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What is This?
Sleep quality in efavirenz-treated Chinese HIV patients – comparing between GT and GG genotype of CYP2B6-516 G/T polymorphisms

Shui Shan Lee1,2, Kin Wang To3, Man Po Lee4, Ngai Sze Wong2, Denise PC Chan2, Patrick CK Li4, Siu Wai Cheung1 and Raphael CY Chan1

Abstract
Seventy-two adult Chinese HIV-positive treatment-naïve patients were recruited in a study to evaluate prospectively the associations between CYP2B6 516 G/T polymorphisms and sleep quality following treatment with an efavirenz-based regimen. Overall, the patients gave an allelic frequency of 0.3 for CYP2B6 516 T, and a genotype frequency of 9.4% for TT. Compared to GG, GT gave a higher median value of plasma efavirenz level at four weeks (3.77 mg/L vs 2.59 mg/L, \( p < 0.001 \)) and 12 months (3.57 mg/L vs 2.97 mg/L, \( p = 0.026 \)). Using generalised estimating equations analysis to track the variance over time, there was poorer Pittsburgh Sleep Quality Index in GT compared to GG, while GT was associated with a higher efavirenz level of \( >4 \) mg/L. There was however no difference in the component sleep scores nor was there direct association between sleep quality and plasma efavirenz levels. The results suggested that CYP2B6 genotype was associated with different patterns of sleep problems, further investigation of which is warranted with the objective of optimizing therapy with efavirenz-based regimens.

Keywords
HIV, AIDS, antiretroviral therapy, efavirenz, CYP2B6, sleep quality, Pittsburgh sleep quality index, pharmacogenetics, therapeutic drug monitoring

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Introduction
For over a decade, efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor, has continued to be a preferred component of highly active antiretroviral therapy (HAART) regimens. Efficacy aside, EFV enjoys the convenience of once-daily dosing, availability of co-formulated single-tablet regimens, and it could be used as an alternative to protease inhibitor drugs. Neuropsychiatric side effects are, however, common and have been reported in over half of the EFV-treated HIV patients.1 Classically, symptoms begin shortly after initiation of therapy, and often resolve by week 4.2 While such toxicity is believed to be short-lived, discontinuation and switching are needed in a proportion of those treated.3 In treatment-naïve patients commenced on Atripla, an EFV-containing single-tablet regimen, cessation was reported in one-fifth.4 Various forms of sleep problems have been found to be associated with EFV treatment, the presentations of which include insomnia, reduced sleep efficiency, vivid dreams, which are also more prevalent in the initial weeks.2,5,6

From published literature and clinical experiences, there is considerable variability in the severity and

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duration of EFV-associated neuropsychiatric toxicity. The influence of host genetics should not be ignored. Of note is the metabolism of EFV by the cytochrome P450 2B6 enzyme system, the 516 G > T polymorphism of which is associated with a significant decrease in EFV metabolism and hence its accumulation in the body.\(^7\) Ethnicity does play a key role, as reflected by the variation of allelic frequency for T across populations, from 0.38 in South Africans, 0.34 in Chinese to 0.22 in Caucasians.\(^8,9\) Homozygocity for 516 G > T is strongly associated with a high plasma level of EFV.\(^10\) Whereas severe dizziness tends to ameliorate over time and with adjustment of sleep time, longer term adverse neuropsychiatric complications may persist.\(^11\) In this study, we explored the pattern of sleep problems in EFV-treated Chinese HIV patients over an observational period of one year, focusing concurrently on its association with plasma EFV levels and CYP2B6 516 G/T genotype.

**Methods**

This is a longitudinal cohort study involving EFV-treated HIV-positive patients attending the Queen Elizabeth Hospital HIV specialist clinic service in Hong Kong. With an active caseload of about 1500, the service is one of the largest in the territory where HAART is prescribed according to standard guidelines established by the local Scientific Committee on AIDS, modeled on recommendations of the United States Panel on Antiretroviral Guidelines for Adults and Adolescents.\(^12\) Over a two-year period, all treatment-naïve HIV patients of Chinese ethnicity initiated on an EFV-containing HAART regimen were invited to join the study, the monitoring period of which was planned to last for one year for each patient. Decision on the use of EFV was made separate from the conduct of the research. The regimen was prescribed on clinical grounds, after discussion with patients concerned. With consent, a blood sample was collected at baseline for CYP2B6 516 G/T genotyping. Blood sampling was repeated at four weeks and 12 months for determining the plasma EFV levels.

