass vials. The extract was evaporated under a nitrogen stream, and the residue was dissolved with 100 μL of an acetonitrile/water mixture and vortexed for 30 s. The extract was then ready for analysis by UHPLC-MS/MS. The limit of detection (LOD) was determined as the minimum detectable amount of analyte with a signal-to-noise ratio ≥ 3. The LODs obtained were 0.05 ng/mL for BP-1, 0.06 ng/mL for BP-3 and 4-OHBP, and 0.10 ng/mL for MeP, EtP, PrP, and BuP.

2.3. RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from 30 mg of endometriotic tissue samples was extracted using QIAzol reagent (Qiagen, Germany) and RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. RNA concentration was determined by spectrophotometer using a NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA) and the A260/A280 ratio was comprised between 1.8 and 2.2 for each sample. Reverse transcription was performed using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California, USA) according to the manufacturer's instructions. For qRT-PCR, cDNA samples, as well as SsoAdvanced Universal SYBR Green Supermix and specific primers (Supplementary Table S1) purchased from Bio-Rad (Bio-Rad Laboratories, Hercules, California, USA) were used. Gene expression levels were detected using a CFX96 RealTime PCR detection system (Bio-Rad Laboratories, Hercules, California, USA), and they were standardized to the levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the $2^{-\Delta\Delta Ct}$ method.

Differential expression levels of 13 genes from 4 different cell signaling pathways was explored: cell cycle [*BMI1* Proto-Oncogene, Polycomb Ring Finger (*BMI1*), Cyclin B1 (*CCNB1*) and Cyclin Dependent Kinase 1 (*CDK1*)], apoptotsis [BCL2-associated X Protein (*BAX*), BCL2 Like 1 (*BCL2L1*), Forkhead Box P3 (*FOXO3*) and Secreted Phosphoprotein 1 (*SPP1*)], cell differentiation [Homeobox *A10* (*HOXA10*), Platelet Derived Growth Factor Receptor Alpha (*PDGFRA*) and SRY-Box Transcription Factor 2 (*SOX2*)], and lipid metabolism [Apolipoprotein E (*APOE*), Phospholipase C Gamma 1 (*PLCG1*) and Phospholipase C Gamma 2 (*PLCG2*)].

2.4. Statistical analysis

Categorical variables were expressed as percentages, while urinary concentrations of individual BP and PB congeners, ΣPBs and ΣBPs, and gene expression levels were expressed as means ± standard deviation and percentiles (25, 50 and 75). Given their non-normal distribution, both exposure and gene expression variables were log-transformed.

Correlations between PB and BP concentrations and gene expression levels were calculated by Spearman's rank correlation coefficient. In addition, bivariate linear regression models were used to explore associations between gene expression levels and PB/BP exposure concentrations. Finally, alternative models were created after exposure categorization into high and low exposure (based on median value of each PB/BP congener), expressing the results as β with 95 % confidence intervals. Those genes expressed in 25–75 % of the samples were considered as dichotomous variables (detected/not detected), and associations with PBs and BPs were evaluated using bivariate logistic regression models. Associations with PB/BPs were not explored for those genes expressed in <25 % of the samples. Moreover, the potential confounding effect of sociodemographic and reproductive characteristics was explored by introducing individual variables in the regression models.

All analyses were performed using SPSS Statistics 23.0 (IBM, Chicago, IL). Associations with *p*-values <0.050 were considered significant, although given the limited sample size, associations yielding p-values between 0.050 and 0.100 were also cautiously discussed The post-hoc analysis to estimate the power (1-β) of the statistical analysis was conducted using G*Power 3.1.9.7 statistical software (Düsseldorf University, Düsseldorf, Germany). Considering the Spearman correlation analyses, the power of the associations found ranged from 65 % to 98 % (ρ coefficients ranging from 0.48 to 0.65; $n = 22$).

3. Results

3.1. Characteristics of the study population and PB and BP concentrations

Sociodemographic and reproductive characteristics of the study population are shown in Table 1. Considering the 33 cases included in this study, the mean (±standard deviation) age was 38.0 (±7.3) years old, and the majority had a normal weight (body mass index <25 Kg/m^2; $n = 20$, 60.6 %). A total of 19 women (57.6 %) lived in rural areas, 24 (72.7 %) hold a job outside home and 12 (36.4 %) had a university degree. A total of 21 women (63.6 %) reported a moderate/heavy menstrual bleeding intensity, 15 (45.5 %) were nulliparous, 25 (75.8 %) had ovarian/peritoneal endometriosis and 21 (63.6 %) were diagnosed with stage I/II endometriosis.

