

**Single-nucleotide polymorphisms
ABCB1 3435C>T, 1236C>T and
CYP2B6 516G>T predict higher plasma
concentrations of nevirapine (NVP).**

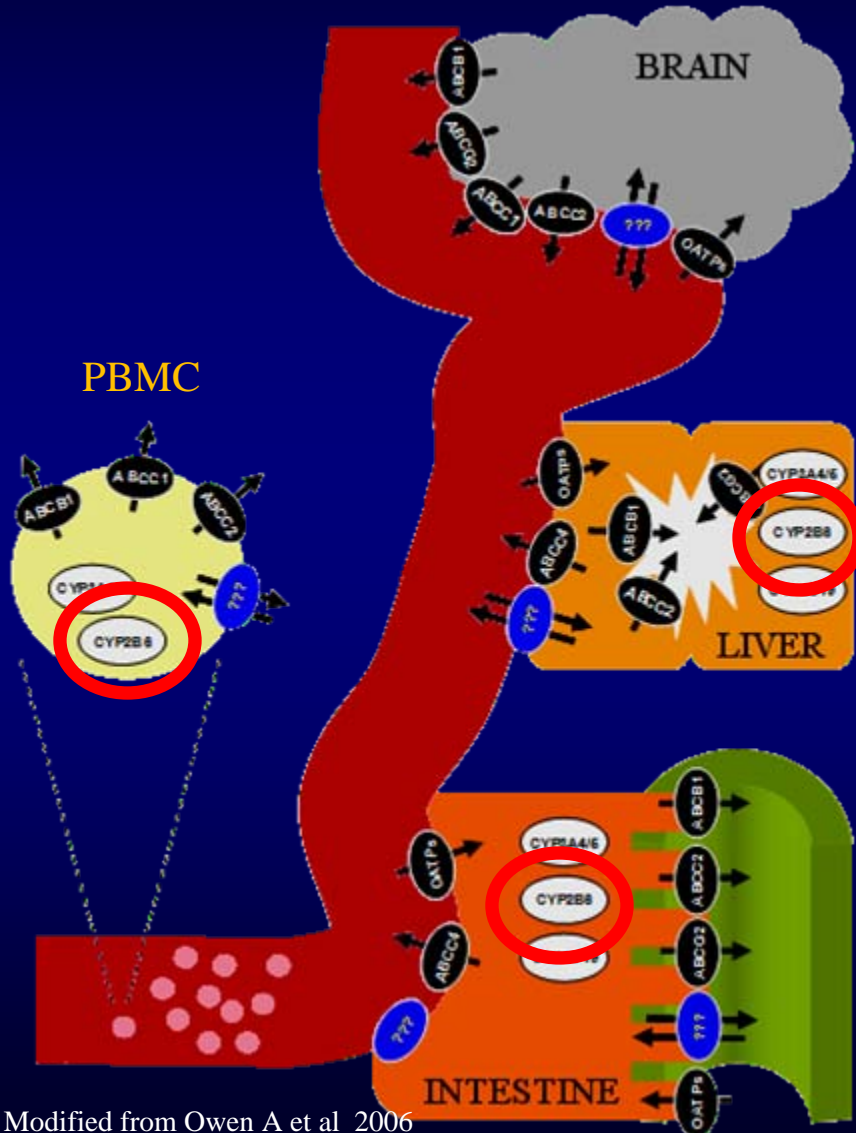
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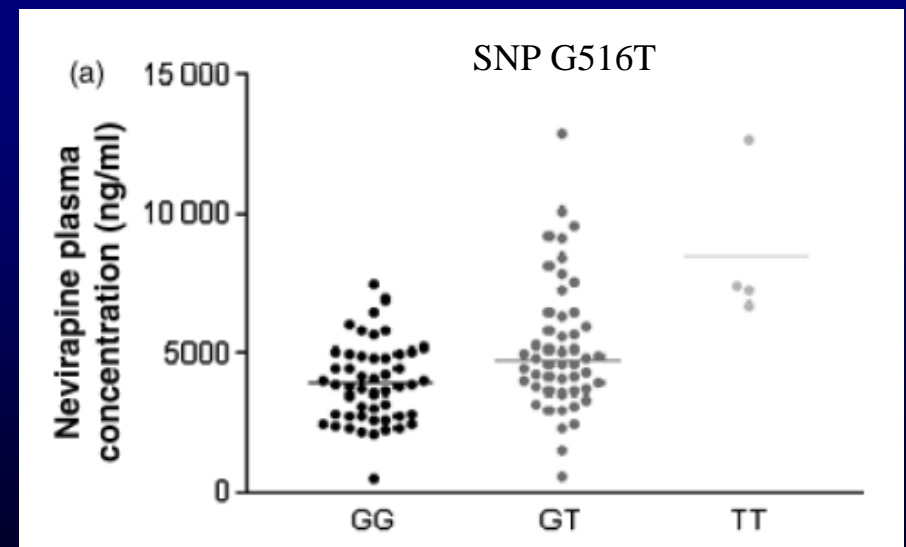
Sorrento (Italy), April 7-9th 2010

