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Pharmacogenetics and pharmacokinetics of CNS penetration of efavirenz and its metabolites

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Background: There are limited data on the pharmacogenetics and pharmacokinetics of the CNS penetration of efavirenz.

Objectives: We investigated genetic polymorphisms associated with CSF concentrations of efavirenz and its metabolites and explored the relationships with neurocognitive performance.

Methods: We included 47 HIV-infected South African black adults with and without HIV-associated neurocognitive disorder on efavirenz/tenofovir/emtricitabine and collected paired plasma–CSF samples. We considered 2049 SNPs, including SNPs known to affect plasma efavirenz exposure, from potentially relevant genes (*ABCC5*, *ABCG2*, *ABCB1*, *SLCO2B1*, *SCLO1A2*, *ABCC4*, *CYP2B6* and *CYP2A6*) and 880 met a linkage disequilibrium (LD)-pruning threshold.

Results: We identified 9 slow, 21 intermediate and 17 extensive metabolizers. The *CYP2B6* 983 genotype in multivariate analyses predicted $\log_{10}$-transformed concentrations of plasma efavirenz ($\beta = 0.38$, $P = 2.7\times10^{-03}$), plasma 7-hydroxy-efavirenz ($\beta = 0.59$, $P = 3.7\times10^{-03}$), plasma 8-hydroxy-efavirenz:efavirenz ratio ($\beta = -0.31$, $P = 1.8\times10^{-04}$) and CSF efavirenz ($\beta = 0.36$, $P = 0.01$). Lower plasma 7-hydroxy-efavirenz concentrations were independently associated with *CYP2A6* rs10853742 ($\beta = -0.55$, $P = 3.5\times10^{-05}$), *ABCB1* rs115780656 ($\beta = -0.65$, $P = 4.1\times10^{-05}$) and *CYP2A6* −48A→C ($\beta = -0.59$, $P = 0.01$). *CYP2A6* −48A→C was independently associated with higher CSF 8-hydroxy-efavirenz:efavirenz ratio ($\beta = 0.54$, $P = 0.048$). *CYP2B6* rs2279345 polymorphism was associated with lower plasma 7-hydroxy-efavirenz:efavirenz ratio in multivariate analyses ($P < 0.05$). No polymorphisms were associated with CSF:plasma ratios of efavirenz, plasma or CSF concentrations of 8-hydroxy-efavirenz or neurocognitive performance.

Conclusions: We identified novel genetic associations with plasma efavirenz, plasma 7-hydroxy-efavirenz, plasma 7-hydroxy-efavirenz:efavirenz ratio, plasma 8-hydroxy-efavirenz:efavirenz ratio, CSF efavirenz and CSF 8-hydroxy-efavirenz:efavirenz ratio.

Introduction

The fixed-dose combination of efavirenz, tenofovir and emtricitabine has been recommended as first-line ART for HIV-infected adults.[1] However, prolonged ART exposure may impact cognitive function if ART neurotoxicity exceeds CNS viral suppression efficacy—a hypothesis supported by preclinical and clinical data.[2] Efavirenz in particular has demonstrated neurotoxicity in *in vitro* studies.[3] Interrupting ART after a median of 4.5 years was associated with improved cognitive function, especially among efavirenz recipients.[4] In a randomized controlled trial (RCT), patients starting efavirenz, tenofovir and emtricitabine rather than PIs or all-NRTI regimens had less neurocognitive improvement after 48 weeks.[5]

Human genetic variants have been associated with antiretroviral pharmacokinetics and pharmacodynamics, but ART CNS-targeted strategies have not considered pharmacogenetics.[6] Efavirenz is primarily metabolized by cytochrome P450 (CYP) 2B6 into 8-hydroxy-efavirenz while CYP2A6 generates the 7-hydroxy-efavirenz metabolite.[7] *CYP2B6* 516G→T (rs3745274) predicts increased plasma efavirenz exposure.[8–13] The *CYP2B6* 516TT genotype is more common in Africans and African Americans than in Caucasians.[8,14,15] Additional polymorphisms that are less frequent than 516G→T in Africans and African Americans, 983T→C (rs28399499) and *CYP2B6* 15582C→T (rs4803419), also predict increased plasma efavirenz exposure.[7,15] The *CYP2B6* 983C allele is found almost exclusively with African ancestry.[15,16] Polymorphisms in genes beyond *CYP2B6* have been infrequently reported to be associated with efavirenz concentrations including polymorphisms in *CYP2A6*.[12,17] Polymorphisms in *CYP2B6* that predict higher efavirenz plasma concentrations predispose to efavirenz-mediated neurotoxicity.[8,18] Patients with *CYP2B6* slow-metabolizer genotypes also have higher CSF efavirenz exposure.[19] *In vitro* studies have implicated efavirenz and especially its metabolite 8-hydroxy-efavirenz in neuronal toxicity.[3,20,21] In a CSF substudy of the ENCORE1 trial, 8-hydroxy-efavirenz exposure correlated with adverse neuropsychiatric outcomes.[19] However, *CYP2B6* 516G→T only predicted efavirenz plasma and CSF concentrations and not 8-hydroxy-efavirenz plasma or CSF concentrations.[19,22,23]

Blood–brain barrier (BBB) and blood–CSF barrier (BCSFB) transporters affect influx and efflux of drugs including antiretrovirals.[24,25] The superfamily of solute carrier (SLC) genes, including *SLCO2B1*, *SLCO1A2* and *SLCO1B1*, influence influx transporter expression in the BBB and BCSFB.[26,27] Efflux transporters in the BBB and BCSFB are influenced by ATP-binding cassette (ABC) genes including *ABCB1* [which encodes P-glycoprotein (P-gp)], *ABCG2*, *ABCC4* and *ABCC5*.[27,28] There are conflicting data regarding whether efavirenz is a P-gp substrate and whether *ABCB1* polymorphisms predict efavirenz concentrations.[10,29,30] *ABCC4* polymorphisms (rs1751034 and rs2274407) have been associated with lower and higher maximum plasma efavirenz concentrations, respectively, and *ABCG2* rs2231142 has been associated with an increased risk of abnormal dreams with efavirenz.[31,32]