From those 33 women that comprises the entire study population, adequate urine sample volume for exposure assessment was only available from 22 cases, and therefore, associations between exposure and gene expression were accomplished in this subsample. No statistically significant differences in sociodemographic and reproductive characteristics were found between this subset of the population included in the analyzes ($n = 22$) and the subset of the population not included in the analyses ($n = 11$), except for the higher percentage of participants who work outside the home in the latter (Supplementary Table S2). Urinary concentrations of PBs and BPs in endometriosis patients participating in the EndEA study were previously reported (Peinado et al., 2021). Regarding our subsample (n = 22), detectable concentrations of the four PB and the three BP congeners were found in all the analyzed samples, with MeP and BP-3 as the PB and BP congeners found in highest concentrations, respectively (Supplementary Table S3).

3.2. Gene expression levels and associations with PB and BP concentrations

Arithmetic means, standard deviations and percentiles of gene expression levels in endometriotic tissue from the total population are shown in Table 2. A total of 7 genes (53.8 %) were expressed in >75 % of the samples, while 3 (23.1 %) were expressed in 50–75 % of samples. In particular, 2 of 5

Table 1
Characteristics of study population (n = 33).

	n		%		n		%
Sociodemographic characteristics				Educational level			
Age (years)[a]	38.0	±	7.3	Less than university degree	21		63.6
Weight (kg)[a]	67.6	±	14.7	University degree	12		36.4
Height (m)[a]	1.6	±	0.1	Working outside home			
Body mass index (kg/m^2)[a]	25.3	±	5.3	Yes	24		72.7
Normal weight (BMI < 25)	20		60.6	No	9		27.3
Overweight/Obese (BMI > 25)	13		39.4	Current smoker			
Residence				Yes	10		30.3
Rural	19		57.6	No	23		69.7
Urban /sub-urban	14		42.4	Urinary creatinine (ng/mL)[a]	152.3	±	68.3
Reproductive characteristics				Endometrioma location			
Parity				Deep infiltrating endometriosis	8		24.2
Nulliparous	15		45.5	Ovarian/peritoneal endometriosis	25		75.8
Primiparous/Multiparous	18		54.5	Endometriosis stage			
Intensity of menstrual bleeding				I/II	21		63.6
Spotting/light	12		36.4	III/IV	12		36.4
Moderate/heavy	21		63.6				

[a] Mean ± standard deviation

genes related to cell cycle (*BMI* and *CDK1*), 3 of 4 genes related to apoptosis (*BAX*, *BCL2L1* and *FOXO3*), 2 out 3 genes related to cell differentiation (*HOXA10* and *PDGFRA*) and 2 of 3 genes related to lipid metabolism (*APOE* and *PLCG2*) were expressed in >50 % of the samples. Gene expression levels in the subsample of patients with both measurements (exposure and gene expression) (n = 22) are summarized in Supplementary Table S4.

Results from the linear (or logistic) regression analyses carried out to assess the associations between urinary concentrations of PBs and BPs and gene expression levels in endometriotic tissue samples are shown in Tables 3-6. Moreover, results from Spearman correlations analyses are shown in Supplementary Table S5.

Urinary concentrations of MeP, BuP and ΣPBs were associated with increased *CDK1* expression levels (Table 3). These associations were also corroborated after exposure dichotomization, with higher expression levels of *CDK1* in those women with higher exposure to these PB congeners. A close-to-significant positive association was also observed between 4-OHBP and *BMI1* when exposure was dichotomized (p-value = 0.088). No significant associations were observed between PB exposure and *CCNB1* gene expression levels. Similarly, no associations were found between exposure to BPs and selected cell cycle-related genes.

Regarding apoptosis-related genes, no association was observed between any PB/BP congener and *BAX*, *BCL2L1*, *BAX/BCL2L1* ratio nor *FOXO3* when exposure was considered as a continuous variable (Table 4). However, we observed a close-to-statistical association between increased expression levels of *BCL2L1* and higher concentrations of BuP (p-value = 0.095). In relation to *FOXO3*, decreased expression levels were observed in those with higher EtP concentrations, although the association was close to the statistical significance (p-value = 0.097). Moreover, Spearman correlation analyses also revealed a close-to-significance inverse correlation between *FOXO3* expression levels and exposure to both EtP and PrP (p-value = 0.074 and 0.096, respectively) (Supplementary Table S5).

