

# Investigation of the association of *CYP2B6* haplotypes with efavirenz plasma concentrations in a Chilean HIV cohort

**Daniel F Carr<sup>1</sup>, Charles JL LaPorte<sup>3</sup>, Deirdre Egan<sup>1</sup>,  
Munir Pirmohamed<sup>1</sup>, Andrew Owen<sup>1</sup>, Claudia P Cortes<sup>2</sup>**

<sup>1</sup>University of Liverpool, Liverpool, UK

<sup>2</sup>The Ottawa Hospital Research Institute and The University  
of Ottawa, Canada

<sup>3</sup>School of Medicine University of Chile and Fundación  
Arriarán, Santiago, Chile

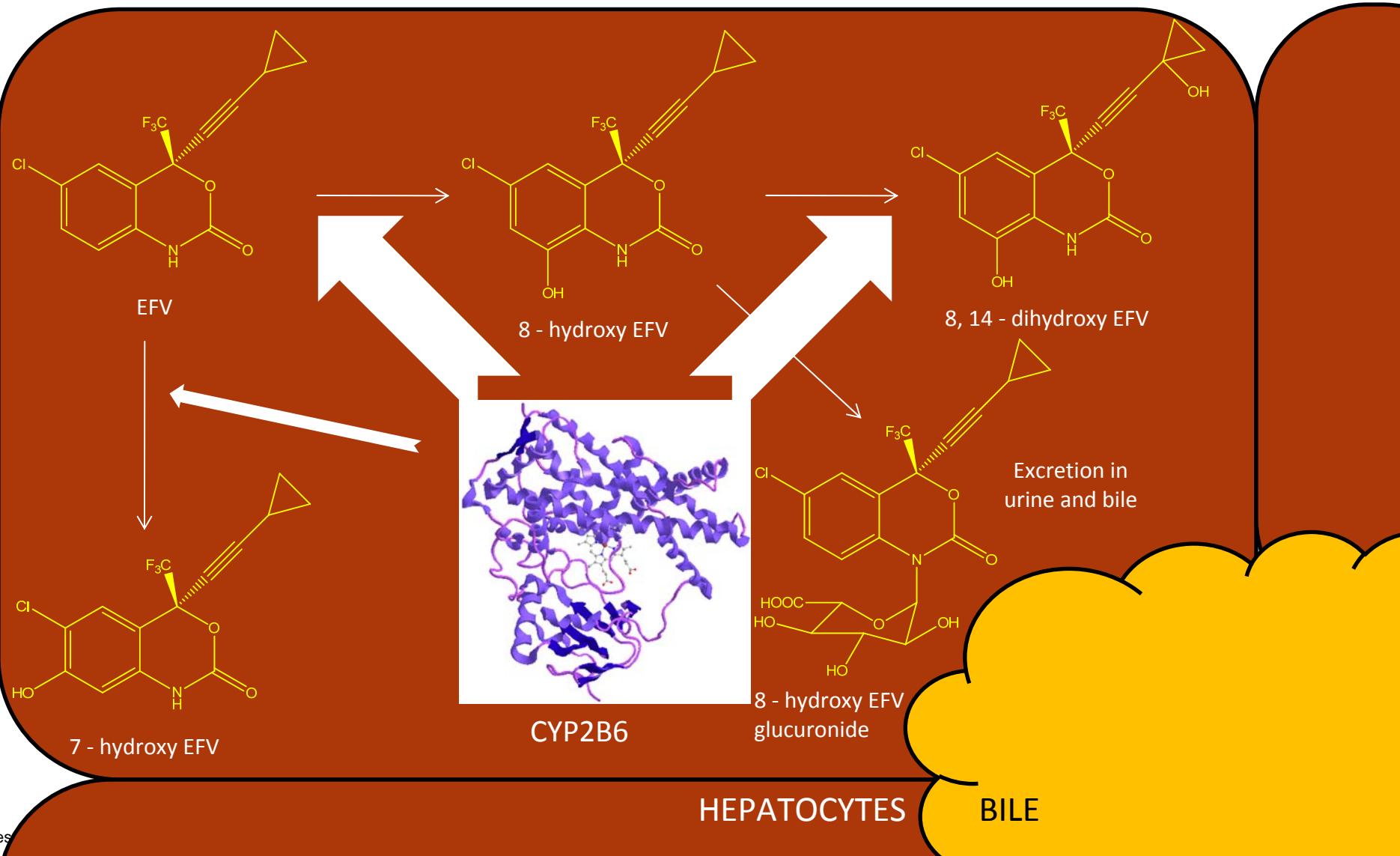
# Background

CNS side effects with efavirenz (EFV) occur in 20 - 50% of patients and generally occur within the first weeks of treatment.