Africans are the most genetically diverse population worldwide.[33] South Africa has the world's largest ART programme, with most patients currently receiving efavirenz/tenofovir/emtricitabine.[34] Genetic polymorphisms that affect metabolizing enzymes or transporters may therefore affect efavirenz CSF penetration. We investigated associations between genetic polymorphisms and CSF exposure of efavirenz and 8-hydroxy-efavirenz in black South Africans. We also explored pharmacokinetic–pharmacodynamic relationships of CSF efavirenz and 8-hydroxy-efavirenz with neurocognitive performance.

Patients and methods

Participants

Adults (aged ≥18 and ≤70 years) who participated in an RCT (PACTR201310000635418) investigating lithium for HIV-associated neurocognitive impairment [global deficit score (GDS) of ≥0.5] were invited to participate in this study.[35] We also invited participants who were screened for the RCT but were excluded based on cognitive impairment criteria. We included participants established on efavirenz-based ART for at least 6 months with suppressed plasma HIV-1 RNA. All participants provided written informed consent. The study was approved by the University of Cape Town Human Research Ethics Committee (HREC 071/2013).

Pharmacokinetic sampling

We collected paired plasma and CSF samples for assays of efavirenz and its metabolites. Participants recorded dosing time the night before and were admitted in the morning for pharmacokinetic sampling. Mid-dosing lumbar punctures were performed. Whole blood was collected within 45 min of CSF sampling, centrifuged within 1 h of collection, aliquoted and stored at −80°C until analysis. CSF was aliquoted and stored at −80°C until analysis.

Measurement of efavirenz and its metabolites

Drug assays were performed at two laboratories. The analytical laboratory in the Division of Clinical Pharmacology at the University of Cape Town quantified total efavirenz in plasma and CSF using validated LC/MS-MS assays. The lower limit of quantification (LLOQ) for plasma and CSF efavirenz was 19.5 and 0.5 ng/mL, respectively. The Bioanalytical Facility, Department of Molecular and Clinical Pharmacology at the University of Liverpool quantified total CSF 8-hydroxy-efavirenz, plasma 8-hydroxy-efavirenz and plasma 7-hydroxy-efavirenz using validated LC/MS-MS assays.[22] We could not quantify CSF 7-hydroxy-efavirenz. The LLOQ for CSF 8-hydroxy-efavirenz, plasma 8-hydroxy-efavirenz and plasma 7-hydroxy-efavirenz was 3.125 ng/mL, 5.0 ng/mL and 5.0 ng/mL, respectively. Concentrations below the limit of quantification were analysed as missing data.

Characterization of genetic polymorphisms

We extracted DNA from buffy coats using QIAsymphony®. Genotyping was done using Illumina MEGA^{EX} (Illumina, San Diego, CA, USA). SNPs that were not genotyped were imputed. SNPs were extracted for seven genes ±50 kb in each direction: *ABCB1* (301 SNPs), *ABCC4* (630 SNPs), *ABCC5* (225 SNPs), *ABCG2* (164 SNPs), *CYP2A6/B6* (202 SNPs), *SLCO1A2* (406 SNPs) and *SLCO2B1* (118 SNPs). SNPs were excluded for genotyping efficiency less than 99%, a 5% minor allele frequency cut-off, and Hardy–Weinberg equilibrium (HWE) *P* values <0.00001. We further performed targeted genotyping of *CYP2B6* 516G→T (rs3745274) and *CYP2A6* −48A→C (rs28399433) by TaqMan™ (Applied Biosystems, Foster City, CA, USA) and of *CYP2B6* 983T→C (rs28399499), *CYP2B6* 15582C→T (rs4803419), *SLCO1B1* 521T→C (rs4149056) and *SLCO1B1* (rs4149032) by MassARRAY® iPLEX Gold (Sequenom Inc., San Diego, CA, USA). All samples were genotyped in duplicate. The final data set included 2049 SNPs from 47 participants. All genotyping was done at Vanderbilt Technologies for Advanced Genomics (VANTAGE). Laboratory personnel with no knowledge of clinical data performed the genotyping. Metabolizer genotype groups for *CYP2B6* were assigned as follows: extensive metabolizer (*CYP2B6* 15882CC-516GG-983TT or *CYP2B6* 15882CT-516GG-983TT), intermediate metabolizer (*CYP2B6* 15882TT-516GG-983TT, *CYP2B6* 15882CC-516GT-983TT, *CYP2B6* 15882CC-516GG-983CT, *CYP2B6* 15882CT-516GT-983TT or *CYP2B6* 15882CT-516GG-983CT) or slow metabolizer (*CYP2B6* 15882CC-516TT-983TT, *CYP2B6* 15882CC-516GT-983CT or *CYP2B6* 15882CC-516GG-983CC). Furthermore, among participants with a slow-metabolizer genotype, additional assessment of *CYP2A6* −48A→C (rs28399433) was assessed to categorize the metabolizer status into an ordinal 12-level metabolizer status as described elsewhere.[7,36]

Neurocognitive performance

We assessed neurocognitive impairment to provide a GDS.[37] We previously reported the domains and tests included.[35] We screened for symptoms of depression using the Center for Epidemiologic Studies Depression (CES-D) scale.[38]