HOXA10 expression levels were increased in those women with higher exposure to MeP and ΣPBs, the latter association being close to the statistical significance (p-value = 0.068) (Table 5). Similarly, *PDGFRA* expression levels were increased in those women with higher concentrations of BuP, while the positive association found with the concentrations of MeP and ΣPBs did not reach the statistical significance (p-values = 0.058 and 0.052, respectively). Finally, no association was observed between PB/BP exposure and *SOX2* expression levels.

A close-to-significant association was found between *APOE* overexpression and 4-OHBP concentrations (p-value = 0.061) (Table 6). A similar but significant association was observed when 4-OHBP exposure was considered as a dichotomous variable. Finally, overexpression of *PLCG2* was found to be associated with PrP concentrations, which was also was corroborated in Spearman correlation analyses (Supplementary Table S5). Moreover, overexpression of *PLCG2* was observed in those women with higher BP-3 concentrations.

Table 2
Gene expression levels in endometriotic tissue.

Cell pathway	Gene	Total cases (n = 33)						
		Expression frequency (n,%)		Mean	St. Dev	Percentiles		
						25	50	75
Cell cycle	BMI1	20	60.6	8.61E+07	1.14E+08	n.e.	7.03E+07	1.22E+08
	CCNB1	9	27.3	1.16E+05	3.02E+05	n.e.	n.e.	9.44E+04
	CDK1	30	90.9	1.66E+08	8.67E+08	7.06E+05	1.97E+06	6.98E+06
Apoptosis	BAX	26	78.8	2.66E+07	2.81E+07	4.78E+05	2.09E+07	4.12E+07
	BCL2L1	29	87.9	2.42E+08	7.47E+08	4.71E+07	1.03E+08	1.89E+08
	BAX/BCL2L1	26	78.8	1.13E+12	6.42E+12	1.38E-02	2.59E-01	3.59E-01
	FOXO3	32	97.0	1.54E+08	4.70E+08	2.44E+07	6.12E+07	1.09E+08
	SPP1	10	30.3	9.65E+06	4.44E+07	n.e.	n.e.	2.69E+04
Cell differentiation	HOXA10	33	100.0	1.49E+08	4.31E+08	2.94E+07	5.67E+07	1.14E+08
	PDGFRA	32	97.0	7.85E+08	3.77E+09	1.13E+07	3.15E+07	1.07E+08
	SOX2	16	48.5	3.23E+06	5.26E+06	n.e.	4.52E+05	5.08E+06
Lipid metabolism	APOE	19	57.6	5.09E+07	1.86E+08	n.e.	1.59E+05	1.29E+06
	PLCG1	1	3.0	3.43E+03	1.91E+04	n.e.	n.e.	n.e.
	PLCG2	25	75.8	1.21E+06	2.44E+06	7.73E+03	1.52E+05	1.49E+06

St. Dev.: standard deviation; BMI1: BMI1 Proto-Oncogene, Polycomb Ring Finger; CCNB1: Cyclin B1; CDK1: Cyclin Dependent Kinase 1; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1; FOXO3: Forkhead Box P3; SPP1: Secreted Phosphoprotein 1; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; SOX2: SRY-Box Transcription Factor 2; ApoE: Apolipoprotein E; PLCG1: Phospholipase C Gamma 1; PLCG2: Phospholipase C Gamma 2; n.e.: not expressed.

Table 3
Association between PB and BP concentrations and expression of genes involved in cell cycle.