11th International Workshop on Clinical Pharmacology of HIV Therapy

CYP450 - 2B6

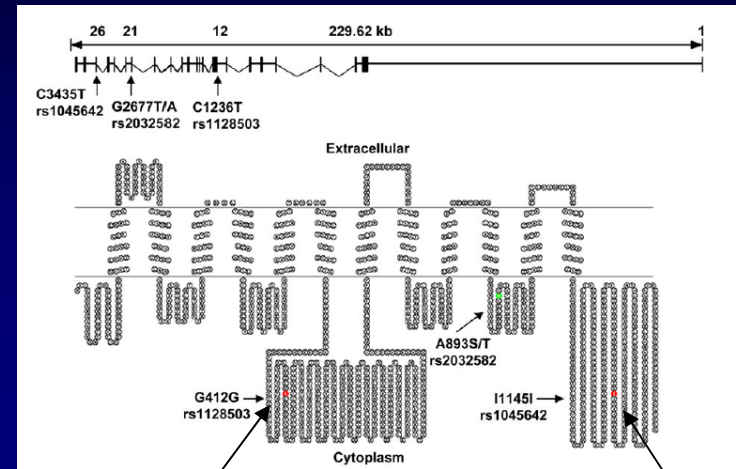
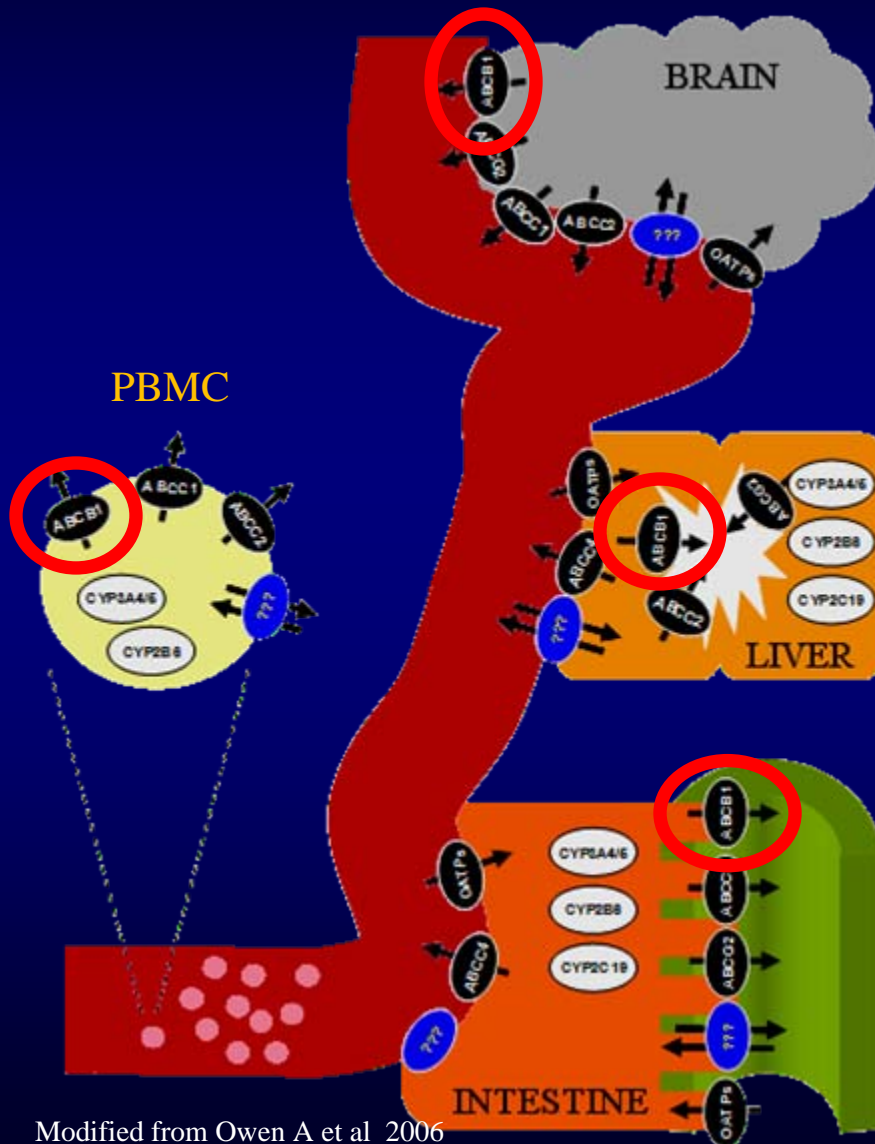


- Nevirapine is primarily metabolized by the CYP450 2B6 enzyme.
- The most important single nucleotide polymorphism is G516T.
- The association of the 516G-T SNP with nevirapine pharmacokinetic is well established.
- The presence of mutation for this SNP cause an higher exposure of plasma concentration of nevirapine.



P-glycoprotein – (MDR1) *ABCB1*

Wang D et al 2006



1236C>T Strong Linkage Disequilibrium (Tang K et al 2002) **3435C>T**

- This protein has an important role in transport of several substrates.
- It plays an important role in first-pass elimination of orally administered drugs.
- It eliminates substrates from the systemic circulation in the kidney and through biliary excretion.
- It plays a defense role in the body by minimizing the intracellular accumulation of xenobiotics.

Modified from Owen A et al 2006

Question:

1. Can P-glycoprotein mediate efflux of Nevirapine?

EVIDENCE

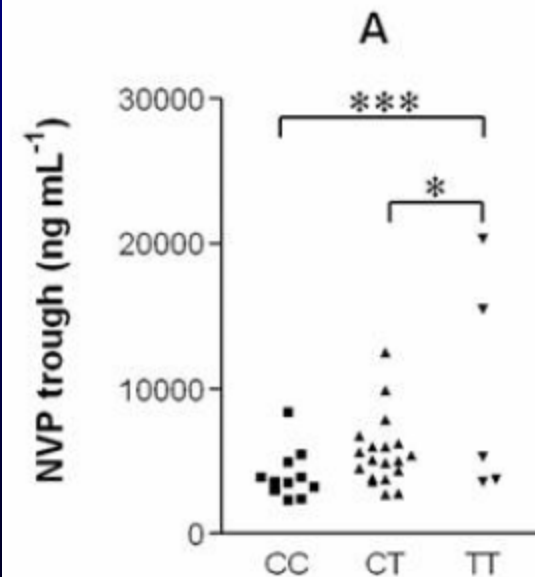
➤ Against

- There is no *in-vitro* test showing Nevirapine as substrate of MDR1 (excluding Haas DW et al. 2005)
- Many papers did not find any significant correlation between MDR1 SNPs and Nevirapine concentrations

➤ PROs

- Haas DW et Al. 2005 (*in-vitro*)
- Possible influence on clearance (Saitho A et al. 2007)
- 3435 association with hepatotoxicity (Cicacci C et al 2010)
- A study found significant influence (*in-vivo*) on C_{trough} (Owen A et al. 2005)

Haas DW, Bartlett J, Anderson J, Sanne I, Wilkinson GR, Quinn J, et al. Pharmacogenetics of nevirapine and hepatotoxicity: NWCS220, an ACTG collaborative study [abstract]. In: Proceedings of the Twelfth Con-



In the discussion and conclusion there are a couple of key points:

Intracellular and plasma pharmacokinetics of nevirapine in human immunodeficiency virus–infected individuals

Background and Objective: Plasma concentrations of nevirapine have been linked to human immunodeficiency virus (HIV) treatment outcome. However, because the site of action of nevirapine is within HIV-infected cells, intracellular concentrations may better relate to antiviral exposure. Investigation of factors that alter the intracellular pharmacokinetics of nevirapine may also aid in our understanding of therapeutic failure. Our objective was to determine intracellular (or cell-associated) nevirapine concentrations over the full dosing interval and to relate protein binding and P-glycoprotein (P-gp) expression to intracellular exposure.