A questionnaire was designed and administered at baseline, four weeks, six months and 12 months after initiation of therapy. The questionnaire was used to capture the occurrence of neuropsychiatric side effects, with a focus on sleep problems. Clinical markers including CD4 count and viral load level at initiation of therapy and the end of 12 months were transcribed from records. The Pittsburgh sleep quality index (PSQI) was used to assess the quality of sleep.\(^13\) The validated Chinese version of the PSQI questionnaire was obtained with permission from Professor PS Tsai in Taiwan.\(^14\) This is an 18-item self-rated questionnaire which was administered by professional nurses at baseline, four weeks, six months and 12 months. The results were used to construct scores for each of the seven components as well as the total score. For component scores, worsening of sleep quality was defined as a score of 2/3 at the respective time-point versus that of 0/1 which was taken as normal. Informed consent was obtained from all participating patients. Approval of the Research Ethics Committee (Kowloon Central/Kowloon East) and The Joint Chinese University of Hong Kong – New Territories East Cluster (CREC) was obtained.

Two laboratory tests were performed on blood samples collected from patients: CYP2B6 516 G/T genotyping and plasma level of EFV. Briefly, blood was sampled from patients at 8–12 hours after the preceding dose of EFV. Plasma was separated from cells and stored at −20°C before testing. Genomic DNA isolated was tested for CYP2B6 516 G/T polymorphism by real-time PCR. Plasma EFV level was determined using high-performance liquid chromatography (HPLC), at 2 time-points – 4 weeks and 12 months after treatment initiation. The chromatographic system consisted of a Waters 1515 Isocratic pump and a Waters autosampler 2707 equipped with a 2489 Dual Wavelength Absorbance Detector set at 240 and 260 nm (Milford, MA, USA).

Genotypically, a patient can be either GT, GG or TT for the CYP2B6 516 G/T locus. The T allelic frequency is the number T alleles expressed as a fraction of all alleles at the locus. Statistically, Mann–Whitney U test was applied for comparing the median plasma EFV levels between genotypes at CYP2B6 516 G/T locus. Odds ratios and Mann–Whitney U test were used for the comparisons of demographics, clinical characteristics and sleep quality. Fisher’s Exact test was used for comparing the component scores of PSQI. Binary logistic generalised estimating equations (GEE) with unstructured working correlation matrix was used for assessing the variation of GG and GT with PSQI and its components scores across time.\(^15\) A p value < 0.05 was generally taken to imply significance in the statistical analyses.

**Results**

**General characteristics of study population**

Between January 2010 and July 2012, a total of 72 treatment-naïve HIV-positive patients of Chinese ethnicity were recruited. All except five were male, with an age of 39.2 ± 10.3 years at diagnosis. The nucleoside reverse transcriptase inhibitor (NRTI) backbone was abacavir plus lamivudine in most cases (n = 59), followed by tenofovir plus emtricitabine/lamivudine (n = 12) or zidovudine plus lamivudine (n = 1).
Criteria for HAART initiation were a CD4 count below 350/μL (n = 67) and/or the presentation of an AIDS-defining illness (n = 15). Seventeen patients were on co-trimoxazole prophylaxis against *Pneumocystis* at the time of treatment initiation. None had psychiatric disorder or had been put on neuro-psychiatric medications. Eight defaulted follow-up before blood sampling could be performed. The allelic frequency of CYP2B6 516T was determined in 64 patients, giving a result of 0.3, with TT genotype present in 9.4% of the tested patients. The genotypic distribution was in Hardy-Weinberg equilibrium (p > 0.05). Of the 64 patients, 15 discontinued therapy before the end of the 12-month follow-up period. A total of 61 plasma samples were tested for EFV level at four weeks, the median of which was 3.13 mg/L (IQR: 2.49–4.50 mg/L). Among 49 plasma samples at 12 months, the median EFV level was 3.29 mg/L (IQR: 2.53–4.09 mg/L). Viral load was suppressed at <100 copies/mL in all (n = 64) patients at 12 months after initiation of therapy.