	BMI1				CCNB1				CDK1			
	OR	95 % CI		p-Value	OR	95 % CI		p-Value	β	95 % CI		p-Value
Parabens												
MeP	0.73	0.44	1.21	0.223	0.71	0.39	1.30	0.711	***0.63***	***0.26***	***1.00***	***0.002***
<39.26 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>39.26 ng/mL	0.45	0.08	2.67	0.379	0.18	0.02	0.92	0.154	***1.99***	***0.42***	***3.56***	***0.016***
EtP	0.94	9.64	1.32	0.747	0.83	0.53	1.31	0.415	0.23	−0.54	1.00	0.535
<4.51 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>34.51 ng/mL	0.45	0.08	2.67	0.379	1.69	0.22	12.81	0.613	1.17	−0.59	2.92	0.179
PrP	1.01	0.67	1.52	0.974	1.21	0.75	1.95	0.438	0.27	−0.18	0.62	0.263
<2.54 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>2.54 ng/mL	2.22	0.38	13.18	0.379	5.71	0.52	62.66	0.154	0.08	−1.57	1.72	0.925
BuP	1.27	0.69	2.36	0.441	0.52	0.20	1.37	0.183	***0.72***	***0.23***	***1.21***	***0.007***
<0.14 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>0.14 ng/mL	1.33	0.23	7.63	0.746	0.47	0.06	3.57	0.463	***2.31***	***0.85***	***3.77***	***0.004***
ΣPBs	0.74	0.45	1.23	0.250	0.70	0.37	1.32	0.267	***0.67***	***0.30***	***1.05***	***0.001***
<53.31 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>53.31 ng/mL	0.45	0.08	2.67	0.379	0.18	0.02	1.92	0.154	***2.05***	***0.50***	***3.61***	***0.012***
Benzophenones												
BP-1	0.66	0.27	1.64	0.369	1.41	0.51	3.96	0.510	−0.25	−1.12	0.62	0.551
<1.42 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>1.42 ng/mL	0.45	0.08	2.67	0.379	1.69	0.22	12.81	0.613	−0.02	−1.83	1.88	0.980
BP-3	0.76	0.37	1.56	0.453	0.91	0.44	1.88	0.792	0.17	−0.54	0.87	0.627
<2.53 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>2.53 ng/mL	2.22	0.38	13.18	0.379	1.69	0.22	12.81	0.613	0.86	−0.95	2.67	0.330
4-OHBP	1.72	0.54	5.50	0.362	2.05	0.56	7.53	0.278	0.26	−0.85	1.38	0.626
<0.73 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>0.73 ng/mL	5.40	0.78	37.51	0.088	5.71	0.52	62.66	0.154	−0.03	−1.88	1.82	0.740
ΣBPs	0.67	0.25	1.84	0.441	1.16	0.38	3.54	0.796	−0.08	−1.13	0.97	0.877
<7.43 ng/mL	1.00	–	–		1.00	–	–		0.00	–	–	
>7.43 ng/mL	1.00	0.18	5.68	1.000	0.59	0.08	4.50	0.613	−0.39	−2.24	1.46	0.661

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; BMI1: BMI1 Proto-Oncogene, Polycomb Ring Finger; CCNB1: Cyclin B1; CDK1: Cyclin Dependent Kinase 1. Bold and italics: p-value<0.05.

Table 4
Association between PB and BP concentrations and expression of genes involved in apoptosis.