High plasma concentrations of EFV increase the likelihood of CNS side effects and a minimum toxic concentration (MTC) of 4µg/mL has been proposed (Marzolini 2001).

*CYP2B6* 516G>T single nucleotide polymorphism (SNP) is associated with higher plasma EFV concentrations and early CNS side effects (Haas 2004).

# Mechanisms for hepatic clearance of efavirenz



# Aims

To investigate the *CYP2B6* haplotype structure in Chilean HIV patients.

To determine whether a *CYP2B6* composite genetic model including multiple SNPs is more strongly associated with EFV plasma concentrations than 516G>T alone.

# Patients (Santiago)

## **Inclusion criteria:**

- >18 years of age
- Documented HIV-1 infection
- Taking EFV >2 weeks
- Able to provide informed consent

## **Exclusion criteria:**

- Unable to provide informed consent
- Concomitant use of rifampicin

## **Demographics:**

- 219 patients enrolled between September and December 2008 at Fundación Arriarán.
- 74% receiving AZT and 3TC as backbone.
- 71% receiving drug(s) other than HAART

# Drug analysis (Ottawa)

- Validated High Performance Liquid Chromatography coupled with tandem Mass Spectrometry.
- The calibration curve ranged from 0.05 to 5  $\mu\text{g}/\text{mL}$ .
- Sample pretreatment with protein precipitation using methanol.
- Assay is enrolled in an international quality program (KKGTT).

# *CYP2B6* Genotyping (Liverpool)

- 30 SNPs with minor allele frequency (MAF)  $>0.05$  in the HapMap CEU population at  $\sim 1$  kB intervals.
- Genotyping conducted by Sequenom iPLEX MALDI-TOF with one 21-plex and one 9-plex assay.
- Genotyping QC:
  - MAF  $>0.01$
  - Hardy Weinberg disequilibrium  $p > 0.01$
  - Call Rate  $>90\%$
- 13 SNPs passed QC



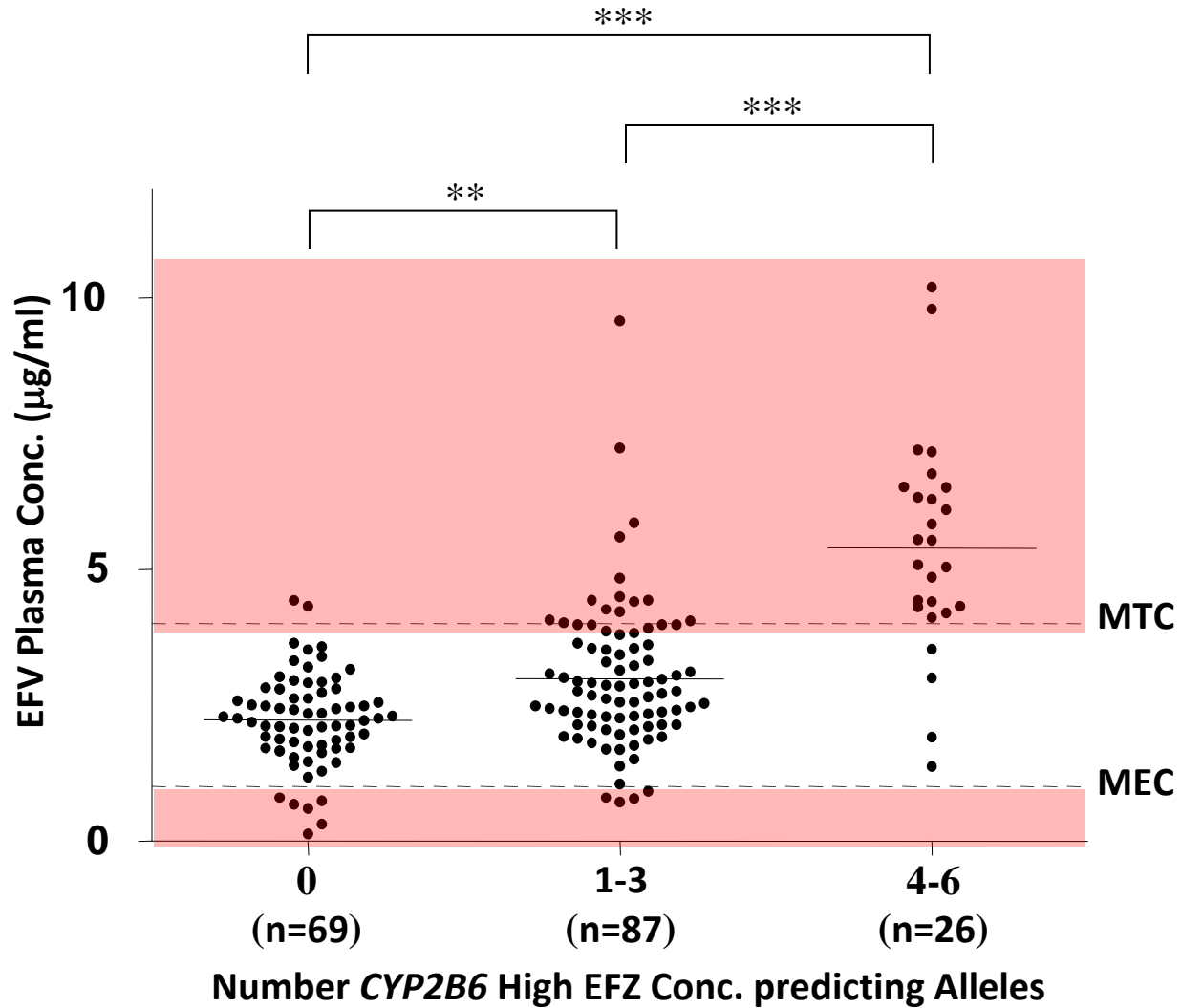
# Association of *CYP2B6* SNPs with EFV plasma Concentrations

