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Full Length Research Paper

Impact of gender, weight and CYP2B6 genotype on efavirenz exposure in patients on HIV/AIDS and TB treatment: Implications for individualising therapy

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Gender, weight and cytochrome P450 2B6 (CYP2B6) 516G>T genetic data of 61 patients on efavirenz containing highly active anti-retroviral therapy (HAART) was collated and analysed. Multivariate data analysis and correlations between variables were done to determine the relative contributions of gender, weight and CYP2B6 genetic polymorphism. Models were derived to guide dose adjustment in patients predicted to have unsafe drug exposure. The data showed that 44% of patients had concentrations above the minimum safe concentrations. Gender, weight and genetics explain 22% of the variation of therapeutic levels efavirenz drug exposure in the model. The model generated indicates that all patients homozygous for the 516G>T variant, irrespective of gender or weight required dose adjustment to 200 mg/day, whilst patients of the G/G genotype should be given the standard 600 mg/day. Patients of the G/T genotype showed mixed outcomes. Analysis of this group showed that females of weight less than 62 kg need dose adjustment to 400 mg/day whereas their male counterparts did not need dose adjustment.

Key words: Efavirenz, CYP2B6 516G>T, weight, gender, dose adjustment, partial least squares.

INTRODUCTION

Work on the exposure levels of efavirenz has received particular attention because this drug is used as a substitute of nevirapine in patients undergoing both HIV/AIDS and TB treatment. Efavirenz is metabolised more by cytochrome P450 2B6 (CYP2B6) than by cytochrome P450 3A4 (CYP3A4) (Ward et al., 2003). In the use of efavirenz, the CYP2B6 516G>T genotype has been associated with high plasma levels of the drug (Leger et al., 2009; Rotger et al., 2005) and also susceptibility to CNS adverse reactions (Gatanaga and Oka, 2009; Haas et al., 2004). Many studies have shown this and other variants of CYP2B6 result in increased exposure of efavirenz in many populations (Gatanaga et al., 2007; Jamshidi et al., 2010; Mukonzo et al., 2009).

Population studies have shown the frequency of 516G>T variant to be relatively high across populations with African populations, 28 to 49%, compared to Caucasian 22 to 29% and oriental populations 14 to 43% (Gatanaga et al., 2007; Nyakutira et al., 2008; Xu et al., 2007; Matimba et al., 2008).

Based on these recent observations, we re-evaluate our data (Nyakutira et al., 2008) for the relative contribution of gender, weight and genetic polymorphism of CYP2B6 on efavirenz exposure levels towards the derivation of a clinical dose adjustment algorithm in the use of the drug. A new analysis of the data using partial least squares (PLS) method was used in order to capture the multivariate nature of the effect of several variables on the efavirenz exposure levels.

MATERIALS AND METHODS

Patient data

Patient data was obtained from our previous work (Nyakutira et al., 2008) and only 61 patients were considered based on their