Methods: Plasma and peripheral blood mononuclear cells were isolated from blood samples taken from 10 HIV-infected patients at 0, 2, 4, 8, and 12 hours after dosing. Intracellular and plasma (total and unbound) concentrations were determined by liquid chromatography–tandem mass spectrometry, and the ratios of intracellular to total plasma exposure (area under the concentration–time curves) were calculated. P-gp expression was measured by flow cytometry.

Results: The median intracellular accumulation ratio was 0.005 (range, 0.001–0.054) and remained unchanged over the dosing interval. There was an association between higher plasma concentrations and lower cellular concentrations of nevirapine (total $r^2 = 0.62$, $P = .007$). There was no relationship between percent unbound nevirapine and intracellular nevirapine. There was a correlation between higher plasma nevirapine exposure and higher P-gp expression ($r^2 = 0.77$, $P = .03$), whereas intracellular nevirapine exposure decreased with higher P-gp expression ($r^2 = 0.62$, $P = .01$).

Conclusions: The intracellular accumulation of nevirapine was low, did not change over the dosing interval, and was not related to protein binding. In this small study, cells with higher P-gp expression had lower cellular concentrations of nevirapine. Further studies are required to explore the influx and efflux transporter profile of this drug. (Clin Pharmacol Ther 2005;78:132–42.)

Lisa M. Almond, PhD, Damitha Edirisinghe, MRCP, Mark Dalton, RGN, Atec Bonington, FRCP, David J. Back, PhD, and Saye H. Khoo, FRCP *Liverpool and Manchester, United Kingdom*

effect of a drug *in-vivo*.

CLINICAL PHARMACOLOGY & THERAPEUTICS
2005;78(2):132–42

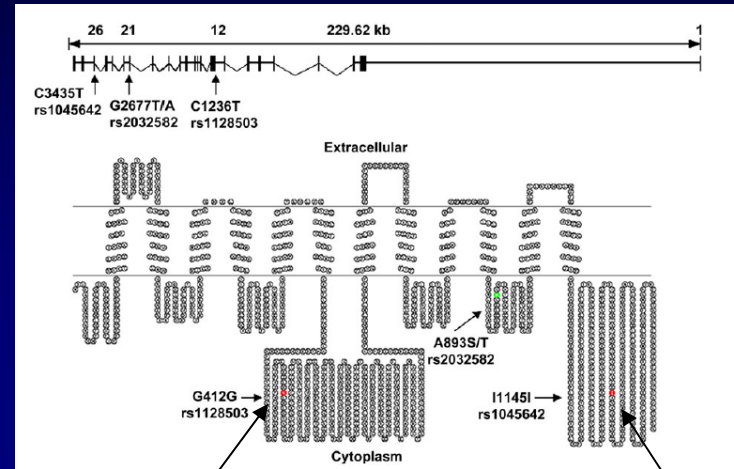
Although nevirapine was not thought to be a P-gp substrate,¹⁴ recent data have provided evidence to the contrary. Studies have reported associations between the C3435T polymorphism in MDR-1 (the gene encoding P-gp) and nevirapine concentrations⁵¹ and hepatotoxicity.¹⁵ Haas et al¹⁵ went on to investigate nevirapine transport *in vitro*, using a polarized cell line, and concluded that nevirapine was transported by P-gp in their model. Here, we show a linear relationship between higher P-gp expression and lower intracellular exposure in these patients. It could be suggested that PBMC P-gp acts as a marker for expression in the gut and that lower intracellular nevirapine concentrations are the result of lower systemic nevirapine concentrations; however, we observed a negative correlation between total and intracellular AUC_{0–12} values. Therefore these data seem to suggest that nevirapine is a substrate for an efflux transporter; if not P-gp itself,

in this study and could explain this variation. However, linear regression analysis showed a correlation between total nevirapine AUC_{0–12} and P-gp expression, where patients with greater exposure to nevirapine had higher P-gp expression. It is, therefore, possible that at higher circulating concentrations of nevirapine the lymphocyte P-gp is up-regulated. Previous studies have found no evidence that nevirapine induced P-gp *in vitro*.²⁰

However, it is important to note that this *in vitro* system was highly simplified. Up-regulation of P-gp can result from binding of the protease inhibitors to the transcription factor pregnane X receptor (PXR, also known as steroid xenobiotic receptor [SXR]),^{48,49} which is expressed in PBMCs.⁵⁰ However, it is possible that nevirapine induces P-gp via a mechanism that cannot occur *in vitro*. These discrepancies highlight the important differences between *in vitro* and *in vivo* systems. Although *in vitro* models are fundamental in the investigation of molecular mechanisms, they can never be used in isolation to predict the full effect of a drug *in vivo*. It is important to note that P-gp expression was not measured in HIV-positive treatment-naïve patients.