SNP	Position	Alleles (A <sub>1</sub> /A <sub>2</sub> )	Frequency		Efavirenz plasma concentration			P
			A <sub>1</sub>	A <sub>2</sub>	A <sub>1</sub> /A <sub>1</sub>	A <sub>1</sub> /A <sub>2</sub>	A <sub>2</sub> /A <sub>2</sub>	
rs4802100	5' UTR	G/C	0.22	0.78	2.57±0.95	2.77±1.11	3.27±1.91	0.082
rs7250601	Intron 1	A/C	0.65	0.35	2.26±0.86	3.16±1.46	5.34±1.93	<b>&lt;0.001</b>
rs16974799	Intron 1	C/T	0.63	0.37	2.20±0.82	3.21±1.52	5.16±1.99	<b>&lt;0.001</b>
rs10500282	Intron 1	T/C	0.63	0.37	2.22±0.84	2.98±1.43	5.32±1.09	<b>&lt;0.001</b>
rs10403955	Intron 1	T/G	0.64	0.36	2.28±0.09	3.08±1.43	5.59±1.87	<b>&lt;0.001</b>
rs3745274	Exon 4	G/T	0.65	0.35	2.20±0.86	3.21±1.42	5.10±1.96	<b>&lt;0.001</b>
rs34083050	Intron 4	A/G	0.14	0.84	2.29±0.00	3.15±1.93	3.08±1.54	0.906
rs2279344	Intron 5	C/A	0.23	0.77	1.91±0.61	2.38±1.12	3.60±1.74	<b>&lt;0.001</b>
rs2279345	Intron 5	T/C	0.23	0.77	1.94±0.69	2.20±0.88	3.59±1.80	<b>&lt;0.001</b>
rs8192719	Intron 8	C/T	0.65	0.35	2.24±0.85	3.09±1.40	5.5±1.86	<b>&lt;0.001</b>
rs11671243	Intron 8	A/C	0.22	0.78	1.91±0.65	2.23±0.92	3.62±1.75	<b>&lt;0.001</b>
rs11673270	Intron 8	A/C	0.66	0.34	2.28±0.89	3.25±1.66	5.07±1.74	<b>&lt;0.001</b>
rs10853744	Intron 8	G/T	0.63	0.37	2.24±0.85	3.08±1.41	5.25±1.94	<b>&lt;0.001</b>

# Composite Genetic Model

- 11/13 SNPs were associated with EFV plasma concentrations ( $p = 4.4 \times 10^{-8}$  to  $3.6 \times 10^{-22}$ ).
- Pair-wise tagging analysis (Haploview;  $R^2 > 0.8$ ) revealed 3 SNPs to be representative of the 11 EFV associated SNPs:
  - rs10403955, rs2279345, rs8192719
- Total number of associated alleles of 3 SNPs tallied for each individual.