Efavirenz and metabolite neurotoxicity

We compared CSF efavirenz and 8-hydroxy-efavirenz concentrations with concentrations reported to be associated with neuronal damage *in vitro* (31.6 ng/mL and 3.3 ng/mL, respectively).[20]

Viral load assessment

We determined HIV-1 RNA concentrations in plasma and CSF using the Abbott RealTime HIV-1 assay (Abbott Park, IL, USA.). We considered participants to be virologically suppressed if the viral load was <400 copies/mL. In plasma and CSF, the lower limit of detection was 40 copies/mL. In CSF, we performed a previously described nested PCR and automated DNA sequencing method to detect HIV-1 transactivator viral protein (Tat) mutations, which have been associated with HIV-1-associated neurocognitive impairment.[39,40]

BBB integrity

We calculated the CSF:blood albumin ratio [CSF albumin (mg/L):serum albumin (g/L)] to determine BBB integrity. The BBB was considered intact if this ratio was less than 6.8 in participants <45 years of age, and less than 10.2 in participants ≥45 years of age.[41]

Pharmacokinetic statistical analysis

Pharmacokinetic data were not normally distributed and were expressed as medians (IQR) and geometric means (95% CI). We corrected for plasma protein binding and estimated protein-free plasma concentrations by multiplying total plasma concentrations by the protein-free concentrations reported in the literature (efavirenz 0.22%).[42] Total CSF concentrations were considered to be similar to CSF protein-free concentrations.[42] Pearson's *r* correlation was used to assess correlations between plasma and CSF concentrations. We performed statistical analysis using STATA version 15.0 (StataCorp, College Station, TX, USA). Graphs were created using GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla, CA, USA).

Genetic associations

Genetic associations with pharmacokinetic parameters were analysed by multivariate linear or logistic regression. Pharmacokinetic data were log-transformed (log_{10}) for genetic analysis. We used ratios of measured concentrations (total in CSF and plasma) without correcting for protein binding. CSF:plasma ratios were calculated using raw concentrations then log_{10}-transformed. For efavirenz analyses we subsequently adjusted for *CYP2B6* 516G→T, 983T→C and 15582C→T. We performed genetic association analyses in PLINK version 1.9 (http://zzz.bwh.harvard.edu/plink/). For the primary analyses, we employed linkage disequilibrium (LD) pruning with an LD r^2 threshold of 0.95 within a 50 kb window at 5 kb increments. The final analysis dataset included 880 SNPs that met the LD-pruning threshold. We used Bonferroni correction to adjust for multiple testing ($P = 0.05$ divided by 880 SNPs). We generated an LD plot using Haploview (https://www.broadinstitute.org/haploview/haploview).

Results

Study participant characteristics

We sampled 47 participants (Table 1), 33 (70%) of whom had mild to moderate neurocognitive impairment. All participants self-identified as black Xhosa and all were virologically suppressed in plasma. Four participants had detectable viral loads, the highest being 128 copies/mL. CSF viral loads were <40 copies/mL in all participants. Five participants had detectable HIV-1 Tat DNA, all of whom had the C30C31S substitution. We were able to determine the CSF:blood albumin ratio in 31 (66%) of 47 participants, with a median value of 2.6 (range 1.1 to 5.2), indicating an intact BBB.

Table 1. Baseline characteristics of study participants ($N = 47$)

Gender, *n* (%)	
male	6 (13)
female	41 (87)
Age (years), median (IQR)	36 (32–43)
CD4+ T cell count (cells/mm^3), median (IQR)	470 (384–586)
Time on ART (months), median (IQR)	38 (18–54)
BMI (kg/m^2), mean ± SD	26.3± 5.3
ART regimen, *n* (%)	
efavirenz/tenofovir/emtricitabine	43 (91)
efavirenz/tenofovir/lamivudine	4 (9)
Neurocognitive impairment	
GDS overall, median (IQR)	0.89 (0.22–1.5)
GDS ≥1, *n* (%)	22 (47)
GDS ≥0.5 to <1, *n* (%)	11 (23)
GDS <0.5, *n* (%)	14 (30)
Neuromedical assessment, *n* (%)	
no disease	26 (55)
mild to moderate disease	21 (45)
severe disease	0
Years in education, *n* (%)	
≥10	24 (51)
<10	21 (45)
missing information	2 (4)
Employment status, *n* (%)	
employed[a]	12 (26)
unemployed	35 (74)
Depression score (CES-D scale), median (IQR)	7 (2–11)

[a]Full-time or part-time work.

Efavirenz pharmacokinetics

Concentrations of efavirenz (plasma and CSF), 8-hydroxy-efavirenz (plasma and CSF) and 7-hydroxy-efavirenz (plasma) are described in Table 2. Plasma 8-hydroxy-efavirenz and 7-hydroxy-efavirenz concentrations correlated with plasma efavirenz concentrations ($P < 0.0001$ for each) (Figure 1a and b). CSF and plasma efavirenz concentrations were correlated ($P < 0.0001$) (Figure 1c). There was no correlation between CSF 8-hydroxy-efavirenz and plasma efavirenz, CSF 8-hydroxy-efavirenz and plasma 8-hydroxy-efavirenz or plasma 7-hydroxy-efavirenz and plasma 8-hydroxy-efavirenz (Figure 1d–f). There was no statistically significant association of CSF:plasma ratios versus time after dosing (Figure 2). CSF efavirenz concentrations were above the IC_{50} (1.3 ng/mL) in all participants.[42–45] CSF efavirenz concentrations and CSF 8-hydroxy-efavirenz concentrations were above the *in vitro* toxic concentration (CSF efavirenz, 31.6 ng/mL; CSF 8-hydroxy-efavirenz, 3.3 ng/mL) in 7 (15.2%) of 46 participants and 14 (29.8%) of 47 participants, respectively.[20]

