

# Mitochondrial disturbances in HIV pregnancies

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**Background:** Mitochondrial consequences from foetal exposure to HIV infection and antiretrovirals could be further investigated.

**Objective:** The main objective of this study was to evaluate maternofoetal mitochondrial disturbances in HIV infection and antiretroviral administration in human pregnancies as the aetiopathogenic basis of suboptimal perinatal-clinical features.

**Design:** Cross-sectional, prospective, observational, exploratory and controlled study.

**Methods:** Clinical/epidemiological data of 35 HIV-infected pregnant women and 17 controls were collected. Mitochondrial DNA (mtDNA) and RNA (mtRNA) content (real time-PCR), enzymatic activities and content (spectrophotometry) were measured in leucocytes. Genetic-functional, maternofoetal and molecular-clinical correlations were assessed.

**Results:** Birth weight was lower in infants from HIV-infected mothers compared with controls. MtDNA values were slightly decreased in HIV cases, although not reaching statistical significance. MtRNA values were lower in HIV-infected mothers. Similarly, binary complex II+III enzymatic activity decreased to 50% in both HIV-infected mothers ( $44.45 \pm 3.77\%$ ) and their infants ( $48.79 \pm 3.41\%$ ) ( $P=0.001$  and  $P<0.001$ ). Global CI+III+IV enzymatic activity was lower in HIV-infected mothers and infants ( $90.43 \pm 2.39\%$  and  $51.16 \pm 9.30\%$ ) ( $P<0.005$  and  $P<0.05$ ). MtDNA content correlated with function in mothers and infants. Maternofoetal parameters correlated at genetic and functional levels.

**Conclusion:** HAART toxicity caused mitochondrial damage in HIV-infected pregnant women and their newborns, being present at a genetic and functional level with a maternofoetal correlation. © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins

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**Keywords:** antiretrovirals, HAART, HIV infection, HIV pregnancies, in-utero exposure, mitochondrial dysfunction and perinatal outcomes

## Introduction

The current implementation of recommendations for universal prenatal HIV counselling and testing, the gestational use of antiretroviral therapy (HAART),

scheduled caesarean section delivery and avoidance of breastfeeding, has led to a reduction in HIV mother-to-child transmission (MTCT) rates from around 20–25% to 1–2% in developed countries [1,2]. Widespread use of antiretroviral drugs has been accepted for the prevention

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of MTCT despite the lack of data related to safety in human pregnancies [3,4]. The potential clinical risks associated with antiretroviral exposure in HIV-infected pregnant women and foetuses have been described by controversial observational studies [5–8] and potential mitochondrial implication has seldom been taken into account. There are limited data on possible toxicities in this population, and the large number of confounding factors limits any conclusions [9]. Antiretrovirals have been associated with adverse pregnancy outcomes such as preeclampsia, foetal death, preterm birth and low birth weight [10]. There are some studies reporting these negative effects of in-utero antiretroviral exposure in animal models [11].

Although antiretroviral therapy is required to suppress viral replication, leading to a decrease in MTCT rates and avoidance of disease progression, its derived mitochondrial toxicity has been widely described in adults, especially concerning the use of nucleoside reverse transcriptase inhibitors (NRTI), which are known to inhibit mitochondrial DNA (mtDNA) polymerase  $\gamma$  [12] and may therefore lead to mitochondrial dysfunction [13]. Other antiretroviral groups included in the backbone of therapeutic regimens such as protease inhibitors and non-NRTI are also known to cause mitochondrial deficiencies mainly through the development of apoptosis [14]. Subclinical mitochondrial molecular consequences from in-utero exposure of foetuses to HIV infection and antiretroviral drugs have not been completely elucidated as well as their association with the perinatal clinical outcomes in human pregnancies. Mitochondrial alterations may entail many important and heterogeneous secondary adverse events such as neuropathies, lactic acidosis, hyperlactataemia, lipodystrophy or myopathies, common to primary mitochondrial disease [15], but they have also been suggested to play a role in fertility [16] and foetal development [10]. Recently, adverse neurochemical and behavioural effects derived from transplacental exposure to zidovudine have been described in a mouse model together with a potential protector role of L-acetylcarnitine on mitochondrial function [17].