	BAX				BCL2L1				BAX/BCL2				FOXO3			
	β	95 % CI		p-Value	β	95 % CI		p-Value	β	95 % CI		p-Value	β	95 % CI		p-Value
Parabens																
MeP	0.01	−0.21	0.24	0.898	−0.04	−1.07	0.99	0.938	−0.43	−2.45	1.59	0.660	−0.11	−0.40	0.17	0.424
<39.26 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>39.26 ng/mL	0.14	−0.75	1.04	0.739	−1.17	−5.08	2.74	0.539	−3.25	−11.28	4.77	0.403	−0.06	−1.51	1.40	0.938
EtP	0.03	−0.18	0.25	0.752	−0.27	−1.11	0.57	0.505	0.21	−1.75	2.17	0.822	−0.15	−0.42	0.12	0.264
<4.51 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>34.51 ng/mL	0.26	−0.63	1.15	0.543	−1.93	−5.78	1.91	0.305	5.17	−2.57	12.91	0.176	−1.12	−2.47	0.22	0.097
PrP	0.00	−0.21	0.21	0.997	−0.02	−0.97	0.93	0.967	−0.91	−2.74	0.93	0.309	−0.22	−0.50	0.06	0.122
<2.54 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>2.54 ng/mL	−0.18	−1.06	0.69	0.661	1.29	−2.62	5.19	0.499	−2.58	−10.52	5.36	0.501	−0.92	−2.30	0.46	0.179
BuP	0.08	−0.19	0.35	0.541	0.89	−0.34	2.12	0.147	−1.20	−3.61	1.20	0.304	−0.50	−0.57	0.46	0.835
<0.14 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>0.14 ng/mL	0.52	−0.31	1.35	0.200	3.11	−0.59	6.81	0.095	−4.12	−11.82	3.58	0.273	0.21	−1.23	1.66	0.760
ΣPBs	0.03	−0.21	0.27	0.803	−0.07	−1.14	1.00	0.898	−0.48	−2.64	1.67	0.642	−0.14	−0.49	0.21	0.418
<53.31 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>53.31 ng/mL	0.01	−0.89	0.90	0.990	−1.28	−5.19	2.62	0.500	−3.26	−11.28	4.77	0.403	−0.74	−2.15	0.66	0.281
Benzophenones																
BP-1	0.15	−0.24	0.54	0.436	0.33	−1.57	2.24	0.717	1.16	−2.45	4.78	0.505	0.15	−0.54	0.85	0.652
<1.42 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>1.42 ng/mL	2.78	−0.98	6.55	0.137	1.81	−2.04	5.67	0.337	3.32	−4.70	11.34	0.393	0.95	−0.43	2.33	0.166
BP-3	−0.11	−0.46	0.24	0.505	−0.10	−1.64	1.44	0.893	2.37	−0.61	5.35	0.111	−0.13	−0.57	0.31	0.530
<2.53 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>2.53 ng/mL	−0.23	−1.10	0.64	0.585	1.56	−2.32	5.44	0.410	2.98	−4.92	10.88	0.436	0.61	−0.81	2.03	0.383
4-OHBP	0.38	−0.16	0.92	0.153	0.70	−1.7365	3.145	0.553	−2.51	−7.59	2.58	0.312	−0.06	−0.69	0.57	0.833
<0.73 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>0.73 ng/mL	0.49	−0.35	1.33	0.236	2.22	−1.59	6.03	0.237	−4.18	−11.93	3.57	0.269	0.12	−1.33	1.57	0.863
ΣBPs	0.00	−0.51	0.50	0.992	0.33	−1.95	2.61	0.766	3.38	−0.86	7.62	0.111	0.09	−0.69	0.87	0.815
<7.43 ng/mL	0.00	–	–		0.00	–	–		0.00	–	–		0.00	–	–	
>7.43 ng/mL	0.46	−0.40	1.33	0.272	1.69	−2.18	5.56	0.372	3.22	−4.81	11.26	0.407	0.60	−0.82	2.02	0.386

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; BAX: BCL2-associated X Protein; BCL2L1: BCL2 Like 1; FOXO3: Forkhead Box P3; SPP1: Secreted Phosphoprotein 1.

Table 5
Association between PB and BP concentrations and expression of genes involved in cell differentiation.

	HOXA10				PDGFRA				SOX2			
	β	95 % CI		p-Value	β	95 % CI		p-Value	OR	95 % CI		p-Value
Parabens												
MeP	0.13	−0.05	0.30	0.145	0.21	−0.09	0.50	0.159	0.75	0.36	1.57	0.446
<39.26 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>39.26 ng/mL	***0.79***	***0.20***	***1.38***	***0.012***	1.04	−0.04	2.11	0.058	0.43	0.06	2.97	0.391
EtP	0.03	−0.12	0.17	0.720	0.11	−0.14	0.35	0.376	1.02	0.58	1.81	0.936
<4.51 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>34.51 ng/mL	−0.05	−0.75	0.66	0.893	−0.10	−1.30	1.09	0.858	0.22	0.03	1.71	0.148
PrP	−0.02	−0.19	0.14	0.785	0.13	−0.12	0.36	0.287	1.23	0.72	2.13	0.450
<2.54 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>2.54 ng/mL	−0.37	−1.06	0.31	0.268	−0.04	−1.23	1.16	0.948	4.50	0.59	34.61	0.148
BuP	0.08	−0.15	0.31	0.459	***0.51***	***0.12***	***0.91***	***0.014***	0.55	0.25	1.17	0.118
<0.14 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>0.14 ng/mL	0.47	−0.20	1.14	0.160	1.02	−0.31	2.35	0.123	0.67	0.10	4.35	0.672
ΣPBs	0.13	−0.06	0.31	0.163	0.22	−0.08	0.52	0.142	0.76	0.35	1.62	0.471
<53.31 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>53.31 ng/mL	0.60	−0.05	1.24	0.068	1.06	−0.01	2.13	0.052	0.43	0.06	2.97	0.391
Benzophenones												
BP-1	−0.28	−0.73	0.18	0.217	−0.56	−1.42	0.29	0.185	2.02	0.74	5.48	0.168
<1.42 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>1.42 ng/mL	−0.45	−1.41	0.50	0.330	−0.78	−2.57	1.02	0.378	4.50	0.59	34.61	0.148
BP-3	−0.13	−0.39	0.14	0.332	−0.09	−0.60	0.43	0.733	0.89	0.47	1.70	0.725
<2.53 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>2.53 ng/mL	−0.39	−1.08	0.29	0.241	0.06	−1.36	1.47	0.936	1.50	0.23	9.80	0.672
4-OHBP	0.03	−0.40	0.46	0.900	−0.27	−1.13	0.58	0.509	1.30	0.39	4.38	0.667
<0.73 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>0.73 ng/mL	0.00	−0.70	0.71	0.997	−0.39	−1.80	1.01	0.564	1.02	0.16	6.42	0.958
ΣBPs	−0.53	−1.24	0.17	0.126	−0.45	−1.04	0.14	0.129	1.42	0.53	3.80	0.479
<7.43 ng/mL	0.00	–	–		0.00	–	–		1.00	–	–	
>7.43 ng/mL	−0.27	−1.52	0.98	0.658	−0.63	−1.78	0.52	0.265	2.50	0.37	16.89	0.347