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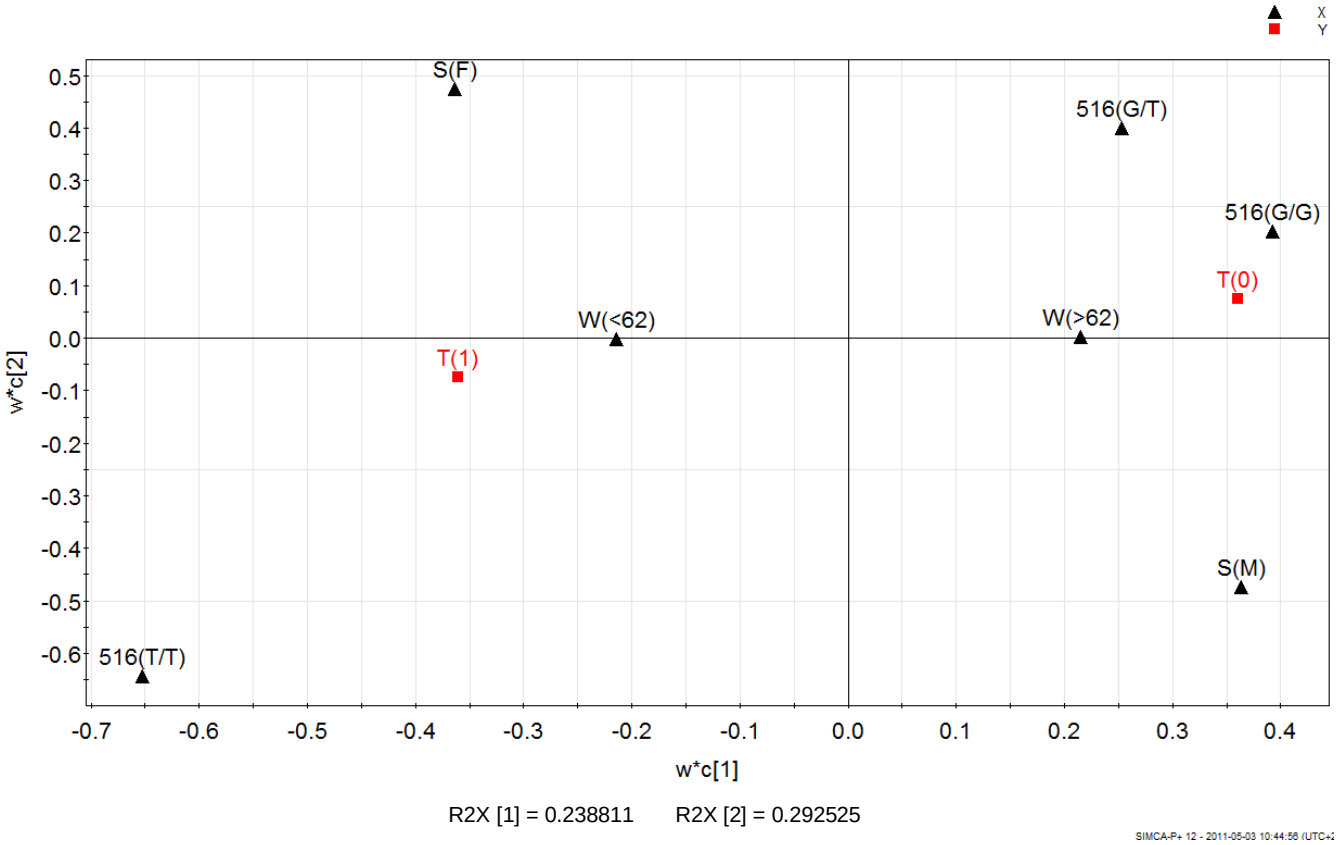

Figure 1. A PLS loading plot of gender weight and genotype and therapeutic margins indicating a positive correlation of 516G>T, T/T, female and weight of below 62 with efavirenz concentrations above 4 µg/ml, T (1), and positive correlation of male, 516G>T, G/G, G/T and weight greater or equal to 62 kg with efavirenz concentration within the 1 to 4 µg/ml range, T (0). The variance explained in this model for efavirenz plasma concentration is 22% (n = 61).

complete data with respect to efavirenz plasma concentration, 516G>T genotype, gender, age and weight. The patients were on TB treatment with regimen containing Rifampcin and Isoniazid, and also on Stavudine and Lamivudine as part of their highly active anti-retroviral therapy (HAART).

Statistical analysis of patient data

Multivariate analysis method of PLS on gender, weight and genotype data against therapeutic exposure levels was done using the Soft Independent Modelling of Class Analogy (SIMCA 12+) software. Principal components 1, 2, and 3 were considered for overall modelling, but only data from PC1 was further considered for PLS because it was the statistically significant component on all the models at 5% level of significance. Age was dropped because of its low contribution to variability in explaining efavirenz levels (R^2 = 0.003). The median age (year) and interquartile range was 39 (36 to 44).

In the analyses, gender, weight and genotype were coded as follows: Gender(S): Males represented by M, Females represented by F; Genotypes for CYP2B6 516G>T(516): G/G,G/T and T/T; Weight(W): less than 62 kg (<62), greater or equal to 62 kg (≥ 62); Therapeutic range(T): within the target therapeutics window (1 to 4 µg/ml (Katsounas et al., 2007) represented by 0; >4 µg/ml -1) and below the maximum effective concentration (MEC) (<1 µg/ml represented by -1). Since there was no patient with concentration below the MEC in this study, only codes T (0) and T (1) were used in the analysis. The weight category cut-off of 62 kg was arrived at after plotting an estimated linear regression line of efavirenz concentration against weight, which gave the equation:

y = -0.403x + 63.183 (y = weight in kg, x = drug concentration in µg/ml).

