CLINICAL STUDY

‘Idiopathic’ partial androgen insensitivity syndrome in 28 newborn and infant males: impact of prenatal exposure to environmental endocrine disruptor chemicals?

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Abstract

Objective: 46,XY disorders of sex differentiation (46,XY DSD) can be due to a testis determination defect, an androgen biosynthesis defect, or androgen resistance (complete or partial androgen insensitivity syndrome (PAIS), or 5 α reductase deficiency). We aimed to evaluate the impact of a prenatal contamination by environmental xenoestrogens in ‘idiopathic’ PAIS-like phenotype.

Subjects: We investigated 28 newborn/infant males with 46,XY DSD, normal androgen production, and no androgen receptor or steroid-5 α R type II enzyme (*SRD5A2*) gene mutations.

Methods: To exclude other genetic defects, we sequenced the steroidogenic factor 1 (SF1) and mastermind-like domain-containing 1 (*MAMLD1*) genes, which were recently found to be associated with the PAIS-like phenotype. Parents were interviewed about their environmental/occupational exposure to endocrine disrupting chemicals (EDCs) before/during the patients’ fetal life. Total estrogenic bioactivity of patient serum was analyzed by ultrasensitive bioassay.

Results: All the patients had normal SF1 sequence and one patient showed a double polymorphism of *MAMLD1*. Eleven (39.3%) of the 28 patients had reported parental fetal exposure to EDCs. The mean estrogenic bioactivity in these 11 patients with fetal EDC exposure (6.65 ± 8.07 pg/ml) versus 17 cases without contamination (1.27 ± 0.34 pg/ml) and controls (1.06 ± 0.44 pg/ml; $P < 0.05$) was elevated.

Conclusions: Our results indicate that the ‘idiopathic’ PAIS-like phenotype may in some cases be related to EDC contamination during fetal life.

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Introduction

Differentiation of the male external genitalia requires normal androgen production and adequate response of target tissues during fetal life (1). Male external genital malformation is related to either insufficient testosterone production by fetal testis or partial androgen resistance of target organs (2–4). The etiology of undervirilization in males includes gonadal dysgenesis, defects in androgen biosynthesis, and, most frequently, androgen resistance (1).

All patients with undermasculinization should be first investigated for the basal or post-human chorionic gonadotropin (hCG) test level of plasma testosterone: a low plasma testosterone level tends to indicate testicular dysgenesis or testosterone biosynthesis defects; conversely, normal/high plasma testosterone orients toward the diagnosis of partial androgen insensitivity syndrome (PAIS) (1, 4–6).

In our experience, the molecular diagnosis of PAIS is confirmed by androgen receptor (AR) gene abnormality in 85–90% of familial cases and only 10–15% of sporadic cases (5), although Ahmed *et al.* (7) reported a positive mutational analysis of the AR gene in 28% of all cases of PAIS (familial+sporadic cases). In addition, the clinical picture is not specific to the AR gene, and other gene alterations may induce undervirilization in XY newborns despite normal androgen secretion (8). For example, steroid-5 α R type II enzyme (*SRD5A2*) deficiency may give rise to the typical clinical picture of PAIS (8). The *SRD5A2* defect is not usually considered, however, because many pediatric endocrinologists assume that it is limited to only certain ethnic groups (Dominican and New Guinean), despite a recent study demonstrating that *SRD5A2* gene analysis should be performed when no AR mutation is identified (8). In addition, steroidogenic factor 1 (*SF1*) or mastermind-like domain-containing 1 (*MAMLD1*) gene abnormalities

have been detected in rare cases of 46,XY disorders of sex differentiation (46,XY DSD) with normal testosterone production.

Unfortunately, a defect in testosterone action cannot be identified in the majority of undervirilized patients with normal androgen secretion (5–7), leading to the diagnosis of ‘idiopathic’ PAIS-like phenotype (1). In these cases, other possible mechanisms should be investigated. It has been demonstrated in animal models and humans that exogenous chemicals with estrogenic and anti-androgenic activities can disturb the androgen/estrogen balance in the developing male fetus and thus impact external genital differentiation (9–11). In addition, most environmental pollutants are known to exhibit both estrogenic and anti-androgenic activities on stable human cell lines expressing the estrogen receptors α (ER α or ESR1) and β (ER β or ESR2), or the AR (12). These chemicals with endocrine disrupting properties are often industrial and agricultural by- and end-products, and they are now referred to as endocrine disrupting chemicals (EDCs). Several authors have documented an increasing trend in male external genital malformations in animals and humans over the last several decades and have focused on these EDCs as suspected causes (9–11, 13–18).

