Maternal Drug Abuse and Human Term Placental Xenobiotic and Steroid Metabolizing Enzymes *in Vitro*

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We evaluated the impact of maternal drug abuse at term on human placental cytochrome P450 (CYP)-mediated (Phase I) xenobiotic and steroid-metabolizing activities [aromatase, 7-ethoxyresorufin *O*-deethylase (EROD), 7-ethoxycoumarin *O*-deethylase (ECOD), pyrene 1-hydroxylase (P1OH), and testosterone hydroxylase], and androstenedione-forming isomerase, NADPH quinone oxidoreductase (Phase II), UDP-glucuronosyltransferase (UGT), and glutathione *S*-transferase (GST) activities *in vitro*. Overall, the formation of androstenedione, P1OH, and testosterone hydroxylase was statistically significant between control and drug-abusing subjects; we observed no significant differences in any other of the phase I and II activities. In placentas from drug-abusing mothers, we found significant correlations between ECOD and P1OH activities ($p < 0.001$), but not between ECOD and aromatase or P1OH and EROD activities; we also found significant correlations between blood cotinine and UGT activities ($p < 0.01$). In contrast, in controls (mothers who did not abuse drugs but did smoke cigarettes), the P1OH activity correlated with ECOD, EROD ($p < 0.001$), and testosterone hydroxylase ($p < 0.001$) activities. Our results (wider variation in ECOD activity among tissue from drug-abusing mothers and the significant correlation between P1OH and ECOD activities, but not with aromatase or EROD activities) indicate that maternal drug abuse results in an additive effect in enhancing placental xenobiotic metabolizing enzymes when the mother also smokes cigarettes; this may be due to enhancing a "silent" CYP form, or a new placental CYP form may be activated. The change in the steroid metabolism profile *in vitro* suggests that maternal drug abuse may alter normal hormonal homeostasis during pregnancy. *Key words:* barbiturates, benzodiazepines, cocaine, cytochrome P450, drug metabolism, gestation, methadone, opiates, pregnancy, smoking, steroids. *Environ Health Perspect* 108:141–145 (2000). [Online 7 January 2000]
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Illicit use of psychostimulants during pregnancy is an increasing problem in modern society, resulting in increased numbers of adverse pregnancy outcomes such as miscarriages, vaginal bleeding, and cognitive effects in newborns. However, the effect of maternal drug abuse on the metabolic characteristics of the human fetoplacental unit has not been evaluated thoroughly; therefore, the risk for underestimations or overestimations of metabolism-based outcomes is obvious.

Human placenta produces and metabolizes estrogenic steroids and metabolizes xenobiotics. Cytochrome P450 (CYP) enzymes participate in the synthesis and catabolism of steroid hormones; they also metabolize vitamins, fatty acids, and a wide range of medicinal drugs and chemical carcinogens (*1,2*). Human placental microsomal CYP enzymes catalyze the synthesis of estrogens (*3*) and metabolize and, in some cases, activate xenobiotic compounds (*4*).

Human placenta at term expresses only a few functional xenobiotic-metabolizing CYP enzymes (*4–6*). Thus far, only cigarette smoke-inducible CYP1A1 (*5*) and ethanol-inducible CYP2E1 (*7*) have been characterized at the protein and mRNA levels in human placenta, although at full term CYP1A1, 2E1, 2F1, 3A4, 3A5, and 3A7 mRNAs can be detected (*6*). Several drug therapies including phenobarbital and phenobarbital-like inducers such as carbamazepine have failed to affect placental CYP-associated activities (*8–10*).

In the present study, we examined the effect of maternal drug abuse on human placental xenobiotic- and steroid-metabolizing phase I and II activities at term *in vitro*. This is the first study to demonstrate that UDP-glucuronosyltransferase (UGT) activity is individually expressed and detectable in all placental samples and that maternal drug abuse may alter or at least enhance the metabolic capacity of the human placenta, which may also affect the phenotype of the newborn.