The mitochondrial genome encodes for proteins of the mitochondrial respiratory chain. The relationship between mitochondrial genetic and functional parameters has already been demonstrated, both in antiretroviral-exposed animals and newborn [18]. Our group carried out a previous study to investigate the role of in-utero antiretroviral exposure in mitochondrial function in mononuclear cells isolated from chord blood from the newborn [10]. In the present study, mitochondrial parameters were measured directly from peripheral blood mononuclear cells of the infant for two purposes: to further confirm our previous results from chord blood, comparing the findings from chord blood with those of peripheral blood and to further investigate the transcriptional level and the general assessment of the global

mitochondrial respiratory chain, as these parameters were not considered in our last work [10].

Our previous findings showed a decrease in global mitochondrial function of the complete respiratory chain in a perinatally HIV-infected paediatric population [19] leading to analysis of this combined general mitochondrial enzymatic activity in infants exposed to antiretroviral drugs during gestation in the present study.

We hypothesized that antiretroviral-derived mitochondrial toxicity is present in HIV-infected and treated mothers and their foetuses exposed *in utero*, that the type and severity of the maternal involvement may be similarly reflected in the newborns and that this mitochondrial damage may underlie the clinical perinatal outcomes in HIV-infected human pregnancies.

The main objective of the present study was to evaluate the subclinical mitochondrial implication within the context of HIV infection and antiretroviral exposure in human pregnancies. We therefore aimed to (i) assess mitochondrial parameters in HIV-infected pregnant women and their infants exposed to antiretroviral *in utero*, (ii) correlate the genetic and functional levels of mitochondrial parameters and (iii) correlate the mother-foetal relationship of the mitochondrial parameters.

## Methods

### Design

We performed a single-site, cross-sectional, prospective, case-controlled observational and exploratory study with an inclusion period from 2007 to 2012.

### Patients

Fifty-two mother-infant couples were recruited in this study. Thirty-five were classified as HIV-infected mothers (with their noninfected infants) and the control group included 17 HIV-uninfected mother-child pairs in follow-up because of other infections susceptible to be vertically transmitted, such as hepatitis C or B virus, syphilis or Chagas. All cases and controls were consecutively included during their routine prenatal care at the last trimester of gestation in the tertiary care Hospital St Joan de Déu of Barcelona (Barcelona, Spain), while the experimental procedures were performed in the Faculty of Medicine, Hospital Clínic of Barcelona (Barcelona, Spain).

### Sample collection and processing

Twenty millilitres of peripheral blood were collected from the mothers in EDTA-tubes, with 2–5 ml being collected from their infants at the age of 6 weeks. Peripheral blood mononuclear cells were obtained by a Ficoll density gradient centrifugation procedure [20] divided into aliquots and stored at  $-80^{\circ}\text{C}$  until analysis. Samples

from mother–child pairs in the control group were eligible only when maternal infection had definitely been ruled out in the infant, usually some months after collection.

### Clinical analysis

Clinical data were collected through detailed questionnaires at inclusion and at delivery. Anthropometric data of the infant were also collected at delivery.

As per protocol, informed consent was obtained and epidemiological and obstetric parameters included information on maternal age, race, parity, and mode of delivery. Information regarding perinatal outcomes for both HIV-infected women and controls included the following data: preeclampsia (new onset of hypertension of >140 mmHg or >90 mmHg of SBP and DBP, respectively, and >300 mg proteins/24 h of urine), gestational age at delivery, preterm birth (<37 weeks of gestation), birth weight, infant small for gestational age (<10th percentile), 5-min Apgar score below 7, time and type of antiretroviral exposure, neonatal admission to ICU and global adverse perinatal outcome.

### Molecular analysis of mitochondrial parameters

Protein content was measured according to the Bradford protein–dye binding-based method [21].