There is evidence of negative correlation between weight and plasma concentration (r = -0.144, P = 0.269). Taking the efavirenz cut-off of 4 µg/ml, to divide the patients into those within the therapeutic range (1 to 4 µg/ml), T (0) and those above 4 µg/ml (T (1)), the weight cut-off was derived as 62 kg.

Dosing algorithm generation

From the PLS global model (Figure 1), patients in various combinations of gender, weight and genotype were evaluated for closeness to either the T (0) or T (1) concentration range. This closeness was captured by values between 0 and 1 with values associated with T (0) being between 0 and 0.5 and those associated with T (1) being between 0.5 and 1. These values are weighted predictions for each group generated by PLS model. For each variable combination of gender, weight and genotype, patients falling in each group had the median of their plasma concentrations

Table 1. Gender, weight, and CYP2B6 516G>T genotype and efavirenz exposure level data of 61 patients on HIV and TB treatment.

Demographics and genetics		Number of patients	Median efavirenz (IQR) µg/ml	Patients with concentrations above 4 µg/ml (%)	VIP for 1st PC for global model
Gender	Male	25	3.44 (2.72,7.23)	8/25 (32)	0.96622
	Female	36	4.53 (2.80,9.06)	19/36 (53)	0.96622
Weight	≥62 kg	29	3.32 (2.23,9.03)	11/29 (38)	0.5676
	<62 kg	32	4.17 (3.09,7.12)	16/32 (50)	0.5676
Genotype (CYP2B6 516G>T)	G/G	13	3.32 (1.62,3.64)	3/13 (23)	1.0382
	G/T	32	3.49 (2.81,6.14)	12/32 (38)	0.6689
	T/T	16	8.70 (5.01,11.40)	12/16 (75)	1.7259

VIP: Very important variable.

calculated. The median drug concentration (*y*) of each category was plotted against the predicted weighted value (*x*), between 0.0 and 1.0, of the fitting of the variable clusters. The data was used to generate the standard curve ($y = 8.9517x + 1.1601$, $R^2 = 0.89761$) from which, efavirenz concentrations associated with various drug doses could be estimated (a direct linear proportional relationship was assumed).

RESULTS

From Figure 1, the plot on the relationship of gender, genotype, weight and 516G>T genotype to efavirenz concentration above 4 µg/ml, coded T(1) showed that, in the first component, PC1, the T/T genotype, female status and weight below 62 kg positively correlated with concentrations above 4 µg/ml. The plot also showed that the G/G and G/T genotype, the male status and weight above or equal to 62 kg correlated with concentration within the therapeutic window [code T(0)] (Figure 1). The variables gender, weight and CYP2B6 genotype were shown to explain 22% of the variation in efavirenz concentration. Following the correlations revealed by PLS (Figure 1), statistical measures of the association of gender, weight categories and 516G>T genotypes were calculated. The order of importance of the variables in determining how the model is formed is as shown in Table 1. The T/T genotype and G/G genotype were shown to be the most important followed by gender. Weight was shown to have the least separation capabilities into the therapeutic window and above 4 µg/ml efavirenz concentration. Furthermore, T/T and G/G genotypes were shown to contribute significantly in how they distinctly relate to plasma efavirenz levels and are very significant along the 1st principal component (Table 1). Separation of efavirenz plasma concentration is relatively significant according to gender and relatively weak according to G/T genotype.

Prediction of dose adjustments

Using the standard curve for 600 mg/day based on a plot of the level of fitting of variables to the T(1) and T(0), efavirenz concentrations were plotted against the median plasma concentration of efavirenz in patients of different variable clusters, after which predictions of plasma concentration of efavirenz when given at different doses were then made. Predictions of efavirenz concentrations for the various variable combination patients were predicted for 200, 400, 600 and 800 mg/day doses (Table 2). The choice of 800 mg/day is based on some recommendations to increase efavirenz concentration to this dose when co-administered with rifampcin (Stohr et al., 2008). The choice of 200 and 400 mg was based on previous modelling and clinical dose adjustment that have been associated with the use of efavirenz (Gatanaga and Oka, 2009; Nyakutira et al., 2008; Gatanaga et al., 2007). The data showed that if given 800 mg, 53/61 patients were predicted to have plasma concentrations above 4 µg/ml. If given 200 mg/day, 8/61 patients (all males of weight >62 kg and of the GG or GT genotype) were predicted to have concentration below the MEC. If given 400 mg/day, 16/61 patients (all of the T/T genotype and of mixed gender and weight categories) are predicted to have concentration above 4 µg/ml. The results we obtained on dose proposition are comparable to the ones from NONMEM (Cabrera et al., 2009; Nyakutira et al., 2008).