Materials and Methods

Subjects. This study was carried out according to the principles of the Declaration of Helsinki. The study protocol and the use of human tissue was approved by the ethics committees of the University of Zürich (Zürich, Switzerland) and the University of Oulu (Oulu, Finland). All women who participated in the study gave their informed consent. Human placental samples were collected at term from drug-abusing ($n = 13$) and non-abusing ($n = 24$) mothers; cigarette smoking was allowed and verified by maternal venous and umbilical cord blood determinations. In the clinical history of drug-abusing mothers, there was extensive use of cannabis, methadone, opiates, cocaine, benzodiazepine, and barbiturates (Table 1). In the urine analyses at term, the following drugs were detected: cannabis, methadone, opiates, codeine, morphine, heroin, benzodiazepine, and barbiturates. Infectious etiology was excluded by hepatitis antibody determinations. Nine out of 14 deliveries were spontaneous vaginal delivery, of which one case was vacuum assisted, and the rest were Cesarean deliveries. The control group consisted of 24 healthy subjects with normal pregnancies. Among these non-drug abusing mothers, we found no evidence of drug use in urine analyses, and self-reported cigarette smoking varied from 0 to 20 cigarettes/day. Fifteen of the cases had normal vaginal delivery (two required forceps and one was vacuum assisted), and nine had Cesarean deliveries because of fetopelvic disproportion. Demographic and perinatal data of the subjects are presented in Table 1.

None of the fetuses presented antenatal or peripartal signs of intrauterine distress (pathologic cardiotocographic or Doppler findings). Postpartal recovery of all mothers and newborns was normal. We found no statistically significant differences between the two groups in weights of fetuses or placentas (data not shown) or in gestation times.

Preparation of placental samples. Immediately after delivery, a physical examination was performed and connective tissues

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and coagulated blood were removed. Placental tissue was rinsed in cold NaCl (0.9%) solution and gently dried between paper towels; small pieces of tissue (10 g) were taken from the central part of each placenta and immersed in liquid nitrogen. Placental samples were stored at -70°C until microsomes were prepared (within 2 months). To minimize decontamination of microsomes by mitchondrial fraction (*11*), microsomes were isolated and washed in 150 mM KCl, 10 mM EDTA buffer (pH 7.4) (*12*) and suspended in 100 mM potassium phosphate, 1 mM EDTA, 20% glycerol buffer (pH 7.4), to give the final protein content of 15–20 mg/mL (*13*).

Biochemical assays. We determined microsomal protein concentrations by the bicinchonic acid method (*14*). The following cytochrome P450-dependent metabolic reactions were determined using standard assays: 7-ethoxycoumarin *O*-deethylase activity (ECOD) was measured by the method of Greenlee and Poland (*15*) using 2 mM 7-ethoxycoumarin as a substrate; 7-ethoxyresorufin *O*-deethylase (EROD; 0.1 mM substrate) activity was determined according to Burke et al. (*16*). We used the method of Waxman et al. (*17*) to determine testosterone hydroxylase activity using a substrate concentration of 50 mM. Four unidentified metabolites and androstenedione were formed in each sample, the intensities of which were comparable in each placenta based on the autoradiography films. For the activity determinations, we selected the chromatographically slowest metabolite band with a relative flow (Rf) value of approximately 0.433. The Rf value for testosterone was 0.867. None of the metabolites comigrated with 6β-hydroxytestosterone. Aromatase activity was determined according to the method of Pasanen (*18*) using a substrate concentration of 10 nM.

The pyrene 1-hydroxylase (P1OH) assay mixture (270 µL final volume) contained 50 mM K-Na phosphate buffer–0.15 M KCl (pH 7.4), 50 µg microsomal protein, 100 µg bovine serum albumin, 1 mM NADPH, and 93 µM pyrene (dissolved in 5 µL DMSO). After incubation at 37°C for 10 min, the reaction was stopped with 0.15% $ZnSO_4 \cdot H_2O$ and cooled on ice. After 600 µL acetonitrile was added to the mixture, it was mixed for 10 sec and centrifuged for 10 min at 10,000 × *g* at 20°C. We analyzed a 50-µL sample for 1-hydroxypyrene using a Spherisorb S3 OD (4.6 × 100 mm) column (Phase Separation Ltd, Deeside Industrial Park, UK), isocratic runs with acetonitrile–0.5% acetic acid in water (75:25), and a Shimadzu HPLC/CLASS VP 5.021 software chromatography data system (Shimadzu, Kyoto, Japan) equipped with an autoinjector and an RF-10A fluorometric detector (λ_{ex} 242 nm, λ_{em} 388 nm). We used 1-hydroxypyrene calibration curves (0, 30, 60, 100, and 200 nM) for peak area quantification. The enzyme reaction was linear up to 30 min.