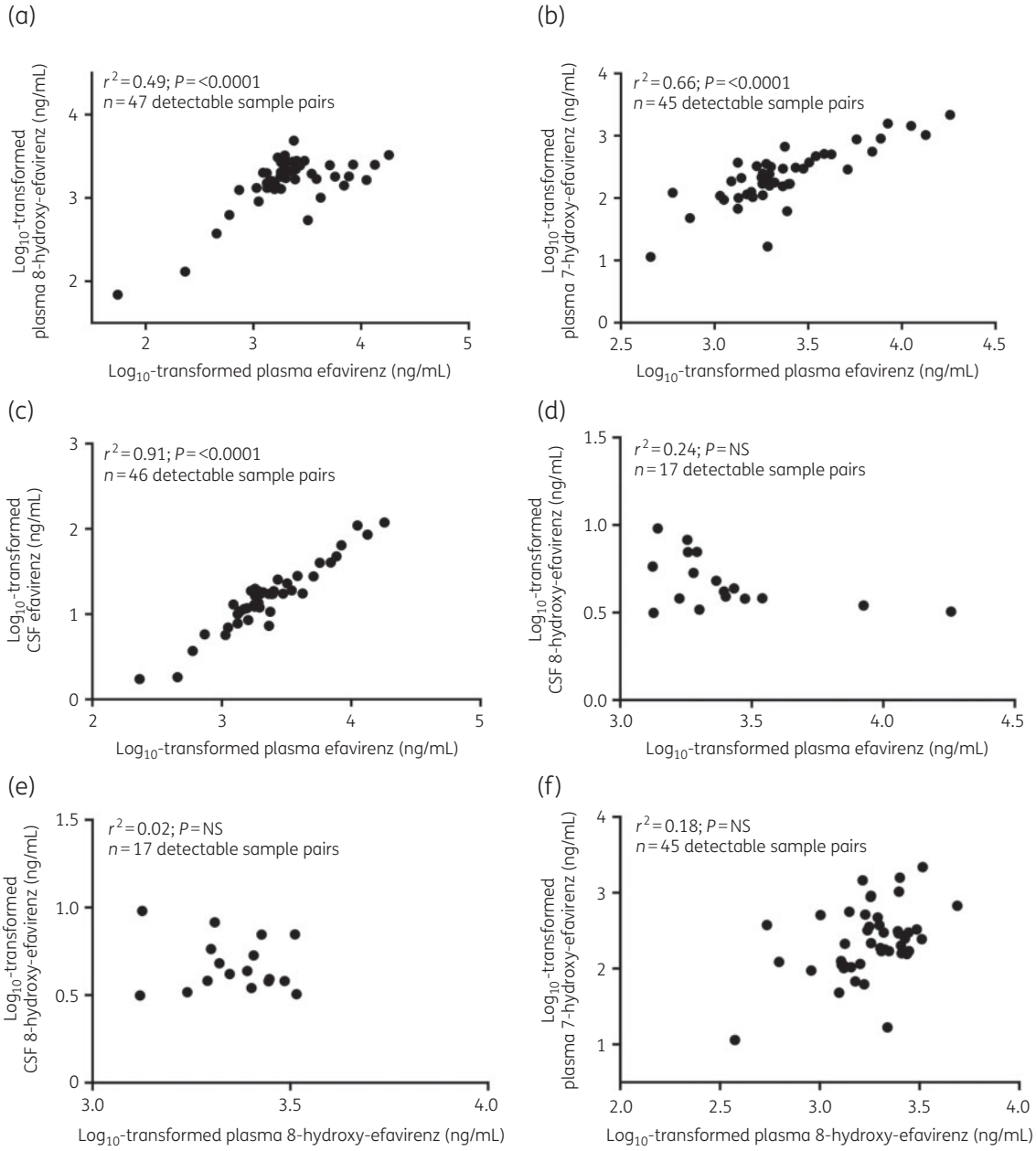

Figure 1. Pearson correlation plots for $\log_{10}$-transformed plasma and CSF concentrations for efavirenz and its metabolites. The relationship between (a) $\log_{10}$-transformed plasma efavirenz concentrations and plasma 8-hydroxy-efavirenz concentrations, (b) $\log_{10}$-transformed plasma efavirenz concentrations and plasma 7-hydroxy-efavirenz concentrations, (c) $\log_{10}$-transformed plasma efavirenz concentrations and CSF efavirenz concentrations, (d) $\log_{10}$-transformed plasma efavirenz concentrations and CSF 8-hydroxy-efavirenz concentrations, (e) $\log_{10}$-transformed plasma 8-hydroxy-efavirenz concentrations and CSF 8-hydroxy-efavirenz concentrations and (f) $\log_{10}$-transformed plasma 8-hydroxy-efavirenz concentrations and plasma 7-hydroxy-efavirenz concentrations. NS, not significant.

Genetic associations for efavirenz

Genotyping of four polymorphisms with known effects on efavirenz (*CYP2B6* 516G→T, 983T→C, 15582C→T and *CYP2A6* −48A→C) and two polymorphisms in *SLCO1B1* (rs4149056 and rs4149032) was successful in all 47 participants. *SLCO1B1* rs4149056 was monomorphic. In 43 (91%) of 47 participants, an additional 2043 polymorphisms from *ABCB1*, *ABCC4*, *ABCC5*, *ABCG2*, *CYP2A6*, *CYP2B6*, *SLCO1A2* and *SLCO2B1* were successfully genotyped. All 2048 polymorphisms were in HWE based on a Bonferroni-adjusted *P* value threshold of 0.00002; 18 had unadjusted *P* values <0.05 (data not shown). The 880 polymorphisms included in the final dataset based on LD pruning were in HWE based on a Bonferroni-adjusted *P* value threshold of 5.7×10^{-05}. Ten polymorphisms had unadjusted *P* values <0.05.