Model validation

Using $PE_i = ODV_i - PDV_i$ where PE_i is the prediction error of the *ith* individual, ODV_i is the observed dependent variable in the *ith* individual and PDV_i is the predicted dependent variable in the *ith* individual. The 95% confidence interval for the mean prediction error for the

Table 2. Predicted 12 h post plasma efavirenz concentration in relation to dose taken.

Weighted prediction for the clusters PLS[1]	n	Patient description	Median (µg/ml)	200 mg/day predicted plasma efavirenz conc. (µg/ml)	400 mg/day predicted plasma efavirenz conc. (µg/ml)	600 mg/day predicted plasma efavirenz conc. (µg/ml)	800 mg/day predicted plasma efavirenz conc. (µg/ml)
0.0583139	3	M,GG,W≥62	2.53	0.56	1.12	1.68	2.24
0.153393	5	M,GT,W≥62	3.27	0.84	1.69	2.53	3.38
0.232465	5	M,GG,W<62	3.52	1.08	2.16	3.24	4.32
0.236611	2	F,GG,W≥62	3.18	1.09	2.19	3.28	4.37
0.327544	5	M,GT,W<62	3.31	1.36	2.73	4.09	5.46
0.33169	9	F,GT,W≥62	3.20	1.38	2.75	4.13	5.51
0.410762	3	F,GG,W<62	3.64	1.61	3.22	4.84	6.45
0.505841	13	F,GT,W<62	4.90	1.90	3.79	5.69	7.58
0.583863	6	M,TT,W≥62	7.23	2.13	4.26	6.39	8.52
0.758015	1	M,TT,W<62	9.25	2.65	5.30	7.95	10.59
0.762161	4	F,TT,W≥62	8.17	2.66	5.32	7.98	10.64
0.936312	5	F,TT,W<62	9.14	3.18	6.36	9.54	12.72

[1]A value close to zero for the values in the first column indicates a gravitation towards the therapeutic window, T(0). As the prediction move toward 1 it indicates weighting towards above 4 µg/ml [T(1)].

model (Figure 1) is given by (-0.1135802, 0.1135802). Since the confidence interval (CI) contain 0, the model has adequate predictability and without significant error (Ette and Williams, 2007). Cross-validation [where one data point is left out (leave one out approach)] showed statistically significant results with the cumulative fraction of the total variation of therapeutic concentrations ($Q^2_{cumulative}$) that could be predicted by components as 0.08 for the model. This was significant since it is greater than 0.05 (n<100).

The percentage positive (correct) predictions for individuals in the model, 74%, for all individuals global are as shown in Figure 1.

DISCUSSION

In this study, we have shown that gender, weight, and 516G>T genotype are important determinants on whether a patient will have efavirenz concentrations within the therapeutic range, T (0), or above 4 µg/ml, T (1). Based on data from the PLS and dose predictions, a clinical dosing algorithm was proposed (Figure 2). This proposes that all patients of the T/T genotype have their dose adjusted to 200 mg/day. Those of the G/G genotype should be given the standard dose of 600 mg/day. Patients of the G/T genotype given mixed results, males generally tolerating the standard dose of 600 mg, whilst their female counterparts of weight <62 kg requires a dose adjustment to 400 mg/day.

Our results agree with observations by a number of research groups. Manosuthi et al. (2009) showed that higher weights were associated with lower efavirenz concentrations. Several works showed that gender affected efavirenz plasma concentration (Burger et al., 2005; Mukonzo et al., 2009; Nyakutira et al., 2008). Without taking 516G>T variant into account, Burger et al. (2005) observed that women were at risk of higher efavirenz concentrations as compared to men and hinted weight difference as a possible cause. Females had mean body weight of 65.3 kg as compared to 75.1 kg of males.

Our findings however differ on conclusions reached by others, who discounted weight and gender as important determinants of plasma concentrations using multiple linear regressions (Cohen et al., 2009; Ramachandran et al., 2009). The use of multiple linear/logistic regressions in these studies, might have failed to identify covariances of these variables as we did by using PLS. The conclusion of Cohen et al. (2009) and Ramachandran et al. (2009) could also have been affected by the fact that their samples were predominantly male. This group is not significantly affected by weight.