P-glycoprotein – (MDR1) *ABCB1*

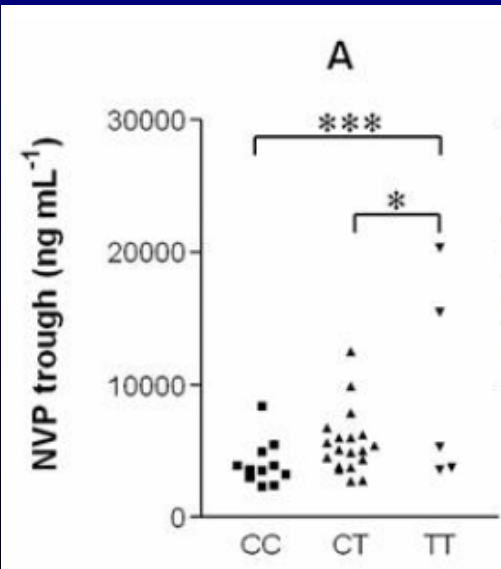
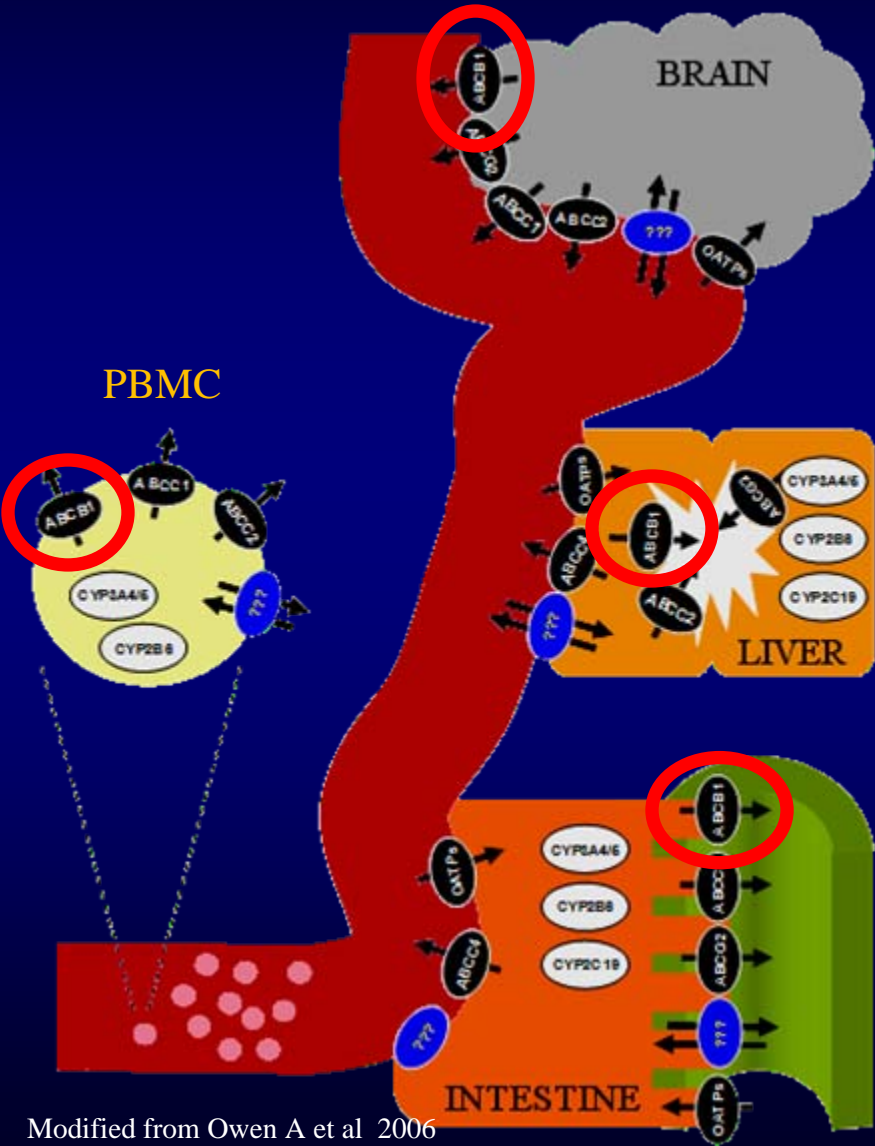
Wang D et al 2006



1236C>T

Strong Linkage Disequilibrium (Tang K et al 2002)

3435C>T



Owen A, Almond L, Hartkorn R, Walsh T, Youle M, Bonington A, et al. Relevance of drug transporters and drug metabolising enzymes to nevirapine: superimposition of host genotype [abstract]. In: Proceedings of the Twelfth Conference on Retroviruses and Opportunistic Infections; Boston, Mass; 2005 Feb 22-25.

Modified from Owen A et al 2006

Table 10. Suggested Minimum Target Trough Concentrations [2-9]
(Updated November 3, 2008)

Drug	Concentration (ng/mL)
Fosamprenavir	400 (measured as amprenavir concentration)
Atazanavir	150
Indinavir	100
Lopinavir	1,000
Nelfinavir ^a	800
Saquinavir	100–250
Efavirenz	1,000
Nevirapine	3,000
Recommendations applicable only to treatment-experienced persons who have resistant HIV-1 strains	
Maraviroc	>50
Tipranavir	20,500

a. Measurable active (M8) metabolite.

DHHS Guidelines 1th December 2009

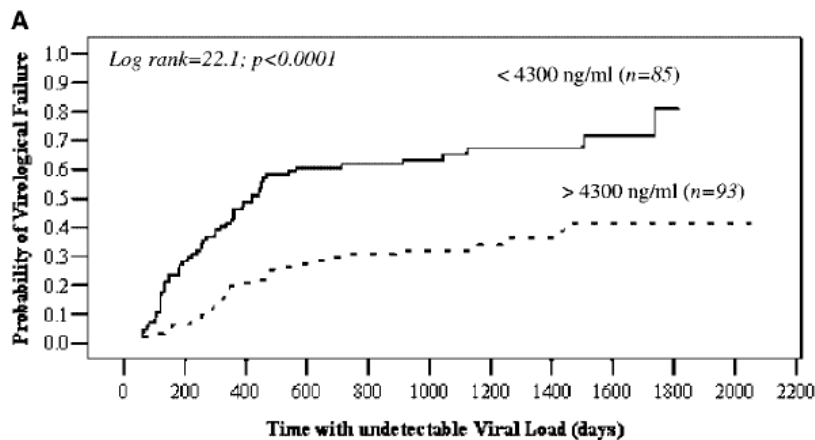
Nevirapine Plasma Exposure Affects both Durability of Viral Suppression and Selection of Nevirapine Primary Resistance Mutations in a Clinical Setting

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Received 8 November 2004/Returned for modification 28 January 2005/Accepted 27 May 2005

The relationship between nevirapine plasma concentrations and the durability of both viral suppression (VS) and selection of nevirapine primary resistance mutations (PRMs) was evaluated. A nevirapine trough concentration (C_{trough}) of $>4,300$ ng/ml was found to predict longer VS. Patients with nevirapine C_{trough} s ranging from 3,100 to 4,300 ng/ml had higher probabilities of developing PRMs than those with nevirapine C_{trough} s below and above this concentration interval.



We have studied PK-PD relationship on NVP

•Not all patients investigated were naive.

CONCLUSION

•Patients with C_{trough} below 4300 ng/ml have higher probability to develop PRMs

To evaluate whether single-nucleotide polymorphisms in these genes, particularly for *MDR1*, may influence nevirapine plasma concentrations, and may predict nevirapine plasma exposure above 4300 ng/ml.

- Patients were recruited in Turin, Italy
- Inclusion criteria were:
 - age of more than 18 years
 - no concomitant interacting drugs
 - no hepatic or renal impairment
 - self-reported adherence more than 95%.
- Genotyping was conducted by real time based allelic discrimination.
- NVP concentrations were quantified using a validated HPLC-PDA method.
- C_{trough} was measured in samples collected 10-14 h after dosing.

Demographic and physical characteristics	
Number of patients	108
Males (%)	77 (71)
Age, median years (IQR)	47 (42-54)
Weight median kg (IQR)	69 (60-80)

Median n. C_{trough} for patients was (\pm SD) 1.59 (\pm 0.85).