Plasma efavirenz concentrations

Relationships between *CYP2B6* slow-metabolizer genotypes and efavirenz concentrations are described in Table 3. Plasma efavirenz concentrations were significantly higher in *CYP2B6* slow metabolizers compared with intermediate and extensive metabolizers (Figure S1, available as Supplementary data at *JAC* Online). In multivariable linear regression analysis adjusted for *CYP2B6* 516G→T, the polymorphism associated at $P < 0.05$ was *CYP2B6* 983T→C ($\beta = 0.38$, 95% CI = 0.14 to 0.61, $P = 2.7\times10^{-03}$). See Table S1.

Plasma 8-hydroxy-efavirenz concentrations

No polymorphisms were significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T, or *CYP2B6* 516G→T and 983T→C (Table S1).

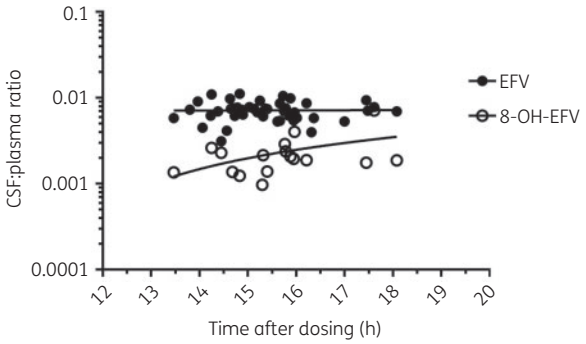

Figure 2. CSF:plasma concentration ratios of detectable pairs of plasma and CSF efavirenz samples versus time after dosing. The lines are linear regression lines and were not statistically significant for efavirenz or 8-hydroxy-efavirenz ($P = 0.09$). EFV, efavirenz; 8-OH-EFV, 8-hydroxy-efavirenz.

Plasma 7-hydroxy-efavirenz concentrations

Plasma 7-hydroxy-efavirenz concentrations were significantly higher in *CYP2B6* slow metabolizers compared with intermediate and extensive metabolizers (Table 3). After adjusting for *CYP2B6* 516G→T, *CYP2B6* 983T→C remained significant ($\beta = 0.59$, 95% CI = 0.22 to 0.96, $P = 3.7\times10^{-03}$) and *ABCB1* rs11578656 became significant ($\beta = -0.71$, 95% CI = −1.01 to −0.42, $P = 2.9\times10^{-05}$). See Table S1. The association between *ABCB1* rs11578656 and $\log_{10}$-transformed plasma 7-hydroxy-efavirenz concentrations remained significant after correcting for multiple testing in multivariate analyses adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C ($\beta = -0.65$, 95% CI = −0.92 to −0.37, $P = 4.1\times10^{-05}$). Two additional *CYP2A6* polymorphisms became significant in multivariate analyses adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C: *CYP2A6* rs10853742 ($\beta = -0.55$, 95% CI = −0.78 to −0.32, $P = 3.5\times10^{-05}$) and the known polymorphism *CYP2A6* −48A→C ($\beta = -0.59$, 95% CI = −1.01 to −0.16, $P = 0.01$).

CSF efavirenz concentrations

CSF efavirenz concentrations were significantly higher in *CYP2B6* slow metabolizers compared with intermediate and extensive metabolizers (Table 3). After adjusting for *CYP2B6* 516G→T, the association between *CYP2B6* 983T→C and $\log_{10}$-transformed CSF efavirenz concentrations persisted ($\beta = 0.36$, 95% CI = 0.10 to 0.62, $P = 1.0\times10^{-02}$). See Table S2.

CSF:plasma efavirenz concentration ratio, CSF:plasma 8-hydroxy-efavirenz concentration ratio and CSF 8-hydroxy-efavirenz concentrations

Linear regression analysis results for associations with CSF:plasma efavirenz concentration ratio, CSF:plasma 8-hydroxy-efavirenz

Table 2. Concentrations of efavirenz and its metabolites in plasma and CSF

	Plasma (ng/mL), total concentration	Plasma (ng/mL), protein corrected	CSF concentration (ng/mL)	CSF:plasma ratio, total concentration	CSF:plasma ratio, protein corrected
Efavirenz pharmacokinetics ($N = 47$)					
undetectable samples, *n*/*N* (%)	0	0	1/47 (2.1%)	1/47 (2.1%)	1/47 (2.1%)
median (IQR)	1960 (1390–3200)	4.31 (3.06–7.04)	17.25 (10.7–19.9)	0.71 (0.61–0.78)	324.34 (278.29–356.44)
range	55–18 100	0.12–39.82	1.73–119	0.31–1.12	142.82–508.66
geometric mean concentration (95% CI)	2081.5 (1557.8–2781.4)	4.58 (3.43–6.12)	15.64 (12.08–20.24)	0.69 (0.64–0.75)	315.54 (291.90–341.10)
8-Hydroxy-efavirenz pharmacokinetics ($N = 47$)					
undetectable samples, *n*/*N* (%)	0	unknown	30/47 (63.8%)	30/47 (63.8%)	unknown
median (IQR)	1808 (1325.5–2498.7)		4.17 (3.80–5.79)	0.20 (0.14–0.24)	
range	68.81–4887.5		3.15–9.56	0.10–0.72	
geometric mean concentration (95% CI)	1570.7 (1255–1965.9)		4.69 (3.93–5.60)	0.21 (0.16–0.26)	
7-Hydroxy-efavirenz pharmacokinetics ($N = 47$)					
undetectable samples, *n*/*N* (%)	2/47 (4.3%)	unknown	not measured	not applicable	not applicable
median (IQR)	216.71 (122.91–375.43)				
range	11.45–2181.73				
geometric mean concentration (95% CI)	229.17 (166.51–315.41)				