We do not discount the importance of CYP2A6 and UGT2B7 as highlighted by Kwara et al. (2009). Other variants commonly found in African populations and associated with reduced enzyme activity include CYP2B6*16 and CYP2B6*18 (Jamshidi et al., 2010). The relative high frequency of 516G>T in African populations as compared to Caucasian and Oriental populations make it a promising biomarker for efavirenz exposure levels in Africa. Follow up studies are important, given the fact that the variables considered in this study account for only 22% of the variability in efavirenz concentration, implying that there are other environmental and/or genetic factors that can improve our proposed algorithm. With our sample size of 61 patients, the mean C_{min} (±SD) was 5.8 (±4.2) µg/ml, with a coefficient of variance (CV)

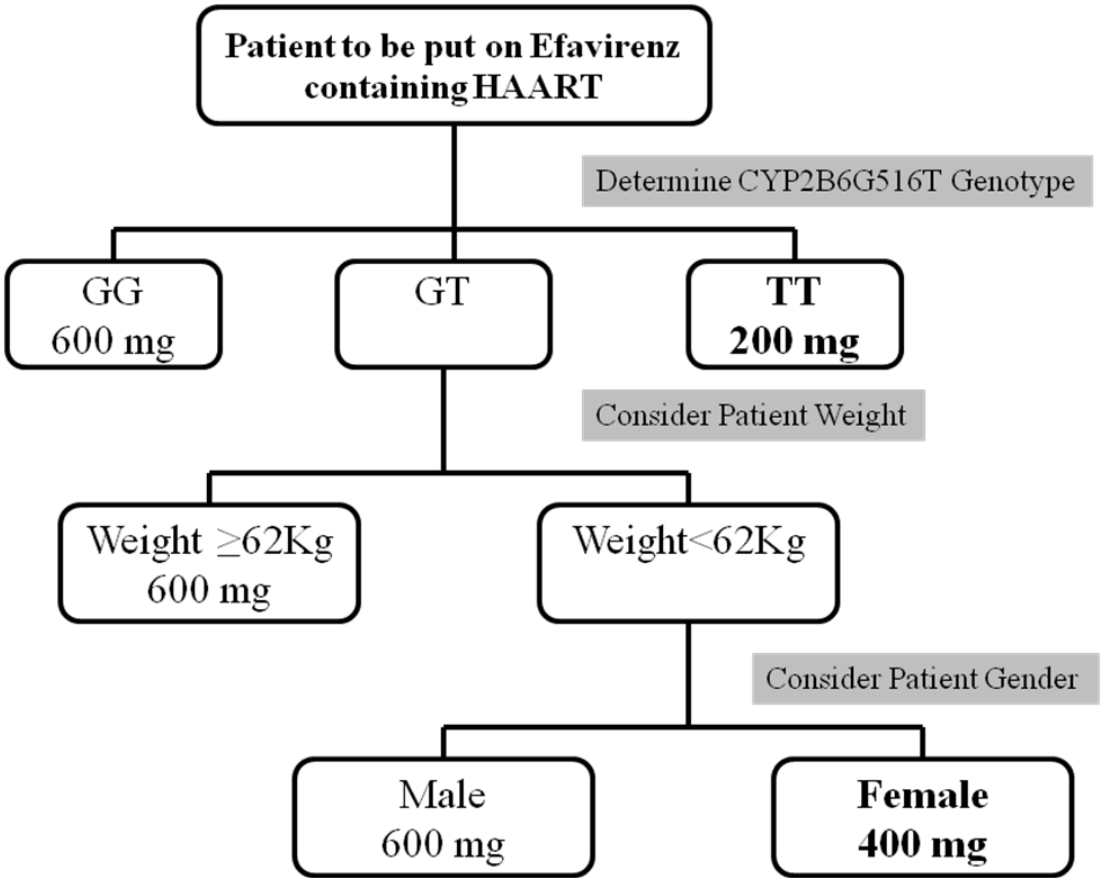

Figure 2. Proposed efavirenz dose adjustment algorithm. CYP2B6 516G>T genotype has the greatest effect on efavirenz concentration with all patients with the poor metaboliser status, T/T, requiring dose adjustment downwards to 200 mg. Female patients of the G/T genotype and of weight lower than 62 kg, also require dose adjustment to 400 mg/day.

of 73%. This level of variation is lower than the 121% observed in 94 patients (Kwara et al., 2009). To capture the large variation of efavirenz exposure levels, a larger patient's sample size would be desirable. A large sample size will also ensure that we have more patients in the various cluster groups we have identified in this study.

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