In this work, we present arguments for the potential role of EDCs in the pathophysiology of ‘idiopathic’ PAIS-like phenotype through investigation of prenatal contamination by EDCs in relation to parental environmental/occupational exposure. In addition, since environmental pollutants are known for their estrogenic activity and can be released progressively from the adipose tissue where they accumulate (10, 11), we assumed that the total estrogenic bioactivity would serve as a good marker for fetal contamination by EDCs. We thus measured it in the patients’ serum with an ultrasensitive bioassay that we developed.

Patients and methods

Participants

Forty-seven males from the neonatal period to 1 year of age with nonsyndromic 46,XY DSD were included in this study. All cases were referred to our Pediatric Endocrine Clinic at the University Hospital of Montpellier (France). The study was approved by the Ethics Committee of the University Hospital of Montpellier, and all participating parents gave written informed consent to all hormonal (included total estrogenic bioactivity) and genetic investigations.

All patients were French with Mediterranean origin (France, Italian, Spanish, Portuguese, or North African) and none presented familial clustering of male external genital malformations, defined as the presence of these malformations in other probands and first-degree relatives. All 47 newborns and infants with undervirilization were oriented toward male gender. Figure 1 shows the distribution of the 47 patients on the basis of endocrine and molecular analysis. In our group, 16 patients presented low basal plasma testosterone and an insufficient response to hCG stimulation. Accordingly, the diagnosis of testicular dysgenesis was raised and confirmed in six cases by the identification of *SF1*, *WT1*, or *SOX9* gene mutations (6/16 cases). The remaining 31 newborns/infants with normal/high plasma testosterone production were diagnosed as PAIS and further investigated for AR and *SRD5A2* gene mutations. We identified three defects in the AR gene (3/31 cases), while no *SRD5A2* gene abnormality was detected. Twenty-eight newborns/infants from this group of 31 PAIS patients were thus considered as presenting an ‘idiopathic’ PAIS-like phenotype and were further investigated in this study.

Figure 1 Distribution of the 47 undervirilized newborns/infants on the basis of endocrine and molecular analysis.

Clinical examination

Pediatric endocrinologists examined the infants in warm conditions (room temperature 20–24 °C) with the infants in supine position. Testicular position was recorded after manipulation of the testis to the most distal position along the pathway of normal descent using firm traction. Patients were diagnosed as cryptorchid if one or both testes were nonpalpable or if they could not be manipulated to a stable position at the bottom of the scrotum (19). Hypospadias was defined as a displacement of the urethral meatus from the tip of the glans penis to the ventral side of the phallus, scrotum, or perineum (20–24). In particular, hypospadias was graded as anterior (glandular and subcoronal locations), medium (distal penile and midshaft), or posterior (penoscrotal and perineal). Micropenis was diagnosed for an anatomically correct penis that was abnormally short (25, 26). Stretched penile length is the most valid measurement and was obtained with the standard method, by placing a ruler against the dorsum of the stretched penis and measuring the distance between the tip of the glans and the pubic symphysis while depressing the supra-pubic fat pad as completely as possible (2). Although -2.5 s.d. has been used as the lower limit of normal penile length for some (2), -2 s.d. is regarded as the lower limit of normal variation for most quantitative traits (27–29) and was thus the definition of micropenis used in this work. According to the standards for mean penile size (27, 30–32), a stretched penile length of <25 mm was thus considered as micropenis.

Data collection

Information on potential risk factors for male genital malformations was obtained from a detailed structured interview with the parents during their visit to the Pediatric Endocrine Clinic. Particular emphasis was placed on the familial environment and parental occupational activities with potential exposure to EDCs. This questionnaire was used in a European Community prospective multi-center cohort study driven by five European countries (Denmark, UK, Finland, Spain, and France) in order to explore the association between fetal exposure to EDCs and male external genital malformations.

Regarding the environment, we gathered information on the habitats of the family units before and during the pregnancies (city or country), since the agricultural area around Montpellier is characterized by extensive cereal, fruit, grape, and rice production with a consequently high 'pesticide impact'. Moreover, residences in proximity to certain settings (i.e. incinerators, water purification stations, and gas stations) may carry an elevated risk of inadvertent contamination by EDCs (33) and thus this type of information was also carefully sought.