Table 3. Efavirenz metabolizer status and detectable efavirenz, 8-hydroxy-efavirenz and 7-hydroxy-efavirenz concentrations in CSF and plasma

		Geometric mean concentration (95% CI)							
		plasma (ng/mL)					CSF (ng/mL)		
Metabolizer genotype	Participants, *n* (%)	EFV	8-OH-EFV	8-OH-EFV:EFV	7-OH-EFV (*n*/*N* = 45/47 detectable)	7-OH-EFV:EFV	EFV (*n*/*N* = 46/47 detectable)	8-OH-EFV (*n*/*N* = 17/47 detectable)	8-OH-EFV:EFV
Slow	9 (19.1)	6896.9 (3984.1–11 939.4)[b]	1860.2 (1421.3–2434.6)	0.27 (0.17–0.42)[b]	810.7 (466.3–1409.6)[b]	0.12 (0.10–0.14)	45.8 (25.0–83.9)[b]	1.8 (1.4–2.4)	0.04 (0.0–3.17)
Intermediate	21 (44.7)	1878.1 (1371.4–2572.0)	1543.8 (1091.4–2183.8)	0.82 (0.64–1.06)	185.5 (108.0–318.5)	0.11 (0.08–0.15)	12.7 (9.3–17.4)	2.7 (2.0–3.5)	0.36 (0.24–0.54)
Extensive	17 (36.2)	1253.48 (778.9–2017.2)	1467.1 (907.9–2370.6)	1.17 (1.03–1.33)	89.6 (45.4–176.7)	0.07 (0.05–0.11)	9.0 (5.0–15.9)	2.2 (1.6–3.0)	0.28 (0.14–0.56)
P[a]		<0.01	NS	<0.01	<0.01	NS	<0.01	NS	NS

EFV, efavirenz; 7-OH-EFV, 7-hydroxy-efavirenz; 8-OH-EFV, 8-hydroxy-efavirenz; NS, not significant.
[a]*P* values were determined by one-way analysis of variance (ANOVA).
[b]$p < 0.01$.

concentration ratio and CSF 8-hydroxy-efavirenz concentrations are displayed in Tables 4, 5 and Table S2, respectively. No polymorphisms were significant in multivariate analyses.

Plasma 8-hydroxy-efavirenz:efavirenz ratios

After adjusting for *CYP2B6* 516G→T, the association between *CYP2B6* 983T→C and $\log_{10}$-transformed 8-hydroxy-efavirenz:efavirenz ratios remained significant ($\beta = -0.31$, 95% CI = −0.45 to −0.16, $P = 1.8\times10^{-04}$). See Table S3.

Plasma 7-hydroxy-efavirenz:efavirenz ratios

In multivariate linear regression models, a previously described *CYP2B6* polymorphism (rs2279345) was significantly associated with lower 7-hydroxy-efavirenz:efavirenz ratios ($\beta = -0.28$, 95% CI = −0.43 to −0.12, $P = 1.2\times10^{-03}$) at $P < 0.05$ (Table S3). The association remained significant at $P < 0.05$ after adjusting for *CYP2B6* 516G→T, *CYP2B6* 516G→T and *CYP2B6* 983T→C.

CSF 8-hydroxy-efavirenz:efavirenz ratios

Linear regression analysis results for genetic associations with $\log_{10}$-transformed CSF 8-hydroxy-efavirenz:efavirenz ratios were available in 17 participants (Table S3). After adjusting for *CYP2B6* 516G→T and *CYP2B6* 983T→C, the association between *CYP2A6* −48A→C and higher $\log_{10}$-transformed CSF 8-hydroxy-efavirenz:efavirenz ratios became significant ($\beta = 0.54$, 95% CI = 0.05 to 1.03, $P = 4.8\times10^{-02}$).

LD

To demonstrate independent associations, the LD plots are displayed in Figures S2 to S8.

Pharmacokinetic–pharmacodynamic associations with neurocognitive performance

We found no significant correlation between GDS and CSF concentrations of efavirenz or 8-hydroxy-efavirenz. Detectable CSF 8-hydroxy-efavirenz tended to be associated with impaired executive function on the Colour Trails Test ($P = 0.043$). Participants with detectable CSF 8-hydroxy-efavirenz had a higher GDS compared with participants without detectable CSF 8-hydroxy-efavirenz (1.0 compared with 0.82), but this was not statistically significant. The GDS in the five participants in whom HIV-1 Tat DNA was detected were similar to those in whom Tat DNA was not detected.

Discussion

We investigated whether genetic polymorphisms are associated with CSF disposition of efavirenz in black South African adults. In multivariate analysis *CYP2B6* 983T→C predicted plasma efavirenz, plasma 7-hydroxy-efavirenz, plasma 8-hydroxy-efavirenz:efavirenz ratio and CSF efavirenz. Lower plasma 7-hydroxy-efavirenz concentrations were independently associated with *CYP2A6* rs10853742, *ABCB1* rs115780656 and *CYP2A6* −48A→C. The *CYP2A6* −48A→C polymorphism was also independently associated with higher CSF 8-hydroxy-efavirenz:efavirenz ratio. The *CYP2B6* rs2279345

Table 4. Genetic associations with detectable $\log_{10}$-transformed CSF:plasma efavirenz concentrations in South African adults