Total DNA was extracted by standard phenol–chloroform procedures. We analysed mtDNA content by the amplification of the mitochondrial gene mt12SrRNA and the nuclear constitutive gene nRNaseP using Applied Biosystems real-time quantitative PCR (Foster City, California, USA) in a 96-well plate and expressed in relative units as the ratio between mtDNA and nuclear DNA (mt12SrRNA/nRNaseP). The amplification procedure was performed as follows:

To determine mitochondrial mtDNA: MtF805 (5'-CCACGGGAAACAGCAGTGAT-3') was used as the 12SrRNA forward primer and MtR927 (5'-CTATGACTTGGGTAAATCGTGTGA-3') was used as the 12SrRNA reverse primer, using a TaqMan sonda 6FAM-5'-TGCCAGCCACCGCG-3'-MGB, of Applied Biosystems.

To determine nuclear DNA a commercial kit was used (RNase P Control Reagent VIC, part no 4316844; Applied Biosystems).

The conditions for the amplification cycles for both genes were: 2 min at 50°C, 10 min at 95°C, 40 denaturalisation cycles of 15 s at 95°C and 60 s of annealing step at 60°C.

Total RNA was extracted by affinity microcolumns of Nucleospin (Düren, Germany), following the instructions of the commercial kit. Reverse transcription was

performed by using random hexamer primers before the RT-PCR experiment.

mtRNA was quantified amplifying a fragment of the conserved mitochondrial gene ND2 (using the forward 5'-GCCCTAGAAATAAACATGCTA-3' primer and the reverse 5'-GGGCTATTCCTAGTTTTATT-3' primer) and the constitutive nuclear gene 18SrRNA (using the forward primer 5'-ACGGACCAGAGCGAAAGCAT-3' and the reverse primer 5'-GGACATCTAAGGGCA TCACAGAC-3' primer). Both genes were quantified separately by real-time quantitative PCR (LightCycler FastStart DNA Master SYBR Green I; Roche Molecular Biochemicals, Mannheim, Germany) and the results were finally expressed by the ratio between mtRNA and nuclear RNA (mtND2/n18SrRNA). The conditions for the amplification cycles were single denaturization–enzyme-activation step of 10 min at 95°C followed by 29 cycles (for the ND2 gene) and 35 cycles (for the 18SrRNA gene). Each cycle consisted in a denaturation step (0 s at 94°C for the ND2 gene and 2 s at 95°C for the 18SrRNA gene), an annealing step (10 s at 53°C for the ND2 gene and 10 s at 66°C for the 18SrRNA gene), and an extension step (10 s at 72°C for the ND2 gene and 20 s at 72°C for the 18SrRNA gene).

Mitochondrial function was measured spectrophotometrically according to the Rustin *et al.* [22] and Miró *et al.* [23] methodologies. We assessed the enzymatic activities of the isolated complexes: complex II (CII), complex IV (CIV) and binary combination enzymatic activities: complex II+III (CII+III), glycerol-3-phosphate dehydrogenase+complex III (G3PDH+CIII) and complete mitochondrial respiratory chain activity: complex I+III+IV (CI+III+IV) of the mitochondrial respiratory chain.

We measured mitochondrial content by citrate synthase activity (EC 4.1.3.7) with spectrophotometric measurements of the absorbance at 412 nm. Citrate synthase is a mitochondrial enzyme of the Krebs cycle which is widely considered as a reliable marker of mitochondrial content [22].

All the enzymatic activities were obtained as absolute values in nanomoles of synthesized product or consumed substrate per minute and milligram of protein (nmoles/min/mg protein) units and afterwards as relative values normalized by citrate synthase activity to relativize the enzymatic activity by mitochondrial content. The remaining genetic or transcriptional analyses were also normalized to citrate synthase activity to relativize parameters to mitochondrial mass.

### Statistical analysis

Epidemiologic, clinical and mitochondrial data of HIV-infected women and their infants were compared with those of uninfected mother–child pairs to assess the

presence of obstetric/perinatal problems and mitochondrial damage due to in-utero exposure to HAART. Additionally, different correlations were sought between: (i) genetic and functional mitochondrial parameters (to ascertain dependence of mitochondrial function on mitochondrial genome content), (ii) mother-to-child mitochondrial parameters (to determine maternal influence on infant cellular condition) and (iii) clinical and experimental data (to assess mitochondrial implication in obstetric problems and perinatal outcomes).