Occupational exposure to environmental pollutants was assessed from the answers to questions on paid employment and jobs that entail the use of pesticides and other EDCs during fertilization for the fathers and before/during patients' pregnancy for the mothers. This concerned farmers, wine and rice growers, tree and fruit farmers, gardeners and florists, as well as commercial painters, workers handling cleaning agents, garbage collectors, shoemakers, chemists, welders, and hairdressers. Parents without a job were considered as having no occupational exposure. Questionnaires were completed during the interviews for 100% of the parents of the 28 patients with an 'idiopathic' PAIS-like phenotype.

Laboratory analysis

The testosterone level was measured by RIA using commercially available reagents from Immunotech (Beckman Coulter, Marseille, France) at baseline and 24 h after the seventh injection of 1500 UI of hCG (one every 2 days). The estradiol level (E_2) was measured by immunosorbent assay using commercially available reagents from bioMérieux (Marcy l'Etoile, France).

Gene sequence analysis

DNA was extracted from peripheral blood lymphocytes using the QIAamp DNA Blood Mini Kit (Qiagen).

After PCR amplification of the *SF1* gene, we performed direct sequencing as described previously (34). Direct sequencing of *MAMLD1* coding exons and their flanking splice sites was performed with the 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using primers as described previously (21). Sequencing reactions were repeated twice with at least two different PCR products. The DNA sequences were compared with the sequences of normal controls and the reference genome from the ensembl.org database (Ensembl: ENSG00000013619) and the Genbank database (MIM. 300120, NCBI Gene ID: 10046).

Evaluation of estrogenic bioactivity

We used a recombinant cell bioassay that we developed (35) for ultrasensitive determination of total serum estrogenic bioactivity in the 11 patients whose parents reported fetal exposure to EDCs. Briefly, this assay is based on human uterine cervix carcinoma cells, HeLa cells, which do not naturally express ER. These cells were stably transfected with plasmids encoding the human ER α along with an estrogen-responsive promoter fused to the luciferase gene, and they were called HeLa estrogen-responsive element luciferase neomycin α (HELN α). HELN α are able to respond to estrogens and various compounds having estrogenic activity. Estrogenic bioactivity was evaluated in

triplicate, by incubation of HELN α cells with the children's serum as described previously (35). The control group ($n=15$), first presented in an earlier work (36), composed of patients matched for age (between birth and 1 year) referred to our clinic for infectious diseases. Information on familial environment and parental occupational activities with potential exposure to EDCs was obtained from the same detailed structured interview that had been administered to the parents of our patients with PAIS-like phenotype. These were newborn/infant males living in downtown Montpellier, thus not considered to be at risk of pesticide contamination. In addition, their parents reported no environmental/occupational exposure to EDCs before/during their child's fetal life. No control presented male genital malformation, nor familial clustering of these abnormalities. Informed consent was obtained from all families.

Statistical analysis

Data are expressed as the arithmetic mean $\pm$ s.d., unless otherwise stated. The Student's *t*-test was used to compare groups and variables. A *P* value <0.05 was considered to be significant.

Results

Among the 28 newborns/infants with 'idiopathic' PAIS-like phenotype, 11 (39.3%) had parents who reported environmental/occupational exposure to EDCs, while the parents of the remaining 17 (60.7%) reported no exposure. Table 1 shows the clinical, endocrine, and genetic findings regarding these 11 male newborns and infants with 'idiopathic' PAIS-like

phenotype and reported EDC exposure. In particular, two of these patients (1, 2) presented cryptorchidism, posterior hypospadias, and micropenis, while four cases (3, 4, 6, 8) showed cryptorchidism and micropenis, four (7, 9, 10, 11) presented anterior or posterior hypospadias and micropenis, and case 5 showed only micropenis (Table 1). All patients thus presented severe micropenis, and the mean stretched penile length was 16.20 ± 5.77 mm.

Endocrine investigation of these 11 patients showed normal testosterone production at all ages, since the mean basal testosterone was 0.50 ± 0.56 ng/ml and the mean testosterone after hCG stimulation (1500 U/2 days $\times 7$) was 5.71 ± 1.86 ng/ml (Table 1). In the patients up to 3 months, the mean basal testosterone was 0.80 ± 0.62 ng/ml and the mean testosterone after hCG stimulation was 6.43 ± 1.87 ng/ml, while in the patients between 4 and 12 months the mean basal and post-hCG stimulation testosterone were 0.13 ± 0.03 and 4.85 ± 1.62 ng/ml respectively. The molecular analysis of the AR gene showed no mutations, while the SRD5A2 gene sequence identified the V89L single nucleotide polymorphism in four patients (3, 5, 7, 10) and the A49T single nucleotide polymorphism in case 4 (Table 1). This normal testosterone production and the absence of AR and SRD5A2 gene mutations were the major criteria for the diagnosis of 'idiopathic' PAIS-like phenotype.