NADPH:quinone oxidoreductase (NQO) is a flavoprotein that catalyzes two-electron reduction of quinones and their derivatives, exerting a protective effect against chemical mutagenicity and toxicity. NQO was recorded at 600 nm as the dicoumarol (10 mM)–sensitive reduction of 2,6-dichlorophenolindophenol with NADPH as the electron donor. The assay mixture (1.2 mL), which contained 0.3–1 mg cytosolic protein from placental samples, was preincubated at 30°C for 5 min before addition of NADPH and the measurement in a Shimadzu 3,000 double beam spectrophotometer (Shimadzu) (*19*).

Glutathione *S*-transferase (GST) activity toward 1-chloro-2,4-dinitrobenzene was recorded spectrophotometrically at room temperature according to Habig et al. (*20*) using 0.04.–0.12 mg cytosolic protein from placental samples in a 2.5-mL assay mixture.

We determined UGT activity toward 1-hydroxypyrene (93 µM) as the rate of microsomal glucuronidation at 37°C in a 10-min assay according to the fluorometric HPLC method of Luukkanen et al. (*21*). We used the Shimadzu HPLC apparatus described above for product identification and calibration of 1-hydroxypyrene-β-D-glucuronide.

Serum cotinine levels from maternal venous blood and umbilical cord blood samples were determined by the method of Kolonen and Puhakainen (*22*) with minor modifications. For the analyses we used a programmable HPLC (Waters 600 E; Waters, Caguas, Puerto Rico) with UV detection (Schoeffel Instrument Group, Labtronic Ltd, Vantaa, Finland) and with a Rheodyne 7125 injector (Rheodyne, L.P., Rohnert Park, CA). Separation was carried out with a silica column (Spherisorb S5 ODS2, 25 cm × 4.6 mm; Phase Separation Ltd) using 40% methanol–60% 0.1 M phosphate buffer, pH 4.5, and a flow rate of 0.5 mL/min. Benzimidazole was used as an internal standard.

All enzyme assays were performed in a blinded fashion as duplicates.

Chemicals. 7-Ethoxycoumarin, 2,6-dichlorophenolindophenol, 1-chloro-2,4-dinitrobenzene, Tween-80, bicinchonic acid, bovine serum albumin, nicotinamide adenine dinucleotide phosphate (reduced), pyrene, 1-hydroxypyrene, and testosterone were from Sigma (St. Louis, MO). Resorufin was obtained from Aldrich (Milwaukee, WI). 7-Ethoxyresorufin was purchased from Pierce (Rockford, IL) and 4-[^{14}C]-testosterone and Hyperfilm MP autoradiography films were obtained from Amersham (Amersham, UK). 1,2-[^{3}H]-androst-4-ene-3,17-dione was obtained from New England Nuclear (Southampton, Hants, UK). Nonradioactive 4-androsten-3,17-dione and testosterone were from Sigma. Silica gel G60 F254 thin layer chromatography plates were obtained from E. Merck (Darmstadt, Germany). All other chemicals were of at least analytical grade. 1-Hydroxypyrene-β-D-glucuronide was synthesized and purified by L. Luukkanen, Helsinki University.

Statistical analysis. Statistical significance was determined by Mann-Whitney *U*-test. *p*-Values < 0.05 were considered statistically significant.

Results

Table 1 presents demographic data from mothers whose placentas were used in this study.

The comparison of xenobiotic-metabolizing activities and maternal venous and umbilical cord plasma cotinine concentrations from the drug-abusing group (the study group) and the cigarette-smoking controls are summarized in Table 2. Briefly, of the phase I enzyme activities assayed in this

Table 1. Demographic and perinatal data of the study and control groups.

Parameter	Study group (*n* = 13)	Control group (*n* = 24)
Maternal age (years)	28.4 ± 6.1 (*n* = 13)	29.7 ± 4.0 (*n* = 24)
Number of previous pregnancies	1.5 ± 0.67 (*n* = 12)	2.3 ± 0.9 (*n* = 23)*
Duration of pregnancy (weeks)	38.3 ± 1.9 (*n* = 13)	38.8 ± 1.7 (*n* = 24)
Consumption of alcohol (self reported)	3 L beer/day (*n* = 1)	None
Drugs of abuse		
Cannabis	1/5[a]	ND/ND
Methadone	7/8	ND/ND
Heroin	(3/2)	ND/ND
Hashish	1/ND	ND/ND
Benzodiazepines	3/4	ND/ND
Neuroleptics	5/NA	ND/ND
Morphine	3/6	ND/ND
Codeine	ND/3	ND/ND
Cocaine	2/ND	ND/ND