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; HOXA10: Homeobox A10; PDGFRA: Platelet Derived Growth Factor Receptor Alpha; SOX2: SRY-Box Transcription Factor 2. Bold and italics: p-value<0.05.

No confounding effect was observed for sociodemographic and reproductive characteristics in associations found, except for educational level and working outside home (Supplementary Table S6).

4. Discussion

To our best knowledge, this is the first study to explore the potential association between exposure to EDCs released from cosmetics and PCPs, and disruption in the expression profiles of genes involved in cell cycle, apoptosis, cell differentiation, and lipid metabolism in the ectopic tissue from women with endometriosis. Most of the genes studied were expressed in the majority of the samples, and exposure to certain PB and BP congeners were related to the overexpression of genes involved in cell cycle promotion, cell differentiation and lipid metabolism disruption.

Cell cycle has been identified as a crucial cell signaling pathway altered in women with endometriosis (Sahraei et al., 2022) with overexpression of cell cycle promoting genes in ectopic tissue (Tang et al., 2009). Recent studies have also related exposure to PBs/BPs with deregulation of a variety of cell cycle genes in *in vivo* models of gynecological disorders such as ovarian cancer (Park et al., 2013) and altered steroidogenesis and antral follicle growth (Gal et al., 2019). In line with these previous studies, we have observed that *CDK1* gene overexpression was associated with exposure to PBs. Lee et al. (2014) also reported a positive association between exposure to bisphenol A (BPA), another estrogenic EDC, and *CDK1* expression levels in breast cancer cell lines. *CDK1* is a crucial gene for cell cycle regulation, orchestrating the G2 to M transition (Jin et al., 1998) and has been reported to be involved in the development of endometriosis (Chen et al., 2021; Tang et al., 2009). Moreover, we have also detected a close-to-significant overexpression of *BMI1* in those women with higher exposure to 4-OHBP. *BMI1* is another key gene involved in cell cycle regulation, which is negatively regulated by p16 and p19 tumor suppressor proteins (Bracken et al., 2007; Bruggeman et al., 2005; Jacobs et al., 1999; Molofsky et al., 2005). Although the role of *BMI1* in the pathophysiology of endometriosis is still unknown, increased *BMI1* levels have been detected in endometriotic tissues (Forte et al., 2009; Ponandai-Srinivasan et al., 2020). In line with these previous findings, our results suggest that exposure to 4-OHBP might contribute to the upregulation of *BMI1* gene, deregulating the cell cycle and, thus, favoring the proliferation of endometriosis. A recent study also described the disruptive potential of BPA in *BMI1* gene regulation (Li et al., 2021).