Statistical analyses were performed by means of the SPSS 15.0 (Chicago, Illinois, USA) program using Mann–Whitney nonparametric tests to search for independent sample differences, chi-square tests were used to calculate odds ratio values (OR; 95% confidence interval [CI]; significance) and the Spearman's rank correlation coefficient was used to correlate parameters ( $R^2$  and significance). Clinical parameters were expressed as mean  $\pm$  SD and experimental results were expressed as mean  $\pm$  SEM or percentages with respect to the means of controls, and the level of significance was set at 0.05 for all the statistical tests.

## Results

### Clinical data

The clinical and epidemiologic characteristics of the HIV-infected mothers and their infants and the control group have been summarized in Table 1.

The maternal, labour, neonatal prophylactic treatments are shown in Table 2. The incidence of preeclampsia in the HIV pregnancies was not higher compared with the controls. The incidence of preterm birth was higher in infants from HIV-infected mothers with respect to those from control pregnant women (36.36 vs. 21.42%), although this was not statistically significant. Additionally, the birth weight was significantly lower in HIV-exposed and antiretroviral-exposed newborn

compared with controls ( $2689.35 \pm 615.92$  vs.  $3292.65 \pm 540.45$ ,  $P=0.001$ ).

### Molecular data of mitochondrial parameters

The mitochondrial mass amount was not compromised in HIV-infected mothers or their infants as shown in Fig. 1. All the absolute mitochondrial parameters were relativized per mitochondrial mass by normalizing per citrate synthase enzymatic activity.

The mitochondrial genome (mtDNA) showed a trend towards depletion in both HIV-infected mothers and their infants with respect to controls ( $1.06 \pm 0.21$  and  $0.97 \pm 0.20$  vs.  $5.05 \pm 2.58$  and  $2.05 \pm 0.95$  mtDNA 12SrRNA/nDNA RNaseP arbitrary ratio units,  $P < 0.1$  for both). At the transcriptional level, mitochondrial RNA showed a decrease in HIV-infected mothers with respect to the control group ( $0.65 \pm 0.16$  vs.  $5.50 \pm 2.63$  mtRNA ND2/nRNA 18SrRNA arbitrary ratio units,  $P=NS$ ), attaining statistical significance in their infants ( $0.29 \pm 0.46$  vs.  $4.78 \pm 2.65$  mtRNA ND2/nRNA 18SrRNA arbitrary ratio units,  $P < 0.01$ ) (Fig. 2a and b).

Mitochondrial function, assessed in isolated complex II and complex IV enzymatic activities, was not compromised in HIV-infected mothers and their infants with respect to controls; CII: ( $0.22 \pm 0.01$  and  $0.21 \pm 0.01$  vs.  $0.25 \pm 0.03$  and  $0.29 \pm 0.04$  nmole/min mg protein,  $P=NS$  in all cases); CIV: ( $0.34 \pm 0.029$  and  $0.34 \pm 0.024$  vs.  $0.87 \pm 0.24$  and  $0.39 \pm 0.055$  nmole/min mg protein,  $P=NS$  in all cases). The isolated enzymatic activity of glycerol-3-phosphate dehydrogenase (G3PDH) was not compromised in any case. G3PDH enzymatic activity combined to CIII was not significantly reduced in cases with respect to the controls. The measurement of binary enzymatic activity CII+CIII decreased to 50% both in HIV-infected mothers and their infants compared with controls ( $0.25 \pm 0.01$  vs.  $0.45 \pm 0.07$ ,  $P=0.001$ ), ( $0.21 \pm 0.01$  vs.  $0.41 \pm 0.059$  nmole/min mg protein,  $P < 0.001$ ) (Fig. 2a and b).

