PHARMACOGENETICS OF EFAVIRENZ EXCRETION INTO HUMAN BREAST MILK AND TRANSFER TO BREASTFED INFANTS

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INTRODUCTION

- The antiretroviral drug efavirenz (EFV) is not licensed for use in children < 3 months old or < 3.5 kg but it is used by nursing mothers (1).
- We previously showed that maternal EFV is transferred to breastfed infants through breast milk (2).
- The influence of single nucleotide polymorphisms (SNPs) in drug metabolizing enzyme, transporter and nuclear receptor genes on plasma EFV concentration is well established (3).
- However, it is not known if they affect its excretion into breast milk and transfer to breastfed infants.

OBJECTIVES

- In this study, we investigated the extent of EFV excretion into human breast milk using a novel dried breast milk spot method.
- The effect of selected SNPs in maternal *CYP2B6*, *CAR*, *ABCB5* and *ABCG2* on breast milk, maternal and infant plasma EFV concentrations was also explored.

METHODS

- **Study Population:** HIV positive nursing mothers (n = 51) receiving regimens containing 600 mg EFV once daily and their exclusively breastfed infants (n = 51) were recruited from 3 Nigerian hospitals.
- **Sample Collection:** Paired dried blood spots (DBS; maternal and infant) and dried breast milk spots (DMS) were collected 12-14 hours post maternal dose.
- **EFV Quantification:** EFV was quantified by a validated LC-MS/MS assay. Plasma EFV concentrations were estimated using $[DBS_{[EFV]}/(1\text{-}HCT_f)]*f_{bpp}$, where $DBS_{[EFV]}$ is EFV concentration in DBS, HCT is average haematocrit and f_{bpp} is fraction bound to plasma protein.
- **Genotyping:** Genotyping for *CYP2B6* rs3745274 (516G>T), *CAR* rs2307424 (c.540C>T) and rs3003596 (g.8784T>C), *ABCB5* rs6461515 (c.1573G>A) and rs2301641 (c.343A>G), and *ABCG2* rs2231164 (c.1728-46G>A) and rs2622604 (6088A>G) was conducted using validated TaqMan assays with assay IDs C_7817765_60, C_25746794_20, C_16194070_10, C_25621077_20, C_2544197_10, C_15922479_10 and C_9510352_10, respectively.
- **Statistical Analysis**: Associations of EFV concentrations with SNPs and demographic factors were investigated by univariate (Mann-Whitney U test) and multivariate analyses (multiple linear regression). P < 0.05 was considered statistically significant.

RESULTS

Maternal age (yrs)	28 (5.3)*
Maternal weight (kg)	58 (8.5)
Baseline CD4 count (cells/mm^3)	402 (217)
Last CD4 count (cells/mm^3)	603 (251)
Maternal regimen	EFV/3TC/AZT, 24; EFV/TDF/FTC, 27
Time post dose	12.3 (6)
Infant age (days)	112 (95)
Infant weight (kg)	5.3 (1.9)
Infant gender	Female, 26; Male, 25

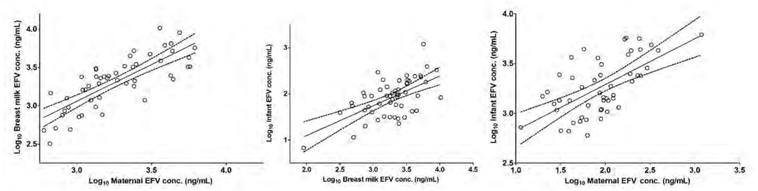
*Mean (standard deviation)

- Of the 20 infants for whom the 6 week-HIV status results were available, none tested positive for HIV. No drug-related adverse reaction or toxicity was observed in any of the infants.

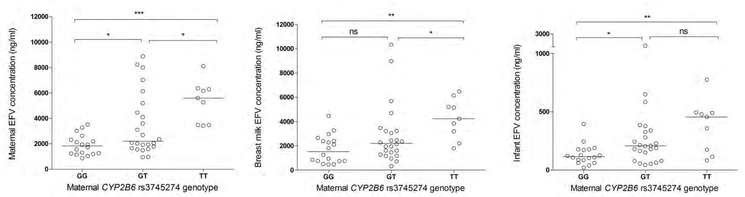
Breast milk EFV conc. (ng/ml)	2280 (1180, 3270)*
Maternal plasma EFV conc. (ng/ml)	2310 (1580, 4460)
Infant plasma EFV conc. (ng/ml)	178 (87.7, 340)
Milk-to-plasma (M/P) ratio	0.82 (0.51, 1.1)