There were 33 patients with T alleles and 31 with non-T in the study population. Patients with TT genotypes gave a much higher EFV level at four weeks and 12 months, with a median of 10.27 mg/L and 10.18 mg/L, respectively. T and non-T patients were compared, after excluding TT genotypes, as the latter accounted for a small proportion of all subjects. There was no statistically significant difference between GG and GT in terms of gender, age (at HIV diagnosis and initiation of therapy), body weight, criteria for HAART initiation, and concurrent therapy (Table 1). At week 4, GT gave a higher median plasma level of EFV at 3.77 mg/L (IQR 2.99–4.67 mg/L) compared to GG at 2.59 mg/L (IQR 2.17–3.26 mg/L) (Mann-Whitney U = 163, p < 0.001). The difference narrowed but remained significant (3.57 mg/L vs 2.97 mg/L, Mann-Whitney U = 163, p = 0.026) at 12 months. Defining a high plasma level of EFV by a threshold of 4 mg/L, GEE analysis was applied to compare between GG and GT patients over time. The results showed that GT was associated with a higher plasma level of EFV (OR = 4.06; 95%CI 1.23 to 13.46; p = 0.02) (Table 2).

### Sleep quality after EFV treatment

PSQI was used as the tool to assess the impacts of EFV treatment on sleep quality. The overall score ranged between 0 and 21, with the higher value indicating poorer quality. The median PSQI was 5 (IQR 4–7), 5.5 (IQR 4–7), 5 (IQR 4–6) and 5 (IQR 4–6) at baseline, four weeks, six months and 12 months, respectively.

### Table 1. Patient characteristics and comparison between GG (n = 31) and GT (n = 27) genotype.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Overall (n = 72)</th>
<th>GG (n = 31)</th>
<th>GT (n = 27)</th>
<th>OR 95%CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>67</td>
<td>30</td>
<td>25</td>
<td>0.42</td>
<td>0.04–4.87</td>
</tr>
<tr>
<td>Median age at initiation of ARV (y)</td>
<td>(40)</td>
<td>38</td>
<td>43</td>
<td>0.71–5.74</td>
<td>0.19</td>
</tr>
<tr>
<td>Age at ARV initiation &gt;40 (y)</td>
<td>(29)</td>
<td>13</td>
<td>16</td>
<td>1.52</td>
<td>0.54–4.28</td>
</tr>
<tr>
<td>Median age at HIV diagnosis (y)</td>
<td>(39)</td>
<td>37</td>
<td>41</td>
<td>1.31</td>
<td>0.46–3.68</td>
</tr>
<tr>
<td>Age at HIV diagnosis &gt;40 (y)</td>
<td>(29)</td>
<td>14</td>
<td>15</td>
<td>1.45</td>
<td>0.50–4.23</td>
</tr>
<tr>
<td>Median body weight (kg)</td>
<td>(62.6)</td>
<td>63</td>
<td>65</td>
<td>0.43</td>
<td>0.11–1.58</td>
</tr>
<tr>
<td>Body weight &gt;63kg</td>
<td>28</td>
<td>14</td>
<td>14</td>
<td>1.13</td>
<td>0.40–3.17</td>
</tr>
<tr>
<td>Median BMI (kg/m²)</td>
<td>22.1</td>
<td>22</td>
<td>22</td>
<td>1.66</td>
<td>0.51–5.38</td>
</tr>
<tr>
<td>BMI &gt;22Kg/m²a</td>
<td>30</td>
<td>14</td>
<td>16</td>
<td>0.55</td>
<td>0.15–1.99</td>
</tr>
</tbody>
</table>

### Baseline clinical status

| Diagnosis of AIDS | 15 | 9 | 4 | 0.43 | 0.11–1.58 | 0.20 |
| CD4 < 200 cells/μl | 33 | 14 | 13 | 1.13 | 0.40–3.17 | 0.82 |
| Viral load ≥ 4 log copies/mL | 54 | 29 | 25 | 0.86 | 0.11–6.57 | 0.89 |

### Treatment history

| Sepritin or Clindamycin | 16 | 7 | 9 | 1.66 | 0.51–5.38 | 0.40 |
| Abacavir-based regimen   | 46 | 26 | 20 | 0.55 | 0.15–1.99 | 0.36 |
| Tenofovir-based regimen  | 12 | 5 | 7 | 1.82 | 0.50–6.59 | 0.36 |

ARV: antiretroviral therapy; BMI: body mass index.

*aThree cases missing*
respectively. Almost half (47%) gave a worsened PSQI at four weeks compared to baseline, dropping to 31% at 12 months. Comparing between GG and GT genotype, there was no difference in the median PSQI at baseline, four weeks and 12 months. At six months, however, the median PSQI was higher for GT than GG (6 vs 4; Mann–Whitney U = 150, p < 0.05) (Figure 1). Using the GEE method, we compared between GG and GT patients longitudinally at the four time-points after classifying PSQI score into two groups (>5 and ≤5), the results of which demonstrated a poorer outcome (PSQI >5) in GT patients (p = 0.04) (Table 2). There was however no correlation between plasma EFV levels and PSQI for all patients at four weeks and 12 months, or when separately evaluated for GT and GG genotype.