**Table 1. Clinical and epidemiologic characteristics of the HIV-infected mothers and their infants included in the study.**

	Control mothers	Control infants	HIV-infected mothers	HIV-exposed infants
Sample size ( <i>n</i> )	17	17	35	35
Age at blood draw (mean $\pm$ SD)	34.3 years $\pm$ 5.7	1.3 months $\pm$ 0.7	31.4 years $\pm$ 7.3	2.0 months $\pm$ 0.7
Female gender (%)	100	58.82	100	41.93
Mean CD4 <sup>+</sup> lymphocyte count (cells/ $\mu$ l <sup>3</sup> $\pm$ SD)	NA	NA	624.8 $\pm$ 316.6	641.75 $\pm$ 304.17
Mean viral load (Log10 viral load copies/ml $\pm$ SD)	NA	NA	0.36 $\pm$ 1.0	NA
Antiretroviral therapy				
3 NRTI ( <i>n</i> )	NA	NA	2	0
2 NRTI + 1 or 2 PI, or 1NNRTI ( <i>n</i> )	NA	NA	22	0
1 NRTI + 1 tNRTI or 1 PI ( <i>n</i> )	NA	NA	5	1
ZDV monotherapy ( <i>n</i> )	NA	NA	0	30
Obstetric parameters				
Preterm birth (%)	NA	21.4	NA	36.4
Birth weight (mean gr $\pm$ SD)	NA	3292 $\pm$ 540	NA	2689 $\pm$ 615*

NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; tNRTI, nucleotide reverse transcriptase inhibitor; ZDV, zidovudine. \* $P=0.001$ .

**Table 2. Maternal, labour and neonatal prophylactic treatments.**

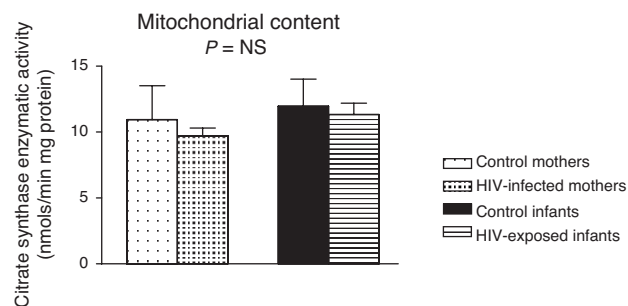
Maternal pregnancy treatments	Maternal labour treatments	Neonatal treatments
Protease inhibitor-based regimens, <i>n</i> = 20	Intravenous ZDV, <i>n</i> = 32	ZDV monotherapy, <i>n</i> = 33
ZDV/3TC+LPV/r, <i>n</i> = 7	Untreated, <i>n</i> = 3	ZDV+NVP, <i>n</i> = 1
ZDV/3TC+SQV/r, <i>n</i> = 3		ZDV+3TC+NVP, <i>n</i> = 1
TDF/FTC+LPV/r, <i>n</i> = 2		
ZDV/3TC+FPV/r, <i>n</i> = 2		
ABC/3TC+LPV/r, <i>n</i> = 1		
ABC+TDF+LPV/r, <i>n</i> = 1		
3TC+d4T+LPV/r, <i>n</i> = 1		
ZDV/3TC+NFV, <i>n</i> = 1		
TDF/FTC+ATV/r, <i>n</i> = 1		
TDF/FTC+SQV/r, <i>n</i> = 1		
NNRTI-based regimens, <i>n</i> = 12		
ZDV/3TC+NVP, <i>n</i> = 6		
ZDV+FTC+EFV, <i>n</i> = 2		
ABC/3TC+NVP, <i>n</i> = 1		
ABC+ddl+NVP, <i>n</i> = 1		
ABC+FTC+NVP, <i>n</i> = 1		
ABC/3TC/ZDV, <i>n</i> = 1		
ZDV monotherapy, <i>n</i> = 1		
Untreated, <i>n</i> = 2		

1st trimester exposure to antiretrovirals, 21/33 mothers (63.6%), median (range) duration of antiretroviral treatment during pregnancy: 33.5 (4–40) weeks. 3TC, lamivudine; ABC, abacavir; ATV/r, atazanavir/ritonavir; d4T, stavudine; ddl, didanosine; EFV, efavirenz; FPV/r, fosamprenavir/ritonavir; FTC, emtricitabine; LPV/r, lopinavir/ritonavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse transcriptase inhibitors; NVP, nevirapine; SQV/r, saquinavir/ritonavir; TDF, tenofovir; ZDV, zidovudine.