*Median (IQR)

- Median (IQR) EFV breast milk concentration was 2280 ng/ml (1180, 3270) and maternal plasma EFV concentration was 2310 ng/ml (1580, 4460). Milk-to-maternal plasma ratio was 0.82 (0.51, 1.1).
- There were significant correlations between EFV concentrations:
  - maternal plasma and breast milk ($p = 1.3 \times 10^{-12}$; rho = 0.80);
  - breast milk and infant plasma ($p = 7.9 \times 10^{-5}$; rho = 0.52);
  - maternal plasma and infant plasma ($p = 2.0 \times 10^{-6}$; rho = 0.62).



- Significant differences in maternal, infant and breast milk EFV concentrations were observed according to maternal *CYP2B6* rs3745274 genotypes.



***, ** and *: statistically significant differences observed at 0.1, 1.0, 5.0% levels; ns: no significant differences

- Only *CYP2B6* rs3745274 was independently associated with breast milk, maternal and infant plasma EFV concentrations in multivariate analysis.

Variable	Univariate analysis		Multivariate analysis	
	B* (95% CI) ng/ml	p value	B (95% CI) ng/ml	p value
***CYP2B6* rs3745274**				
Maternal plasma conc.	1180 (662, 1700)	3.2×10^{-5}	1240 (684, 1790)	4.7×10^{-5}
Breast milk conc.	1240 (474, 2010)	0.002	1240 (474, 2010)	0.002
Infant plasma conc.	67.1 (0.075, 134)	0.050	67.1 (0.075, 134)	0.050
***CAR* rs2307424**				
Maternal plasma conc.	-92.5 (-616, 431)	0.724		
Breast milk conc.	98.1 (-618, 814)	0.784		
Infant plasma conc.	-52.8 (-110, 4.38)	0.070		
***CAR* rs3003596**				
Maternal plasma conc.	16.6 (-564, 597)	0.955		
Breast milk conc.	-66.1 (859, 727)	0.868		
Infant plasma conc.	38.5 (-26.1, 103)	0.237		
***ABCB5* rs6461515**				
Maternal plasma conc.	-255 (-979, 469)	0.481		
Breast milk conc.	340 (-628, 1310)	0.482		
Infant plasma conc.	-56.4 (-140, 27.5)	0.182		
***ABCB5* rs2301641**				
Maternal plasma conc.	-901 (-1560, -246)	0.008		
Breast milk conc.	-228 (-1190, 729)	0.633		
Infant plasma conc.	-34.4 (-117, 47.7)	0.403		
***ABCG2* rs2231164**				
Maternal plasma conc.	297 (-464, 1060)	0.437		
Breast milk conc.	988 (-18.8, 1990)	0.054		
Infant plasma conc.	68.3 (-15.7, 152)	0.109		
***ABCG2* rs2622604**				
Maternal plasma conc.	-66.2 (-879, 746)	0.871		
Breast milk conc.	-134 (-1240, 976)	0.809		
Infant plasma conc.	9.56 (-82.1, 101)	0.835		
Time post dose				
Maternal plasma conc.	58.2 (-12.8, 129)	0.106		
Breast milk conc.	41.5 (-57.5, 141)	0.404		
Infant plasma conc.	7.75 (-0.173, 15.7)	0.055		

*B is the regression coefficient and represents incremental change in EFV concentration per allele carried

CONCLUSIONS

- EFV is excreted into human breast milk and transferred to breastfed infants. Milk-to-plasma ratio was 0.82.
- Using 150ml/kg/day as average milk intake, this equates to a ~340 ug/kg/day (177, 491) infant dose. This represents < 2% of the paediatric dose for children weighing ≥ 3.5 kg and ≥ 3 months old and achieved 178 ng/ml (87.7, 340) in infant plasma, ~18% of the minimum target therapeutic concentration.
- In addition to its effect on maternal plasma EFV concentration, *CYP2B6* rs3745274 was independently associated with EFV concentrations in breast milk and plasma of breastfed infants.
- Further study is warranted to define the clinical significance of long-term exposure of breastfed infants to maternal EFV through breast milk in larger cohorts, especially as it relates to toxicity, prophylactic benefits and possible drug resistance.

REFERENCES

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