Nevirapine concentrations were not correlated with any demographic factors.

- The allele frequencies for *ABCB1* 3435T, *ABCB1* 1236T, *CYP-2B6* 516T and were 44.9%, 40.7% and 30.5% respectively.

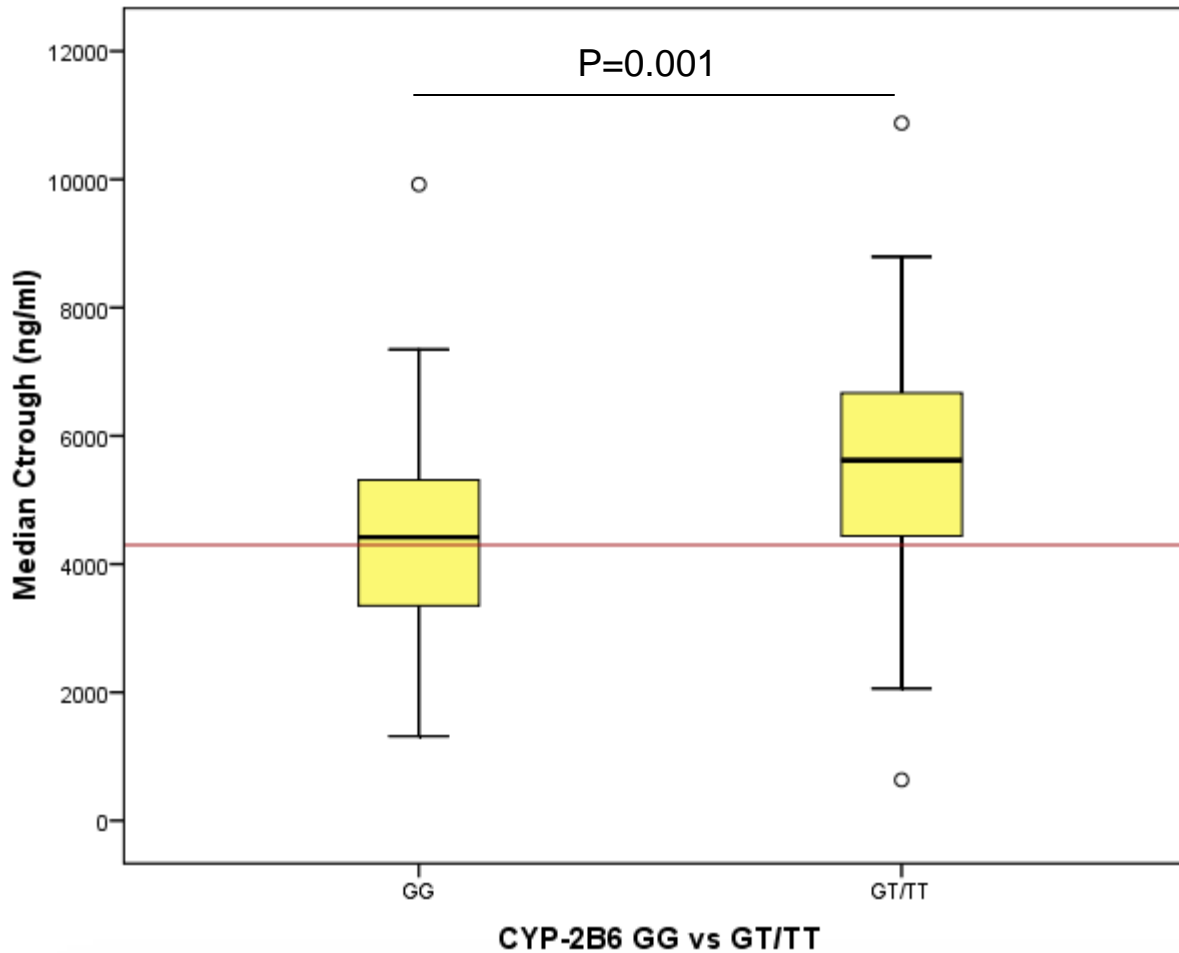
-All SNPs were in Hardy-Weinberg equilibrium.

CYP450 - 2B6

RESULTS

As expected, median Ctroughs of NVP were higher for 2B6 mutate genotypes, with a significant P value.

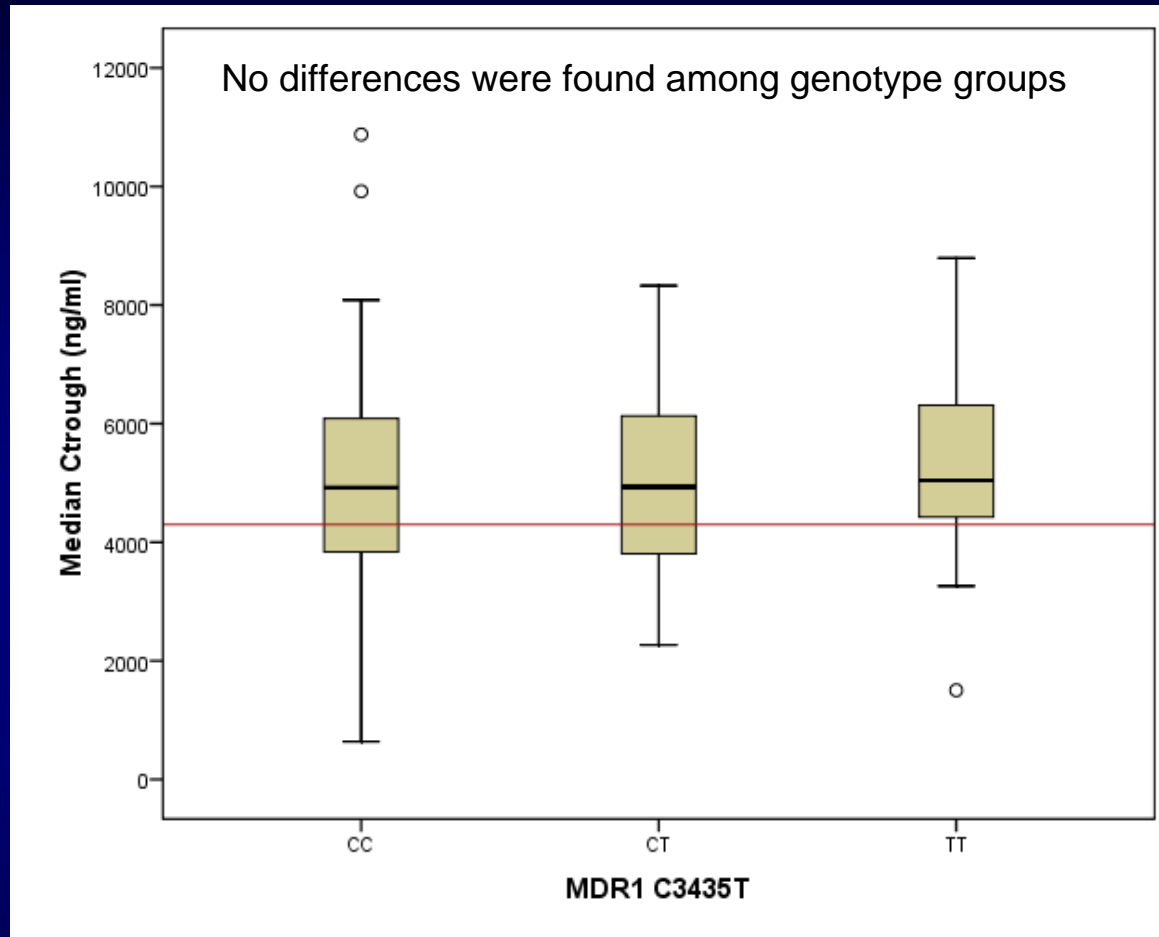
These two groups of patients (GT and TT) have very close concentrations.