Cell differentiation is another important process in the endometriosis pathophysiology, with some theories suggesting that the origin of this disease might be the result of the differentiation of various cell types into endometrial-like cells (Klemmt and Starzinski-Powitz, 2018). Several genes could be involved in this process, including the *HOXA10* gene. *HOXA10* gene has been related to the differentiation of embryonic tissues to endometrial tissue and it was shown to be abnormally expressed in different types of endometriotic lesions (Browne and Taylor, 2006; Van Langendonckt et al., 2010; Zanatta et al., 2015). The results of our study showed increased expression in endometriotic tissues from those women with higher concentrations of MeP. *PDGFRA* gene, that encodes a protein that participates in the activation of a series of proteins involved in a variety of signaling pathways, such as multipotent stem cell differentiation as well as cell growth, proliferation and survival (Bartoletti et al., 2020), is also overexpressed in ectopic tissues in comparison with eutopic endometrium (Matsuzaki et al., 2006). Our findings showed a positive association between exposure to BuP and *PDGFRA* and close-to-statistical associations with exposure to MeP and ΣPBs. Thus, taken together, these findings might support the hypothesis of a potential role of exposure to cosmetic- and PCP-released EDCs favoring differentiation of multiponent stem cells into endometriotic cells.

Alterations in lipid metabolism have also been related to the onset and progression of endometriosis (Lu et al., 2023), identifying the deregulation of several lipids such as phosphatidylcholines, sphingomyelins, phosphatidylethanolamines, and triglycerides (Adamyan et al., 2018; Dutta et al.,

Table 6
Association between PB and BP concentrations and expression of genes involved in lipid metabolism.

	APOE				PLCG2			
	OR	95 % CI		p-Value	β	95 % CI		p-Value
Parabens								
MeP	0.90	0.57	1.42	0.652	0.02	−0.62	0.66	0.942
<39.26 ng/mL	1.00	–	–		0.00	–	–	
>39.26 ng/mL	0.31	0.05	1.85	0.200	0.72	−1.33	2.78	0.462
EtP	1.01	0.69	1.47	0.967	−0.05	0.54	0.44	0.826
<4.51 ng/mL	1.00	–	–		0.00	–	–	
>34.51 ng/mL	1.46	0.26	8.05	0.665	−0.50	−2.57	1.58	0.617
PrP	1.09	0.73	1.63	0.682	***0.50***	***0.02***	***0.99***	***0.043***
<2.54 ng/mL	1.00	–	–		0.00	–	–	
>2.54 ng/mL	3.20	0.54	18.98	0.200	1.73	−0.12	3.58	0.064
BuP	0.79	0.45	1.392	0.412	0.59	−0.28	1.47	0.167
<0.14 ng/mL	1.00	–	–		0.00	–	–	
>0.14 ng/mL	0.93	0.17	5.15	0.937	1.07	−0.94	3.07	0.272
ΣPBs	0.89	0.55	0.42	0.612	0.05	−0.60	0.70	0.872
<53.31 ng/mL	1.00	–	–		0.00	–	–	
>53.31 ng/mL	0.69	0.12	3.78	0.665	1.29	−0.69	3.27	0.185
Benzophenones								
BP-1	1.63	0.67	3.97	0.283	0.20	−0.77	1.17	0.667
<1.42 ng/mL	1.00	–	–		0.00	–	–	
>1.42 ng/mL	1.46	0.26	8.05	0.665	0.08	−2.03	2.19	0.935
BP-3	1.05	0.56	1.97	0.887	0.20	−0.47	0.87	0.538
<2.53 ng/mL	1.00	–	–		0.00	–	–	
>2.53 ng/mL	3.20	0.54	18.98	0.200	***1.93***	***0.14***	***3.73***	***0.037***
4-OHBP	4.13	0.94	18.21	0.061	0.28	−0.87	1.43	0.613
<0.73 ng/mL	1.00	–	–		0.00	–	–	
>0.73 ng/mL	***7.88***	***1.11***	***56.12***	***0.039***	0.15	−1.95	2.24	0.884
ΣBPs	1.55	0.59	4.11	0.378	0.31	−0.71	1.32	0.530
<7.43 ng/mL	1.00	–	–		0.00	–	–	
>7.43 ng/mL	3.20	0.54	28.98	0.200	0.68	−1.37	2.74	0.487

CI: confidence intervals; MeP: methylparaben; EtP: ethylparaben; PrP: propylparaben; BuP: buthylparaben; PB: paraben; BP-1: benzophenone-1; BP-3: benzophenone-3; 4-OHBP: 4-hydroxibenzophenone; BP: benzophenone; APOE: Apolipoprotein E; PLCG2: Phospholipase C Gamma 2. Bold and italics: p-value<0.05.