NS, not detectable; NA, not analyzed. Values shown are mean ± SD.
[a]Self reported/urine analysis. *$p < 0.05$.

study, pyrene hydroxylation ($p < 0.000$), testosterone hydroxylation ($p < 0.011$), and formation of androstenedione ($p < 0.037$) exhibited statistically significant differences between the study and control groups. Phase II activities were not enhanced concomitantly with the phase I activities in the study group. Both groups consisted of active cigarette smokers, as indicated by blood cotinine analysis. However, we did not find significantly higher cotinine concentrations in the blood of drug-abusing mothers than in controls.

Table 3 is a summary of the correlation matrix of all the determinations in the study group. Significant correlations existed only between P1OH and ECOD activities ($p < 0.01$), and between blood cotinine and microsomal UGT levels ($p < 0.01$). We found no significant correlation between P1OH and aromatase, P1OH and EROD, or P1OH and testosterone hydroxylation.

The correlation matrix containing determinations in the cigarette smoking control group is summarized in Table 4. In this group, we found significant positive correlations among ECOD, EROD, and P1OH activities ($p < 0.001$). Testosterone hydroxylase activity correlated with aromatase, ECOD, and EROD activities ($p < 0.05$).

Discussion

This is the first study to demonstrate that maternal drug abuse significantly affects human placental xenobiotic- and steroid-metabolizing activities; steroid-metabolizing and pyrene 1-hydroxylation activities significantly differed between the drug-abusing study group and the cigarette-smoking controls. Moreover, EROD activity, a well-known CYP1A1 marker activity in the human placenta, did not correlate with P1OH ($r = 0.019$) activity measurements in drug abusers, whereas it did correlate in cigarette-smoking controls ($r = 0.984$; $p < 0.001$) suggesting the expression of a possibly new catalytic characteristic (or a "silent" form in normal conditions) in the placentas of drug abusers. The observed trend, reduction of GST and UGT activities among abusers, may also refer to increased metabolism-related chemical and oxidative stress in the fetal tissues caused by maternal illicit drug use.

Thus far, placental xenobiotic-metabolizing activities have been characterized primarily using cigarette smoking as a xenobiotic load (*23*), whereas placentas from drug abusers have received only minor interest. However, contaminated food stuffs (*24*) and polluted air (*25*) may enhance placental xenobiotic-metabolizing activities, namely, CYP1A1 expression. The present study group consisted of individuals with multiple drug loads and uncommon and varied lifestyles. Nevertheless, the overall metabolizing characteristics in these individuals did not differ significantly from controls when the classical indicators of placental drug induction were used. This study suggests some new biochemical marker activities, such as testosterone hydroxylase, P1OH, and UGT, which until now have not been intensively studied in placenta, and responds to maternal chemical exposure.

In rodents, the hepatic GST activity increases due to xenobiotics. However, human placental GST does not seem enhanced, at least not because of cigarette smoking. We have shown that GST levels reflect the activity of steroid-metabolizing/synthesizing functions (*23*) rather than indicate an increase in the xenobiotic load. The present results agree with our earlier study (*23*); placental GST levels decreased rather than increased in response to the maternal drug load. The phenomenon was identical when humans were exposed to industrial chemicals or radioactive ionization, both factors that enhance oxidative stress and decrease placental GST levels (*26*).

UGT activity has been detected with a wide interindividual variation in human liver (*27*) and placenta (*28*). An early study demonstrated that placental UGT activity does not positively correlate to maternal cigarette-smoking status (*28*). According to our study in placenta, placental UGT activity responds to maternal cigarette smoking, detected by blood cotinine determinations, as decreased absolute enzymatic activity levels and a marginal positive correlation. However, in placentas from drug-abusing mothers, a strong correlation was observed. This is the first report to describe such a causality, suggesting that in the placenta the UGT activity does not readily respond to maternal cigarette smoking and, like the

Table 2. Comparison of the biochemical data between the study and control groups.