2B6-516	N (%)	NVP Ct Mean (\pm SD)	P value
GG	54 (50.0)	4468 (\pm 1568)	0.0021
GT	42 (38.9)	5549 (\pm 1777)	
GT+TT	54 (50.0)	5624 (\pm 1812)	
TT	12 (11.1)	5888 (\pm 1988)	

P-glycoprotein - *MDR1* (C3435T)

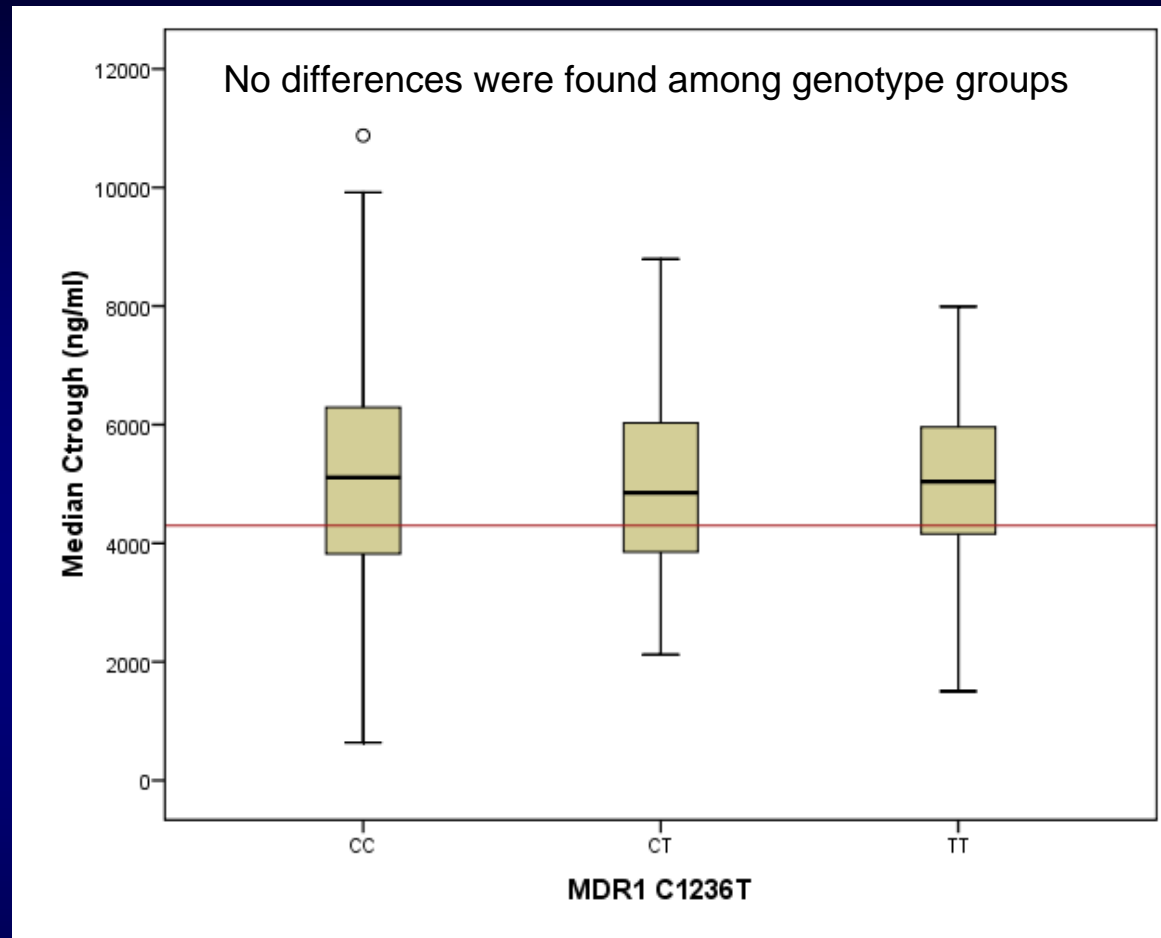
RESULTS



MDR1-3435	N (%)	NVP Ct Mean (\pm SD)	P value
CC	33 (30.6)	4989 (\pm 2242)	0.806
CT	53 (49.0)	4988 (\pm 1502)	
TT	22 (20.4)	5271 (\pm 1784)	

P-glycoprotein – *MDR1* (C1236T)