General assessment of the whole mitochondrial respiratory chain enzymatic activity through analysis of CI+III+IV showed a significant decrease in both HIV-infected mothers and their infants compared with the control groups ( $0.20 \pm 0.05$  and  $0.21 \pm 0.03$  vs.  $2.09 \pm 0.82$  and  $0.43 \pm 0.11$ ;  $P < 0.01$  and  $P = 0.05$ ) (Fig. 2a and b).

### Mitochondrial genetic and functional associations

Mitochondrial genome content correlated with mitochondrial function measured as CII+CIII/citrate synthase enzymatic activity (Fig. 3a) in both groups of mother–child pairs, whether HIV-infected or not.



**Fig. 1. Mitochondrial content measured by citrate synthase enzymatic activity of mothers and infants in both the control and case groups.** Mann–Whitney nonparametric test.

### Maternofoetal associations

The mitochondrial parameters of the mothers and their infants positively correlated at a genetic level, measured as mtDNA content, as well as at the functional level, with CII+CIII/citrate synthase measurement (Fig. 3b and c) in both cohorts. No consistent molecular and clinical associations were found in the present study (Fig. 3d and e).

### Discussion

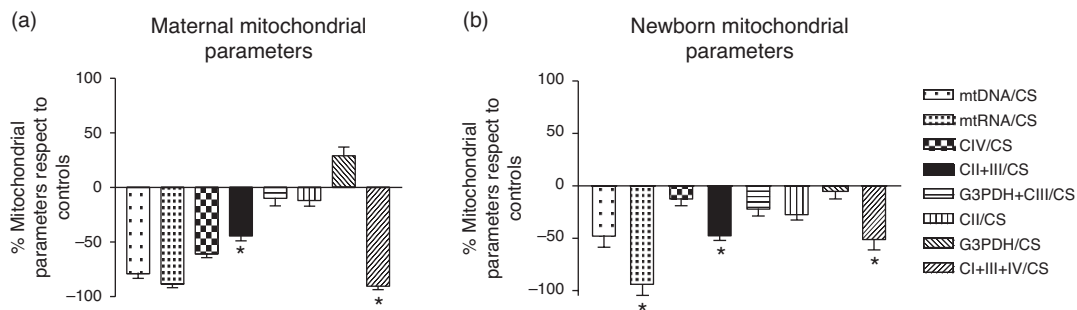
Although mitochondrial toxicity has been demonstrated in in-utero exposure to antiretroviral drugs in animal models, specifically secondary to NRTIs [24], more information on this subject in human HIV pregnancies could be addressed.

Previous studies have demonstrated HAART-induced mtDNA depletion in oocytes from HIV-infected women that may impair their reproductive capacity [16].

The present study does not show a higher incidence of preeclampsia in HIV pregnancies. There were no cases of foetal death in any of our cohorts. However, the incidence of preterm birth tended to be higher and birth weight was significantly lower in infants born to HIV-infected mothers with antiretroviral treatment. Previous studies have demonstrated the implication of mitochondria in abnormal perinatal foetal weight in non-HIV or HAART-exposed mothers [25]. The clinical data in our study suggest an association of HAART toxicity in the context of HIV infection and the presence of perinatal outcomes in human pregnancies. We therefore wished to determine whether the mitochondrion remains behind these clinical manifestations, as the aetiopathogenic basis.

As the viral load of the HIV-infected mothers was undetectable and the infants were confirmed to be HIV-negative, the mitochondrial toxicity observed in our HAART-exposed cohorts was attributed to antiretroviral exposure but not to HIV infection itself.