Sleep quality was then analyzed by components constructed from self-rated items. Seven components could be differentiated in this study – duration of sleep, sleep disturbance, sleep latency, day dysfunction, sleep efficiency, perceived overall sleep quality, and the need for sleeping medications. The number of patients with a high PSQI score >5 or a score of 2/3 (denoting a poorer score) at the four time-points for the seven component scores are shown for GG and GT patients in Figure 2. At four weeks, there was a poorer score in at least 10% of the EFV-treated patients as regards sleep disturbance, sleep efficiency and perceived sleep quality, the persistence of which at six months and 12 months was only seen for the perceived self-rated overall sleep quality. Compared to GG, a higher proportion of GT patients had poorer score for day dysfunction at both four weeks (13% vs 7%) and six months (8% vs 0%), but the differences were not statistically significant when evaluated at the respective time points (Fisher’s Exact test, p > 0.1). There was poorer sleep latency at baseline for both groups, which normalized at 12 months. GEE analysis was again applied to test the variance of component scores with genotype and time as factors. Comparison of the component scores did not reveal any significant difference between GG and GT (results not shown). Overall, there was a higher rate of statistically insignificant worsening of sleep disturbance at 4 weeks (OR = 1.70; p = 0.13), but significantly lower rate of sleep disturbance at six months (OR = 0.10; 95%CI 0.01 to 0.87; p = 0.04). The perceived overall sleep quality at six months (OR = 2.46; 95%CI 1.25 to 4.82; p = 0.01) and 12 months (OR = 2.99; 95%CI 1.46 to 6.16; p = 0.003) were worse than that at baseline, while sleep latency improved at 12 months (OR = 0.42; 95%CI 0.20 to 0.89; p = 0.02).

Other adverse reactions associated with EFV

Enrolled patients were asked to rank the severity of adverse reactions referable to the neurological system (e.g. headache, dizziness/giddiness, vivid/strange dreams), gastrointestinal system (e.g. vomiting, diarrhea) and skin (e.g. pruritus, rash). Presence of such experiences after EFV, which were otherwise absent at baseline, or their occurrence with severity higher than that at baseline, were noted. The proportion of patients experiencing adverse reactions at the three time-points, i.e. four weeks, six months and 12 months, was then assessed. Compared to baseline,
dizziness/giddiness was commonly reported at four weeks (60% in GG; 58% in GT), dropping to around 30% at six months and 12 months, irrespective of the genotype. Overall, there was no difference in adverse neuropsychiatric reactions between the two groups with GEE analysis (statistics not shown). For skin reactions, GT gave a higher rate compared to GG in GEE analysis, which just reached statistical significance (OR = 2.6; 95%CI 1.06 to 6.40; \( p = 0.04 \)).

**Discussion**

With the popularity of including EFV in first-line HAART regimens, the occurrence of neuropsychiatric complications with treatment has continued to be an important clinical problem. In this study, our results of EFV-associated dizziness/giddiness presenting at week four compared to baseline confirms previous findings.\(^{16}\) Discontinuation of antiretroviral therapy due to neuropsychiatric adverse effects is higher with EFV-based regimens.\(^{17}\) This constitutes a cause for concern, as the association between plasma EFV level and treatment discontinuation has been reported in some though not all studies.\(^{3,18–20}\) Using PSQI, we managed to examine longitudinally the sleep quality of EFV-treated patients so that not just the incidence but timing of the adverse reaction can be evaluated. Our results suggested the association of CYP2B6 516G/T genotype with PSQI following EFV treatment. There were, in parallel, higher levels of plasma EFV in GT compared to GG genotype, at four weeks and 12 months. Overall, the median PSQI was higher at six months for GT than GG, while there was no difference at other time-points. From GEE analyses, we demonstrated poorer outcome in terms of the proportion with a PSQI score >5 in GT patients compared to GG over time. Our results did not however support any direct correlation between plasma levels and sleep quality. The positive association of genotype with EFV levels on one hand versus the lack of association of plasma level with sleep quality or other neuropsychiatric adverse reactions on the other would need to be confirmed in larger studies.

In our study, the differentiation of sleep quality by PSQI components offered a convenient means of characterizing sleep problems. A similar approach using PSQI in combination with other assessment tools has been reported. In one study, for example, sleep latency was prolonged in 34% of a cohort of 290 HIV patients, 70% of which were on antiretroviral therapy.\(^{21}\) Our results suggested that poorer day dysfunction was commoner in GT than GG though the difference did not reach statistical significance. The association of

**Figure 1.** PSQI of GG and GT patients at baseline and four weeks, six months and 12 months after initiating efavirenz. PSQI: Pittsburgh sleep quality index.