Parameter	Study group ($n = 13$)	Control group ($n = 24$)	p-Value[a]
Androstenedione formation (pmol/mg × min)	12.3 ± 4.75 ($n = 13$)	9.22 ± 6.15 ($n = 22$)	0.037
Aromatase (pmol/mg × min)	27.4 ± 12.1 ($n = 13$)	24.6 ± 10.5 ($n = 24$)	0.479
ECOD (pmol/mg × min)	89.7 ± 72.2 ($n = 13$)	76.0 ± 62.7 ($n = 23$)	0.253
EROD (pmol/mg × min)	119 ± 182 ($n = 13$)	113 ± 226 ($n = 23$)	0.454
P1OH (pmol/mg × min)	46.4 ± 50.5 ($n = 13$)	11.8 ± 22.0 ($n = 23$)	0.000
Testosterone hydroxylase (pmol/mg × min)	10.7 ± 8.43 ($n = 12$)	5.43 ± 3.31 ($n = 23$)	0.011
Cotinine, umbilical cord (mg/mL)	4.38 ± 5.44 ($n = 13$)	3.97 ± 4.67 ($n = 24$)	0.672
Cotinine, maternal vena (mg/mL)	4.64 ± 5.87 ($n = 13$)	3.37 ± 3.78 ($n = 24$)	0.79
GST (nmol/mg × min)	179 ± 72 ($n = 13$)	206 ± 53 ($n = 23$)	0.149
NQO (pmol/mg × min)	2,359 ± 1,785 ($n = 12$)	2,388 ± 1,305 ($n = 22$)	0.929
UGT (pmol/mg × min)	3.97 ± 2.88 ($n = 13$)	13.2 ± 23.5 ($n = 23$)	0.626

Values shown are mean ± SD.
[a]As determined by Mann-Whitney *U*-test.

Table 3. Correlation matrix for the study group.

	Androstenedione formation	Aromatase	ECOD	EROD	P1OH	Testosterone hydroxylase	Cotinine (UC)	Cotinine (MV)	GST	NQO	UGT
Androstenedione formation	1	-0.0150	0.107	-0.193	0.207	0.445	-0.377	-0.330	-0.044	0.063	0.289
Aromatase	0.336	1	0.493	-0.006	0.217	0.622*	-0.114	-0.015	0.164	-0.452	0.337
ECOD	0.107	0.493	1	0.332	0.773**	-0.150	-0.147	-0.150	0.390	0.099	-0.030
EROD	-0.193	-0.006	0.332	1	0.019	-0.330	0.252	0.197	-0.205	0.001	0.086
P1OH	0.207	0.217	0.773**	0.019	1	-0.281	-0.073	-0.126	0.302	0.266	0.035
Testosterone hydroxylase	0.445	0.622*	-0.150	-0.330	-0.281	1	-0.142	-0.067	0.051	-0.401	0.302
Cotinine (UC)	-0.377	-0.114	-0.147	0.252	-0.073	-0.142	1	0.982#	0.383	0.141	0.733**
Cotinine (MV)	-0.330	-0.015	-0.150	0.197	-0.126	-0.067	0.982#	1	0.405	0.039	0.767**
GST	-0.044	0.164	0.390	-0.205	0.302	0.051	0.383	0.405	1	0.409	0.302
NQO	0.063	-0.452	0.099	0.001	0.266	-0.401	0.141	0.039	0.409	1	0.048
UGT	0.289	0.337	-0.030	0.086	0.035	0.302	0.733**	0.767**	0.302	0.048	1

Abbreviations: MV, maternal vena; UC, umbilical cord.
*$p < 0.05$; **$p < 0.01$; #$p < 0.001$.

Table 4. Correlation matrix for the control group.