The SF1 sequence was normal in all patients, while one double polymorphism of MAMLD1 (P286S/htz+N589S/htz) was identified in case 6 (Table 1).

We documented the parents' environmental/occupational exposure to EDCs before/during patients' fetal life (Table 1). In particular, all parents reported environmental exposure to EDCs before/during patients' fetal life, since the family units lived over a gas station

Table 1 Clinical, endocrine, and molecular evaluations of 11 newborns/infants with 'idiopathic' PAIS-like phenotype and reported fetal exposure to EDCs.

Patients	Age (months)	Cryptorchidism	Hypospadias	Micropenis (mm)	Testosterone basal/post-hCG (ng/ml)	Sequence		Family units' environment before/during PFL	Mothers' occupation before/during PFL	Fathers' occupation during fertilization	EB (pg/ml)
						SRD5A2	MAMLD1				
1	1.5	B	P	7	1.2/9.3	N	N	Gas station	Gas station attendant	Driver	2.70
2	0.1	B	P	15	0.64/4.10	N	N	Water purification station	Pharmacist	Teacher	3.26
3	7.1	L	No	20	0.10/5.36	N+V89L htz	N	Countryside	Secretary	Farmer	16.40
4	6.3	R	No	16	0.13/3.22	N+V89L htz	N	Countryside	Unemployed	Wine grower	5.40
5	6.7	No	No	20	0.16/3.80	N+A49T htz	N	Countryside	Cook	Wine grower	0.80
6	2.1	R	No	15	1.24/5.46	N	P286S/htz+N589S/htz	Countryside	Teacher	Commercial painter	27.00
7	2.5	No	P	10	0.10/5.70	N+V89L htz	N	Countryside	Tailor	Farmer	3.80
8	2.2	L	No	10	0.10/6.10	N	N	Countryside	Unemployed	Commercial painter	4.00
9	1.1	No	P	25	1.54/7.90	N	N	Countryside	Secretary	Farmer	1.67
10	7.3	No	A	20	0.16/7.38	N+V89L htz	N	Countryside	Pharmacist	Agronomist	7.60
11	9.8	No	P	20	0.10/4.50	N	N	Countryside	Unemployed	Farmer	0.50

N, normal; P, posterior; A, anterior; B, bilateral; L, left; R, right; PFL, patients' fetal life; EB, estrogenic bioactivity. AR and SF1 sequences are normal for all patients. Plasma estradiol measured for all patients was < 9 pg/ml except for patient 10 with 11 pg/ml.

(case 1), near (<100 m) a water purification station (case 2), or in the countryside near Montpellier, where extensive agriculture implies high 'pesticide impact' (Table 1). In addition, fathers of cases 3–11 also reported occupational exposure to EDCs, since before fertilization they had worked at jobs carrying a risk of contamination, while only one mother (case 1) had occupational exposure to EDCs (gas station attendant) before/during pregnancy (Table 1).

Table 1 shows the estrogenic bioactivity in these 11 patients. The mean was elevated (6.7 ± 8.1 pg/ml, range 0.50–27.00 pg/ml) versus the 17 'idiopathic' PAIS-like phenotype newborns/infants without fetal EDC exposure (1.27 ± 0.34 pg/ml, range 0.56–2.15 pg/ml; $P < 0.05$), as well as controls matched for sex and age (i.e. between birth and 1 year; 1.1 ± 0.4 pg/ml, range 0.54–2.01 pg/ml; $P < 0.05$; Fig. 2). Nine of them (81.82%) presented increased estrogenic bioactivity (Table 1, Fig. 2). Please note that in most newborns/infants, the plasma E_2 was lower than the limit of detection of the assay (<9 pg/ml; Table 1). Table 2 presents the clinical, endocrine, and molecular data of the 17 infants with 'idiopathic' PAIS-like phenotype and none reported fetal exposure to EDCs. The plasma E_2 measured in these 17 newborns/infants with 'idiopathic' PAIS-like phenotype and no reported fetal EDC exposure was lower than the limit of detection of the assay (<9 pg/ml) and their estrogenic bioactivity was not elevated (1.27 ± 0.34 pg/ml, range 0.56–2.15 pg/ml) versus controls matched for sex and age (i.e. between birth and 1 year; 1.1 ± 0.4 pg/ml, range 0.54–2.01 pg/ml; $P > 0.05$). In particular, only two of them (11.76%) presented slightly increased estrogenic bioactivity (Fig. 2).