<table>
<thead>
<tr>
<th>Median (IQR)</th>
<th>GT</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (4.8-7.3)</td>
<td>5  (4-7)</td>
<td>5 (4.5-7)</td>
</tr>
<tr>
<td>6 (4-8)</td>
<td>6 (4-6.5)</td>
<td>4 (4-6)</td>
</tr>
<tr>
<td>6 (4-6)</td>
<td>5 (4-6)</td>
<td>5 (4-6)</td>
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</table>

Mann-Whitney U

<table>
<thead>
<tr>
<th>p-value</th>
<th>GT</th>
<th>GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.27</td>
<td>197</td>
<td>197</td>
</tr>
<tr>
<td>0.03</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>0.59</td>
<td>219</td>
<td>219</td>
</tr>
</tbody>
</table>

Error bars: 95% CI
insomnia, another sleep component, with a higher plasma EFV level of >3.5 mg/L has been previously reported, but this relationship was not elicited in our study. In interpreting these observations, it should be cautioned that poorer sleep quality could be related to HIV infection per se instead of antiretrovirals, and that there may be associations with stress and weakened immunity. Overall, our demonstration of the variability of sleep quality across time could be a reflection of the combined effects of adverse reactions.

**Figure 2.** Comparison between GG (n = 31) and GT (n = 27), expressed as the number of patients showing worse score (2 or 3) for PSQI components (a) to (g), and (h) the number with overall worse score (PSQI > 5), at baseline, four weeks, six months and 12 months.
to EFV on one hand and the body’s response to effective antiretroviral therapy on the other. In view of the variations in the pattern of reported sleep quality in different studies, ethnic variation would need to be further examined to determine its specific impacts of EFV treatment.

Our study carries some limitations. Foremost, a relatively small number of patients on longitudinal follow-up were studied, some of whom had discontinued therapy. While PSQI offers a more objective means for evaluating treatment impacts, the self-reported nature of the results could have led to biased observations. On the other hand, our exclusive focus on CYP2B6 516 G/T locus might have neglected the influence of other polymorphisms like 983TC and rs4803419, as reported in another study. In exploring the possible association between sleep quality and genotypes, we have deliberately excluded TT because of the latter’s association with a very high plasma level of EFV. We reckoned that TT could only account for a small proportion of all EFV-treated patients in any population because of the low allelic frequency. Their associations with adverse reactions could not explain all neuropsychiatric problems arising from EFV treatment. By treating TT as a unique sub-group, we have turned our attention to an analysis of the clinical importance of GT genotype, in view of the reported differences in EFV pharmacokinetics between GG and GT. The exclusion of TT genotype in comparing between T and non-T groups did further reduce the sample size, but the results were more robust in studying the genotypic association with sleep quality.

In conclusion, pre-screening for CYP2B6 516 G/T polymorphism may be considered in populations with a high genotype frequency for TT, which would in turn support the identification of patients with a strong tendency of treatment withdrawal. While plasma EFV level is important, it may however not give high distinguishing power for predicting drug-related complications. In our study, we have shown that CYP2B6 516 G > T constitutes a characterizing feature for distinguishing a subgroup of EFV-treated patients. Not only is the plasma EFV level higher in GT genotype but also there is a tendency for sleep problems to emerge as a marker of neuropsychiatric complications. Even if the higher plasma level and short-lived sleep problems are proved to be harmless, patients should be well-prepared before putting on an EFV-containing regimen, so as to reduce the tendency for treatment withdrawal. In the rolling out of HAART in countries around the world, retention of patients on treatment remains a challenge, and attrition can be high in the first year. Low retention is associated with a whole range of factors, including its association with toxicity from specific regimens. Nevertheless, an individualized approach would be important for improving retention, through innovative strategies like the alternation of regimens. Finally, the lower yet effective plasma level of EFV (as reflected by viral suppression) in patients with GG genotype, compared to GT, calls for a re-examination of the optimal daily dose for achieving virus suppression with fewer side effects. In populations with a higher frequency of T allele for CYP2B6 516 G/T, the standard daily dose of 600 mg for EFV may be too high for a proportion of patients. As our study has not addressed clinical outcomes specifically, such strategy would need to be further evaluated in future research.

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Conflict of interest
The authors declare no conflict of interest.

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