Mitochondrial amount was not affected in HIV-infected mothers and their in-utero antiretroviral-exposed newborns. However, the mitochondrial genome showed a trend towards depletion in both HIV-infected mothers and their infants with respect to controls. These findings are in accordance with previous studies reporting mtDNA depletion in newborn from HIV-infected and treated mothers [26–29]. The trend to mtDNA depletion observed in the present study was reflected downstream at the transcriptional and functional levels, displaying an organelle dysfunction with significantly lower mtRNA levels in HIV-exposed infants and a decrease in the combined binary and global enzymatic activities of the mitochondrial respiratory chain, respectively. Despite



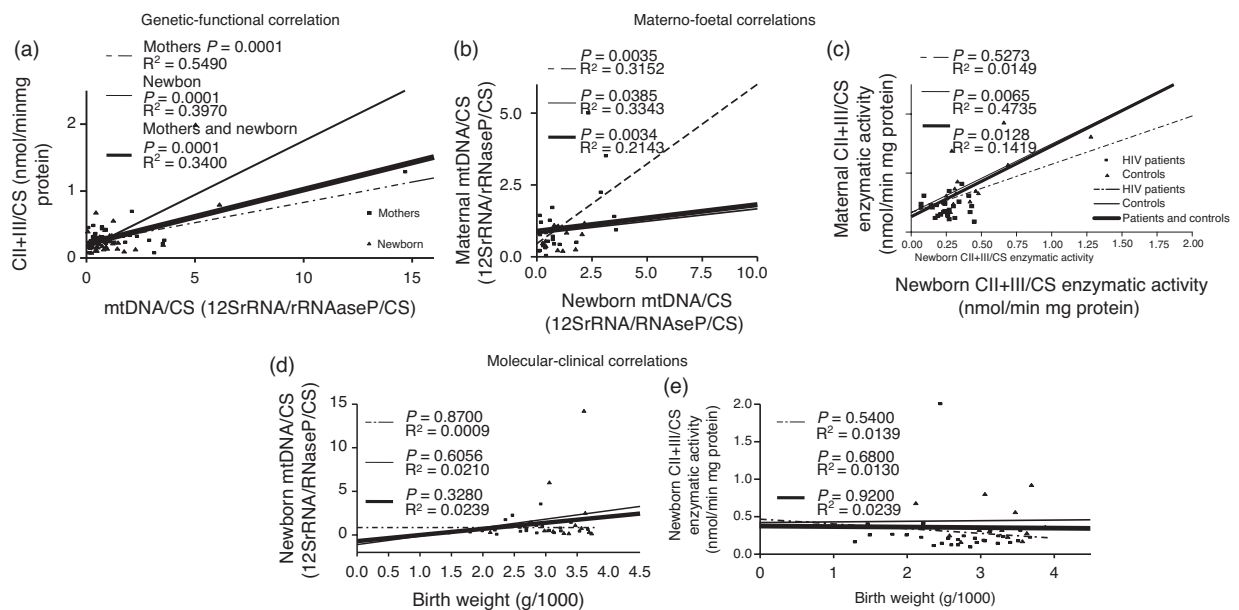
**Fig. 2. (a) Maternal mitochondrial parameters and (b) Mitochondrial parameters of the infants.** Percentage of increase/decrease of the mitochondrial parameters in HIV-infected mothers with respect to the mean values of uninfected controls. CIII, complex III; CII+III, complex II+III; CI+III+IV, complex I+III+IV; CIV, complex IV; G3PDH, glycerol-3-phosphate dehydrogenase; MtDNA, mitochondrial DNA; mtRNA, mitochondrial RNA. \* $P=0.05$ . Mann-Whitney nonparametric test.

the general involvement of these global enzymatic activities, isolated complexes were not compromised. These findings are consistent with our previous studies in perinatally HIV-infected paediatric patients in which we found significant alterations of the global enzymatic activity of mitochondria in the absence of any suboptimal function of single complexes [19]. Our results suggest the presence of mild alterations in individual complexes of the mitochondrial respiratory chain which are only noticeable through the measurement of binary or global enzymatic activities, thus, reaching a detectable threshold as a summatory effect of these mild alterations.

The positive and significant correlation found between mitochondrial genetics and functional binary enzymatic

activity strengthens the idea of a genetic defect affecting the general function of the mitochondria and, subsequently, cell viability. This fact demonstrates the dependence of mitochondrial function on mitochondrial genetics.