	Androstenedione formation	Aromatase	ECOD	EROD	P1OH	Testosterone hydroxylase	Cotinine (UC)	Cotinine (MV)	GST	NQO	UGT
Androstenedione formation	1	-0.354	-0.037	-0.197	-0.188	-0.178	0.021	0.008	-0.011	-0.199	0.022
Aromatase	-0.354	1	0.033	0.355	0.355	0.700#	0.159	-0.064	0.135	0.206	-0.148
ECOD	-0.037	0.033	1	0.806#	0.821#	0.210	-0.162	-0.275	0.082	-0.099	-0.138
EROD	-0.197	0.355	0.806#	1	0.984#	0.471*	-0.104	-0.156	0.064	-0.095	-0.043
P1OH	-0.188	0.355	0.821#	0.984#	1	0.479*	-0.051	-0.098	0.129	-0.169	-0.013
Testosterone hydroxylase	-0.178	0.700#	0.210	0.471*	0.497*	1	0.244	-0.053	0.097	-0.008	0.274
Cotinine (UC)	0.021	0.159	-0.162	-0.104	-0.051	0.244	1	0.884#	0.013	0.148	0.055
Cotinine (MV)	0.008	-0.064	-0.156	-0.141	-0.098	-0.053	0.884#	1	-0.069	0.017	0.019
GST	-0.011	0.135	0.082	0.064	0.129	0.097	0.013	-0.069	1	0.111	0.242
NQO	-0.199	0.206	-0.099	-0.095	-0.169	-0.008	0.148	0.017	0.111	1	-0.176
UGT	0.022	-0.148	-0.138	-0.043	-0.013	0.274	0.055	0.019	0.242	-0.176	1

Abbreviations: MV, maternal vena; UC, umbilical cord.
*$p < 0.05$; #$p < 0.001$.

GST activity, may reflect changes in oxdative stress or hormonal homeostasis rather than classical xenobiotic-metabolizing capacity.

We detected an effect caused by maternal drug abuse that was additive as compared to the effect detected after maternal cigarette smoking. Moreover, we detected the existence of a new catalytic activity, P1OH, which correlated consistently with the ECOD activity, but correlated with the EROD or testosterone hydroxylase activity only in cigarette-smoking controls. Our earlier study demonstrated that the ECOD activity could serve as a general marker for enhanced placental xenobiotic activities correlating with EROD and aromatase activity levels in placentas of smokers and nonsmokers (*23,29*). In the present study group, neither the ECOD nor the P1OH levels correlated with EROD determinations or with any other catalytic determination including aromatase activity. However, we cannot exclude the possibility that, in normal conditions, a "silent" CYP form could be responsible for P1OH activity because at least 11 distinct human CYP forms can catalyze ECOD activity (*30*). Interestingly, aromatase (CYP19) accepts cocaine, aflatoxin B1, or 7-ethoxycoumarin as substrates (*31–34*). However, the *CYP19* gene is not affected by maternal cigarette smoking (*23*) or drinking of alcohol (*35*). There is no significant correlation between P1OH and aromatase activities; this supports the view of the expression of a new catalytic activity.

Our earlier studies have shown that human placenta expresses more *CYP* genes at the mRNA level in the first trimester than at term (*6,36*). This may mean that at the phase of early development and growth of the fetoplacental unit, the expression of those *CYP* genes which are not urgently needed for the well-being of the human fetus could be switched off. However, maternal drug abuse could serve as a relevant enhancing stimulus to maintain the expression of specific CYP form(s) throughout the time of gestation.

Could these metabolic disturbances be reflected in later development of the affected fetus or child? An outstanding example of a compound that affected gestational hormonal homeostasis is diethylstilbestrol, with well-known health effects appearing at adolescence. However, in these cases, no evaluation of placental xenobiotic or steroid-metabolizing capacity was carried out at delivery but only afterward, and no association for adverse events and placental metabolism could be demonstrated (*37*).

During the 9 months of pregnancy, the human placenta passes through all its ontogenic developmental phases with the associated changes in its metabolic characteristics. During that period of growth and specialization, the human placenta is a potential target for unwanted metabolic responses caused by maternal diseases or xenobiotics. For example, maternal intrahepatic cholestasis of pregnancy has been shown to decrease overall placental metabolic capacity at term (*38*), and maternal clinical use of corticosteroids dramatically suppresses placental aromatase at term (*39*). However, the final clinical responses may be recorded only after a long delay, as with diethylstilbestrol. Responses such as these among drug abusers may be shown in future cohort studies.

Are there any implications to the current drug therapy during pregnancy? Results in the present study, generated using classical analytical methods, show no dramatic differences in metabolic characteristics or macroscopic characteristics between the study and control groups. However, in our extended panel of analysis, some significant correlations were observed. Maternal drug abuse caused an additive effect in xenobiotic-metabolizing characteristics as compared to the cigarette-smoking control group. It is possible that the combination of drug abuse with any other medication could complicate the expression of placental metabolizing enzymes; these issues are still to be resolved. Therefore, all term placentas from mothers who use any relevant medication or abuse drugs should be evaluated in terms of total xenobiotic-metabolizing activity *in vitro*.

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