2016; Ortiz et al., 2021). In fact, some of these deregulated lipids have proposed as potential biomarkers for early diagnosis of endometriosis (Li et al., 2018). In this sense, ApoE protein, that plays an important role in lipid metabolism (Chen et al., 2005) have been found to be highly expressed in the follicular fluid of patients with endometriosis in comparison with controls without endometriosis (Liu et al., 2021). The results of our study showed increased odds of *APOE* gene expression in endometriotic tissue in women with higher concentrations of 4-OHBP. Similarly, we have also observed that women exposure to PrP and BP-3 was associated with increased expression levels of the *PLCG2* gene, which encodes a crucial enzyme in the phosphoinositide signal transduction system. This is in agreement with previous studies that demonstrated that *PLCG2* was upregulated in endometrial cancer cell lines in response to exposure other EDCs used in cosmetics such as phthalates (Song and Cho, 2014). Therefore, our findings suggest that exposure to different congers of PBs and BPs might deregulate lipid metabolism in endometriotic lesions (Lu et al., 2023).

Apoptosis, *i.e.* the efficient elimination of cells from tissue without eliciting an inflammatory response (Kerr et al., 1972), is another physiological process that has been found to be deregulated in endometriotic tissues (Béliard et al., 2004). Hence, various genes involved in apoptosis have been shown to be deregulated in endometriotic tissues, with a decreased expression of the pro-apoptotic genes *BAX* (Meresman et al., 2000), and an overexpression of the anti-apoptotic gene *BCL2L1* (Meresman et al., 2000). Despite a variety of apoptosis-related genes have been explored in this study, only few close-to-significant associations was detected between exposure to BuP and overexpression of *BCL2L1* anti-apoptotic gene and lower expression of the pro-apoptotic *FOXO3* gene suggesting that apoptosis might not be crucially deregulated by women exposure to these chemicals.

The findings of this study should be taken with caution because it has some limitations: firstly, the sample size was relatively small that could decrease the statistical power of explored associations. However, it is worth to mention that it is highly challenging to gather endometriotic tissue samples for investigation. Secondly, the fact that undergoing elective surgery for endometriosis was considered one of the inclusion criteria might have introduced a selection bias. Therefore, despite our study population included 63.6 % of women with stage I/II endometriosis and 37.4 % with stage III/IV endometriosis, the extrapolation of these study findings should be made with caution. Moreover, the assessment of exposure through a spot urine specimen prevented consideration of the variability in daily exposure to analytes with a relatively short elimination half-life. Nevertheless, samples were all first-morning urine samples, evidenced to be representative of the exposure (Deng et al., 2023), taken during hospitalization before endometriosis surgery. In addition, only two families of EDCs were measured, with no evaluation of the combined effect of PBs and BPs alongside other EDCs. This is of relevance, given the increasing interest in the mixed impact of multiple EDC exposures on human health. Regarding outcome assessment, the random bias that might be attributable to the selected genes was reduced, at least in part, using a wide panel of genes from four different cell signaling pathways. Finally, the epidemiological findings of this study were not corroborated through *in vivo* studies. This study have some strengths that should be also considered such as the measurement of numerous genes involved in cellular pathways related to the pathophysiology of endometriosis in endometriotic tissue. Moreover, this study has identified plausible and consistent associations between gene expression and exposure to certain PB/BP congeners. The combined investigation of biomarkers of exposure and potential biomarkers of effect yielded evidence of different pathways for adverse outcomes in endometriosis.

Taken together, our findings suggest that women exposure to cosmetic- and PCP-released EDCs (including PBs and BPs) might be associated with altered gene expression profiles related to the cell cycle, cell differentiation and lipid metabolism in endometriotic tissues. Given the novelty of these results, further studies with larger sample sizes and *in vivo* corroboration of findings are warranted to shed some light on the impact of human exposure to EDCs on the pathophysiology of endometriosis.

CRediT authorship contribution statement

F.M. Peinado: Investigation, Formal analysis, Writing – original draft. **A. Olivas-Martínez:** Investigation, Formal analysis. **L.M. Iribarne-Durán:** Investigation. **A. Ubiña:** Resources, Writing – review & editing. **J. León:** Investigation, Writing – review & editing. **F. Vela-Soria:** Investigation. **J. Fernández-Parra:** Resources, Writing – review & editing. **M.F. Fernández:** Writing – review & editing. **N. Olea:** Writing – review & editing. **C. Freire:** Investigation, Writing – review & editing. **O. Ocón-Hernández:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **F. Artacho-Cordón:** Conceptualization, Methodology, Supervision, Formal analysis, Funding acquisition, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

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