Chromosome	Gene	Polymorphism (minor allele)	MAF	516G→T adjusted		516G→T and 983T→C adjusted		516G→T and 983T→C and *CYP2B6* 15582C→T adjusted	
				β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
13	*ABCC4*	rs9584273 (T)	0.07	0.14 (0.06–0.22)	2.2×10^{-03}	0.14 (0.05–0.22)	2.8×10^{-03}	0.14 (0.06–0.22)	2.5×10^{-03}
13	*ABCC4*	rs9590160 (A)	0.09	−0.12 (−0.19 to −0.04)	3.2×10^{-03}	−0.12 (−0.19 to −0.04)	3.5×10^{-03}	−0.12 (−0.19 to −0.04)	4.3×10^{-03}
13	*ABCC4*	rs74107818 (G)	0.10	−0.11 (−0.18 to −0.04)	3.2×10^{-03}	−0.11 (−0.19 to −0.04)	3.3×10^{-03}	−0.11 (−0.18 to −0.04)	4.0×10^{-03}
13	*ABCC4*	rs74107809 (A)	0.07	−0.13 (−0.21 to −0.04)	5.8×10^{-03}	−0.13 (−0.21 to −0.04)	4.8×10^{-03}	−0.13 (−0.21 to −0.04)	5.7×10^{-03}
19	*CYP2B6*	rs8100458 (C)	0.19	0.07 (0.02–0.12)	6.8×10^{-03}	0.08 (0.03–0.14)	6.4×10^{-03}	0.10 (0.03–0.16)	5.4×10^{-03}
3	*ABCC5*	rs7610724 (G)	0.07	0.10 (0.03–0.18)	7.7×10^{-03}	0.10 (0.03–0.17)	7.6×10^{-03}	0.11 (0.03–0.18)	9.8×10^{-03}
7	*ABCB1*	rs2235023 (T)	0.42	−0.06 (−0.11 to −0.02)	7.8×10^{-03}	−0.06 (−0.11 to −0.02)	7.6×10^{-03}	−0.06 (−0.11 to −0.02)	9.2×10^{-03}
19	*CYP2G1P*	rs142357867 (T)	0.03	−0.16 (−0.28 to −0.05)	8.9×10^{-03}	−0.17 (−0.29 to −0.06)	5.5×10^{-03}	−0.18 (−0.30 to −0.06)	5.1×10^{-03}
13	*ABCC4*	rs200689258 (AC)	0.09	0.11 (0.03–0.19)	8.0×10^{-03}	0.11 (0.03–0.18)	0.01	0.11 (0.03–0.19)	0.01
3	*ABCC5*	rs7427051(A)	0.24	−0.07 (−0.11 to −0.02)	8.6×10^{-03}	−0.07 (−0.12 to −0.02)	0.01	−0.07 (−0.12 to −0.02)	0.01
13	*ABCC4*	rs73548889 (C)	0.06	0.13 (0.04–0.22)	9.9×10^{-03}	0.13 (0.03–0.22)	0.01	0.14 (0.05–0.24)	6.9×10^{-03}
13	*ABCC4*	rs9524925 (G)	0.17	−0.07 (−0.12 to −0.02)	7.6×10^{-03}	−0.07 (−0.13 to −0.02)	0.01	−0.07 (−0.12 to −0.02)	0.01
19	*CYP2B6*	composite *CYP2B6* 516/983 (C)	0.41	NA	NA	NA	NA	NA	NA
19	CYP2B6	*CYP2B6* 516G→T[a]	0.29	NA	NA	NA	NA	NA	NA
19	CYP2B6	*CYP2B6* 983T→C[b]	0.13	−0.03 (−0.10–0.06)	0.60	NA	NA	NA	NA
19	CYP2B6	*CYP2B6* 15582C→T[b]	0.10	−0.03 (−0.10–0.07)	0.68	−0.03 (−0.12–0.07)	0.57	NA	NA
19	CYP2A6	*CYP2A6* −48A→C[b]	0.09	−0.00 (−0.09–0.09)	0.97	0.01 (−0.09–0.12)	0.78	0.01 (−0.09–0.12)	0.80

MAF, minor allele frequency; NA, not applicable.
The targeted SNPs (*CYP2B6* 516G→T, *CYP2A6* −48A→C, *CYP2B6* 983T→C, *CYP2B6* 15582C→T, *SLCO1B1* 521T→ C and *SLCO1B1*) included 47 patients and the rest 43 patients.
[a]$P < 0.05$ accepted as significant for SNPs with a previously described association.
[b]SNP of interest but did not meet criteria of $P < 0.01$; Bonferroni-corrected *P* value 5.68×10^{-05}.

Table 5. Genetic associations with detectable $\log_{10}$-transformed CSF:plasma 8-hydroxy-efavirenz concentrations in 16 South African adults