RESULTS



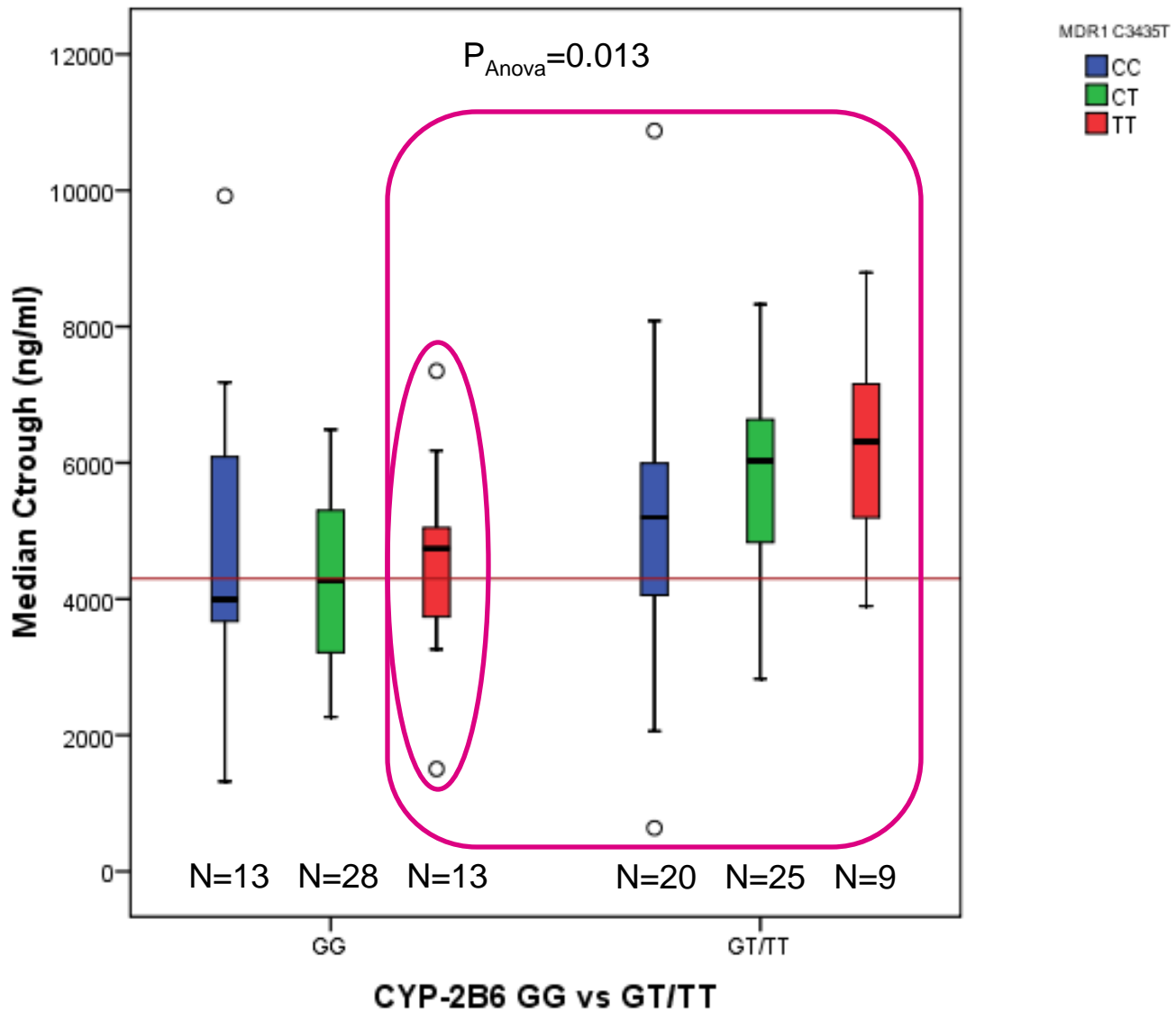
MDR1-1236	N (%)	NVP Ct Mean (\pm SD)	P value
CC	39 (36.1)	5134 (\pm 2151)	0.895
CT	50 (46.3)	4959 (\pm 1568)	
TT	19 (17.6)	5093 (\pm 1557)	

CYP450 - 2B6/MDR1 (3435)

RESULTS

When consider the two groups based on 2B6 genotypes, ... stratifying 3435 we observed an Anova for trend with significant P value.

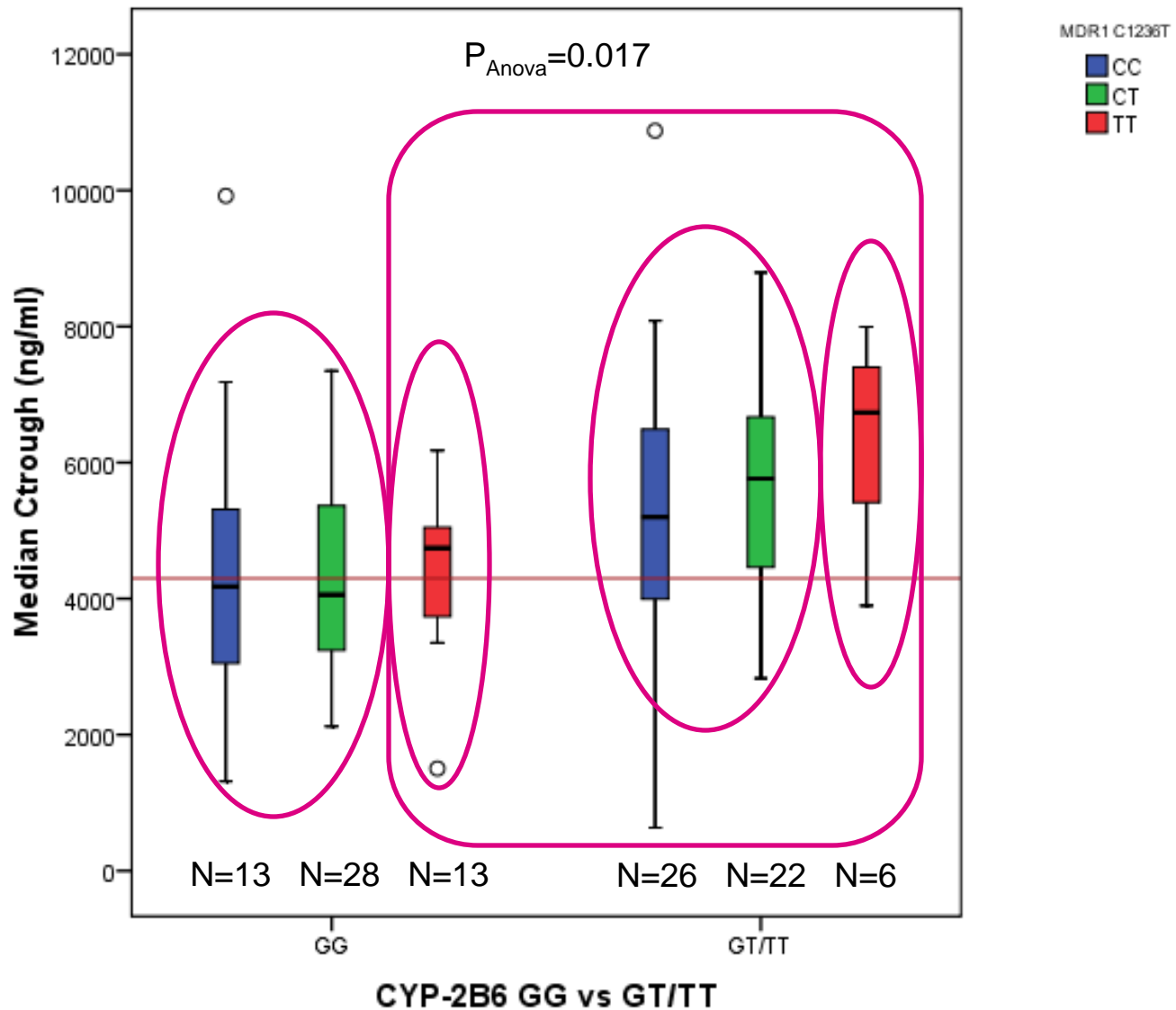
Median Ctrough was above 4300 ng/ml in 2B6 WT (GG) when 3435 is homozygote mutata.