# Composite Genetic Model



# Association with concentrations within the proposed therapeutic range

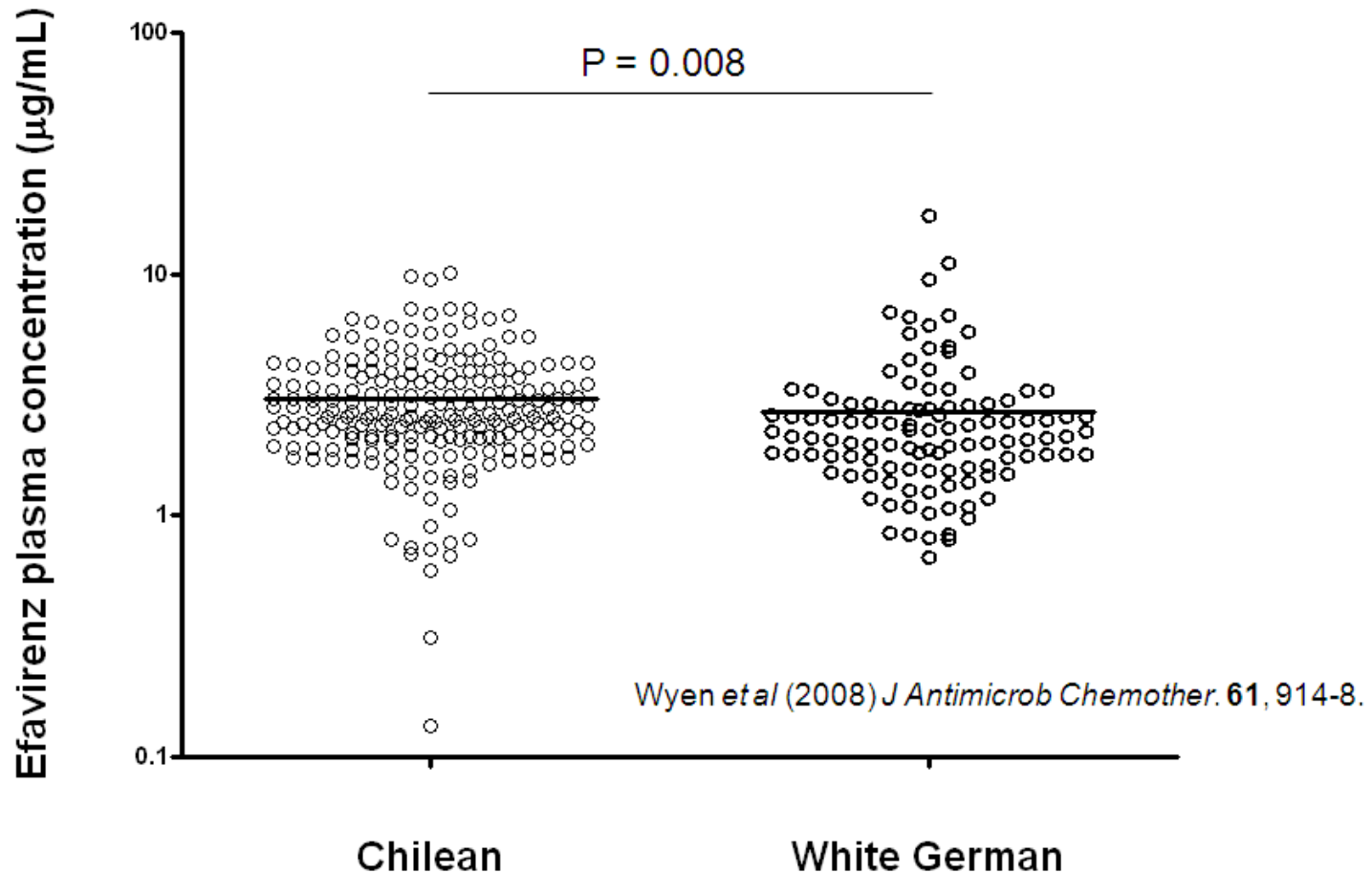
**Plasma EFV**  
**>Minimum Toxic**  
**Concentration**  
**(4µg/ml)**

	EFZ Plasma Conc.> MTC	EFZ Plasma Conc.< MTC	
0-3 High EFZ Conc. Alleles	16	140	Negative Predictive Value= <b>89.7%</b>
4-6 High EFZ Conc. Alleles	22	4	
	Sensitivity= <b>57.9%</b>	Specificity= <b>97.2%</b>	Positive Predictive Value= <b>84.6%</b>

**Plasma EFV**  
**<Minimum Effective**  
**Concentration**  
**(1µg/ml)**

	EFZ Plasma Conc.< MEC	EFZ Plasma Conc.> MEC	
0-3 High EFZ Conc. Alleles	10	146	Positive Predictive Value= <b>6.4%</b>
4-6 High EFZ Conc. Alleles	0	26	
	Sensitivity= <b>100%</b>	Specificity= <b>15.1%</b>	Negative Predictive Value= <b>100%</b>

# Discussion



- No data are available for one of these SNPs in the Hapmap MEX population but allelic frequencies for other *CYP2B6* polymorphisms appear to be comparable.

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## The Liverpool team

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Antonia Weirzbicki  
Helen Reynolds  
Kay Seden  
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Yuan Zhang  
Beth Williamson  
James Hobson

## Collaborators

Santiago  
Claudia Cortes

Ottawa  
Charles la Porte

Torino  
Stefano Bonora  
Giovanni Di Perri  
Marco Siccardi  
Antonio D'Avolio

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