Discussion

The diagnosis of external genital malformations in 46,XY newborns is of major importance, and several investigations will be required to identify the cause and orient subsequent management. Unfortunately, the etiological cause of 46,XY DSD cannot be identified in many cases (5–7). In this work, we investigated 28 newborns and infants with undervirilization and normal androgen production to identify potential etiological factors.

In order to exclude other molecular defects, we explored the most recent candidate genes associated with a PAIS-like phenotype, *SF1* and *MAMLD1*. In particular, recent observations by Coutant *et al.* (37), as well as our own experience (38), suggest that an *SF1* gene mutation may be associated in newborn/infant males with PAIS-like phenotype. In addition, we recently identified two mutations of *MAMLD1* responsible for severe hypospadias and micropenis in male children with PAIS-like phenotype (39). Accordingly, we investigated the 28 patients with PAIS-like phenotype for *SF1* and *MAMLD1* gene mutations, but none was identified.

Figure 2 Estrogenic bioactivity (pg/ml) in the 28 children with 'idiopathic' PAIS-like phenotype who presented no genetic abnormality, but 11 were known to have fetal exposure to EDCs versus 17 without fetal exposure to EDCs and 15 controls matched for age (36). Each point corresponds to the value of estrogenic bioactivity obtained in PAIS-like phenotype children with or without fetal exposure to EDCs as well as in controls; the dashed line represents the mean value.

Several epidemiological studies have documented a deterioration in male reproductive health in the last few decades (13, 14, 16, 40–47), and a similar phenomenon has been reported in numerous wildlife species (48–52). In addition, the evidence from wildlife observations, registers of genital malformations and diethylstilbestrol (DES)-exposed human male fetuses, and experimental data from animals and cells all have increased the suspicion that fetal exposure to EDCs could adversely affect fetal male sex differentiation (9–11, 15, 23, 49–53).

Table 2 Clinical, endocrine, and molecular evaluations of 17 newborns/infants with 'idiopathic' PAIS-like phenotype and no reported fetal exposure to EDCs.

Patients	Age (months)	Cryptorchidism	Hypospadias	Micropenis (mm)	Testosterone basal/post-hCG (ng/ml)	Mothers' occupation before/during PFL	Fathers' occupation during fertilization	EB (pg/ml)
1	0.3	B	P	17	1.60/3.80	Secretary	Driver	1.12
2	0.4	No	M	15	0.60/4.21	Doctor	Doctor	0.56
3	3.1	L	No	20	0.92/4.13	Unemployed	Policeman	1.28
4	5.4	R	A	16	0.65/3.34	Teacher	Teacher	1.37
5	4.2	No	A	22	0.52/3.96	Secretary	Cook	0.91
6	6.8	R	No	12	0.44/3.10	Student	Unemployed	1.41
7	3.7	No	M	11	0.90/5.10	Nurse	Doctor	1.09
8	2.2	L	No	8	1.11/3.94	Pharmacist	Pharmacist	0.93
9	1.8	No	P	23	0.71/4.44	Secretary	Butcher	1.35
10	0.4	R	A	11	0.65/4.32	Nurse	Engineer	1.70
11	0.9	L	M	13	0.45/5.10	Tailor	Teacher	1.36
12	2.5	No	P	9	0.90/3.34	Fishwife	Policeman	1.35
13	1.7	B	A	10	0.70/4.12	Cook	Cook	2.15
14	6.6	L	No	21	0.42/4.10	Student	Nurse	1.25
15	4.5	R	A	17	0.71/3.12	Unemployed	Butcher	1.22
16	4.8	No	No	13	0.91/3.60	Unemployed	Driver	1.39
17	7.9	B	P	25	0.41/4.50	Secretary	Engineer	1.19

P, posterior; M, medium; A, anterior; B, bilateral; L, left; R, right; PFL, patients' fetal life; EB, estrogenic bioactivity. AR, SRD5A2, SF1 and MAMLD1 sequences are normal for all patients. For all patients Downtown was the Family units' environment before/during patients' fetal life. Plasma estradiol measured for all patients was < 9 pg/ml.