CYP450 - 2B6/MDR1 (1236)

RESULTS

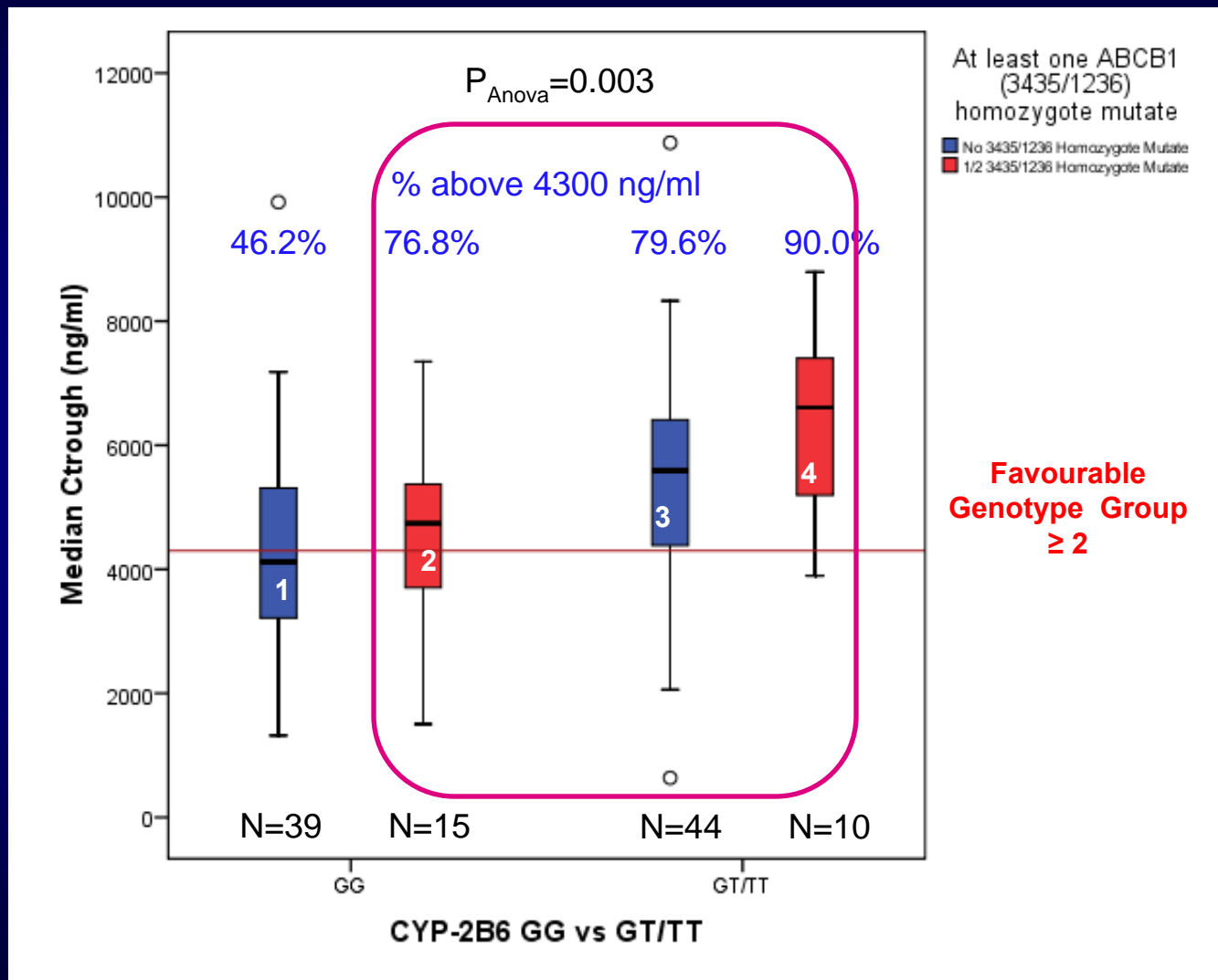
In order to assess the additive impact of predictive genotype, patients were assigned to four groups. ...stratifying 1236 we observed an Anova for trend with significant P value.



CYP450 - 2B6/MDR1

RESULTS

at least one (3435/1236) homozygote mutate



...based on 2B6 genotype and on the presence at least one MDR1 homozygote mutate SNP, predictor of nevirapine Ctrough above 4300 ng/ml.

- CYP-2B6 polymorphism has been repeatedly found to be a significant genetic driver of nevirapine plasma exposure.
- P-glycoprotein SNPs might contribute to interpret inter-individual variability.
- Integrated CYP-2B6 and MDR1 SNPs may better predict nevirapine plasma exposure.
- Further studies are now warranted to confirm this composite association and to evaluate whether a pharmacogenetic approach can be adopted to optimise selection of NVP intakers.



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A screenshot of the TDM (Therapeutic Drug Monitoring) website. The page features a header with the TDM logo and the title 'Therapeutic Drug Monitoring'. Below the header, there is a navigation menu with 'Links' and two items: 'Linee Guida BHIVA' and 'Linee Guida DHHS'. The main content area is titled 'Terapia anti HIV: ogni paziente è unico' and contains a section 'Il ruolo del Therapeutic Drug Monitoring (TDM)'. This section includes a 'Certified Laboratory' badge (EN UNI ISO 9001:2000) and a paragraph explaining the role of TDM in HIV therapy. A 'Web Site' link with a UK flag icon is also visible. The footer of the page contains the URL 'www.tdm-torino.org'.

www.tdm-torino.org