The significant positive correlation between the maternofoetal mitochondrial parameters suggests that the toxicity caused by HAART in the context of HIV infection in human pregnancies has a similar impact on both the mother and fetus. Consequently, our findings show that the mtDNA depletion, the decrease in mtRNA content and the general dysfunction of the mitochondrial respiratory chain observed in HIV-infected mothers also occurred in their newborn.



**Fig. 3. Mitochondrial genetic-functional, maternofoetal and molecular-clinical correlations.** (a) Mitochondrial genome and function in mothers and newborn; (b) Mitochondrial genome; (c) Mitochondrial function; (d) Mitochondrial genome and birth weight; (e) Mitochondrial function and birth weight. CII+III, complex II+III enzymatic activity; CS, citrate synthase; MtDNA, mitochondrial DNA.

As the use of chord blood may be questioned as a proper model of study due to a possible contamination with maternal cells, we assessed all the molecular parameters in samples from the peripheral blood (specifically monocytes and lymphocytes) of infants obtained at the age of 6 weeks to further confirm the results of our previous study performed in chord blood cells from mother–newborn couples [10]. The findings derived from the present work confirm the presence of a mitochondrial lesion following the same general pattern of maternofetal correlation.

Although abnormal perinatal outcomes and mitochondrial alterations were more prevalent in HIV-infected mothers and their infants with respect to uninfected controls, the results obtained from this study do not confirm an association between the mitochondrial defects and the clinical manifestations observed (preterm birth and low birth weight). Further studies in larger cohorts are necessary to confirm a potential relationship between antiretroviral exposure and clinical morbidities in HIV-infected mothers and newborn.

Finally, the presence of a mitochondrial lesion derived from antiretroviral intake both in HIV-infected pregnant women and in their newborns exposed *in utero* indicates that the toxic effects associated with HAART cross the placenta and affect the HIV-negative, but antiretroviral in-utero exposed newborn in a similar manner. As the antiretroviral intake ensures that viral loads are decreased to undetectable levels, it is expected that the damage in mitochondrial function in the HIV-infected mothers should be rather attributed to antiretroviral drugs. However, it is generally considered that the infant may sustain some mitochondrial dysfunction caused by the maternal virus infection, in the absence of any drug therapy, [28]. The HAART-derived toxicity in fertility or pregnancy context confirms previously documented results in chord blood cells from HIV-uninfected but HAART-exposed newborn [9].

Some limitations of this study are worthy to be mentioned. In spite of the previously mentioned fact that the mitochondrial toxicity in our cohort has been attributed to antiretroviral exposure rather than to HIV infection itself, it is not possible to completely dissect the role of mother's HIV infection and HAART because all HIV-infected-pregnant women are currently treated. Furthermore, the heterogeneity of therapeutic schedules and the clinical history of each patient, with differential cumulative antiretroviral drug intake, hampers the possibility of further specific descriptions of the molecular mechanisms together with more focalized antiretroviral-specific conclusions.

In summary, the findings of the present study demonstrate a significantly lower birth weight and genetic and functional maternofetal mitochondrial toxicity in HIV-infected mothers and their infants, although no

relationship was found among these clinical and molecular parameters.

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Role of each of the authors in the study reported: C.M. is the main author of the manuscript as she was in charge of most of the experimental procedures; A.N.J. is the coordinator of the Infectious Disease Unit of the paediatric hospital Sant Joan de Déu. He organises the inclusion of the patients; G.G. is the postdoctoral research who coordinates the experimental research procedures; N.R. is the person in charge of the inclusion of the controls; M.C. is responsible for the enzymatic activities measurement; M.B. is responsible for the mitochondrial DNA content quantification; M.G.M. is responsible for the analysis of the total cell protein content; E.T. is the laboratory technician in charge of the preparation and cryopreservation of the samples; S.H. is the gynaecologist in charge of the management of the obstetric data; F.C. is the head of the Internal Medicine Department who is in charge of the final review of the study; O.M. is the clinician in charge of the management and analysis of the clinical data; C.F. is the head of the Infectious Disease Unit of the paediatric hospital who coordinates the inclusion of the samples and the management of the project.

## Conflicts of interest

There are no conflicts of interest.

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