In particular, some studies in the literature have dealt with the association between male genital malformations and parents' environmental/occupational exposure to EDCs (54–66). The reported results are not totally conclusive and cannot always be compared, since the newborns were sometimes explored only for cryptorchidism or hypospadias and sometimes for external genital malformations in general (58). Nevertheless, in most of them, a significantly increased risk of genital malformations was found to be associated with parental exposure to EDCs (58–66).

This study is original because it deals with both environmental and occupational exposure of family units to EDCs before or during patients' fetal life. In addition, to our knowledge, there is no report dealing with the association of micropenis and prenatal EDC contamination, whereas in experimental animals (67, 68), as well as in our recent clinical experience, this is the malformation most likely to be associated with such exposure. The medical histories actually indicated that within this group of infants with an 'idiopathic' PAIS-like phenotype, 11 newborns/infants – that is, nearly 40% – had parents who reported environmental or occupational exposure to EDCs before/during the patients' fetal life and all patients presented severe forms of micropenis (16.20 ± 5.77 mm; Fig. 1). In particular, it is likely that all patients could have been exposed to EDCs during fetal life, since the mothers reported living before and during pregnancy in the country regions surrounding Montpellier, known to have a high pesticide impact, as well as in proximity to settings at risk of contamination, such as gas or water purification stations. In addition, 9/11 fathers reported occupational exposure to EDCs, which is an additional risk factor for male genital malformation in offspring

(61, 64). Although an epigenetic mechanism is likely to be involved in this father–son transmission, the exact mechanisms remain to be elucidated.

Unfortunately, the study of chemical exposure during fetal sexual differentiation is extremely difficult: it is impossible for both ethical and technical reasons to biopsy fetal tissue at the time of toxic effect on sexual differentiation, i.e. between weeks 6 and 12. The one tissue that mostly reflects fetal tissue is the placenta. However, placental conservation would be possible only as part of a prospective epidemiological study of all male newborns or when ambiguous genitalia are detected by ultrasonography before birth. Several EDCs are known to be lipophilic and thus concentrated in lipid-containing tissues and breast milk. Unfortunately, none of the mothers of the 28 patients with 'idiopathic' PAIS-like phenotype had breastfed her child, so we could not perform this search in breast milk. The search for these products in newborn adipose tissue would also have been very interesting but, from an ethical point of view, it was obviously impossible to sample this tissue. Despite these difficulties, we nevertheless suspected that we would find evidence of these pollutants in serum after birth, since they are known to be stocked in adipose tissue during fetal life and progressively released from adipocytes into the serum. Because several EDCs have both estrogenic and anti-androgenic activity, we assumed that detection of high estrogenic activity in serum, in a context of low plasma E_2 and no breastfeeding, would be a good marker for fetal xenoestrogen contamination.

To evaluate the potential role of EDCs in external genital malformations, a focused exposure assessment methodology was required with more specific markers of fetal exposure to xenoestrogens (10, 58, 69, 70).

In this context, immunosorbent assay for E₂ measurement is a limited method, since it takes into account mainly E₂ and not other estrogenic molecules, such as xenoestrogens. For this reason, we used a recombinant cell bioassay that we developed (35) for ultrasensitive determination of serum total estrogenic bioactivity. Because it is the only assay carried out on total serum, it is a sensitive marker for evaluating xenoestrogen contamination (35). We thus used this method to investigate all the 28 newborn and infant males with 'idiopathic' PAIS-like phenotype and we found elevated mean estrogenic bioactivity (6.7 ± 8.1 pg/ml), despite a low level of E₂, in the 11 patients with fetal EDC exposure versus the 17 cases without fetal EDC contamination (1.27 ± 0.34 pg/ml, range 0.56–2.15 pg/ml; $P < 0.05$) and the controls matched for age and sex (i.e. between birth and 1 year; 1.1 ± 0.4 pg/ml, range 0.54–2.01 pg/ml; $P < 0.05$; Fig. 2). In addition, the difference in estrogenic bioactivity between the 17 infants with 'idiopathic' PAIS-like phenotype and no fetal EDC exposure and the controls matched for age and sex was not statistically significant ($P > 0.05$).

In conclusion, the discrepancy between the low plasma E₂ and the increase in total estrogenic bioactivity found in most of the EDC-exposed patients in a context of no breastfeeding – up to 20 times the mean value in the unexposed patients with PAIS-like phenotype and controls – is likely due to the presence of prenatal xenoestrogen contamination and further supports the hypothesis of fetal exposure to EDCs as a possible risk factor for 'idiopathic' PAIS-like phenotype.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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