				516G→T adjusted		516G→T and 983T→C adjusted		516G→T and 983T→C and *CYP2B6* 15582C→T adjusted	
Chromosome	Gene	Polymorphism (minor allele)	MAF	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
3	*ABCC5*	rs6762938 (T)	0.31	0.23 (0.14–0.33)	3.9×10^{-04}	0.22 (0.12–0.32)	1.1×10^{-03}	0.22 (0.11–0.33)	0.02
13	*ABCC4*	rs11343244 (T)	0.10	0.41 (0.23–0.59)	6.2×10^{-04}	0.39 (0.20–0.49)	1.1×10^{-03}	0.41 (0.23–0.59)	8.5×10^{-04}
13	*ABCC4*	rs7997839 (G)	0.14	0.41 (0.23–0.59)	6.2×10^{-04}	0.39 (0.20–0.49)	1.1×10^{-03}	0.41 (0.23–0.59)	8.5×10^{-04}
3	*ABCC5*	rs10937161 (T)	0.20	0.36 (0.21–0.51)	4.1×10^{-04}	0.36 (0.22–0.49)	2.3×10^{-04}	0.35 (0.21–0.50)	5.0×10^{-04}
3	*ABCC5*	rs36092077 (A)	0.16	0.39 (0.21–0.56)	8.9×10^{-04}	0.39 (0.23–0.54)	3.7×10^{-04}	0.39 (0.21–0.56)	7.1×10^{-04}
3	*ABCC5*	rs6807271 (A)	0.31	0.21 (0.10–0.32)	2.6×10^{-03}	0.21 (0.10–0.32)	2.6×10^{-03}	0.20 (0.08–0.31)	5.8×10^{-03}
3	*ABCC5*	rs59309690 (A)	0.09	0.60 (0.27–0.93)	3.7×10^{-03}	0.58 (0.26–0.90)	4.1×10^{-03}	0.57 (0.24–0.90)	6.5×10^{-03}
4	*ABCG2*	rs2728108 (A)	0.08	0.60 (0.27–0.93)	3.7×10^{-03}	0.58 (0.26–0.90)	4.1×10^{-03}	0.57 (0.24–0.90)	6.5×10^{-03}
13	*ABCC4*	rs1678392 (A)	0.19	0.32 (0.14–0.51)	4.9×10^{-03}	0.30 (0.11–0.50)	0.01	0.31 (0.11–0.51)	0.01
13	*ABCC4*	rs116336902 (A)	0.07	0.32 (0.13–0.51)	5.1×10^{-03}	0.30 (0.10–0.50)	0.01	0.31 (0.11–0.51)	0.01
13	*ABCC4*	rs147385814 (C)	0.07	0.32 (0.13–0.51)	5.1×10^{-03}	0.30 (0.10–0.50)	0.01	0.31 (0.11–0.51)	0.01
13	*ABCC4*	rs4771904 (T)	0.27	−0.27 (−0.44 to −0.10)	7.8×10^{-03}	−0.26 (−0.45 to −0.07)	0.02	−0.27 (−0.44 to −0.10)	0.03
3	*ABCC5*	rs6794223 (G)	0.14	0.27 (0.10–0.43)	6.7×10^{-03}	0.25 (0.09–0.41)	9.0×10^{-03}	0.25 (0.08–0.42)	0.01
11	*SLCO2B1*	rs57141326 (A)	0.08	0.34 (0.12–0.56)	9.6×10^{-03}	0.31 (0.09–0.54)	0.02	0.33 (0.11–0.56)	0.02
7	*ABCB1*	rs28401781 (T)	0.24	0.19 (0.07–0.32)	9.0×10^{-03}	0.19 (0.06–0.31)	5.8×10^{-03}	0.22 (0.11–0.33)	2.4×10^{-03}
3	*ABCC5*	rs56889675 (T)	0.26	0.24 (0.09–0.38)	7.6×10^{-03}	0.27 (0.14–0.39)	1.1×10^{-03}	0.26 (0.13–0.39)	2.1×10^{-03}
3	*ABCC5*	rs10470524 (T)	0.22	0.24 (0.09–0.38)	7.6×10^{-03}	0.27 (0.14–0.39)	1.1×10^{-03}	0.26 (0.13–0.39)	2.1×10^{-03}
13	*ABBC4*	rs4148551 (T)	0.36	−0.25 (−0.41 to −0.10)	7.4×10^{-03}	−0.23 (−0.39 to −0.08)	0.01	−0.23 (−0.40 to −0.07)	1.8×10^{-03}
19	*CYP2B6*	composite *CYP2B6* 516/983	0.41	NA	NA	NA	NA	NA	NA
19	*CYP2B6*	*CYP2B6* 516G→T[a]	0.29	NA	NA	NA	NA	NA	NA
19	*CYP2B6*	*CYP2B6* 983T→C[b]	0.13	−0.18 (−0.45–0.08)	0.19	NA	NA	NA	NA
19	*CYP2B6*	*CYP2B6* 15582C→T[b]	0.10	0.02 (−0.24–0.28)	0.88	−0.05 (−0.33 to 0.22)	0.70	NA	NA
19	*CYP2A6*	*CYP2A6* −48A→C[b]	0.09	−0.04 (−0.32–0.25)	0.81	0.10 (−0.23 to 0.43)	0.55	0.09 (−0.26–0.52)	0.61

MAF, minor allele frequency; NA, not applicable.
For the CSF 8-hydroxy-efavirez analysis, the targeted SNPs (*CYP2B6* 516G→T, *CYP2A6* -48A→C, *CYP2B6* 983T→C and *CYP2B6* 15582C→T) included 17 patients and the rest 16 patients.
[a]$P < 0.05$ accepted as significant for SNPs with a previously described association.
[b]SNP of interest but did not meet criteria of $P < 0.01$; Bonferroni-corrected *P* value 5.68×10^{-05}.

polymorphism was associated with lower plasma 7-hydroxy-efavirenz:efavirenz ratio in multivariate analyses adjusting for *CYP2B6* 516G→T and 983T→C ($P < 0.05$). No polymorphisms were associated with CSF:plasma ratios of efavirenz, plasma or CSF concentrations of 8-hydroxy-efavirenz or neurocognitive performance.

We expected CSF 8-hydroxy-efavirenz concentrations to be higher in extensive metabolizers as it is formed via the *CYP2B6* enzymatic pathway. However, similar to the findings of others, 8-hydroxy-efavirenz concentrations in plasma and CSF remained constant irrespective of metabolizer status.[19,22,23] It is possible that the small number of participants (17 of 47) with detectable CSF 8-hydroxy-efavirenz concentrations contributed to the lack of association between the metabolizer status and CSF 8-hydroxy-efavirenz concentrations. Winston *et al.*[19] proposed that CSF 8-hydroxy-efavirenz concentrations are independent of metabolizer status as it is 8-hydroxy-efavirenz spillover from the plasma, or that efavirenz is metabolized to 8-hydroxy-efavirenz in the CNS, trapping 8-hydroxy-efavirenz within the CNS compartment. We found that *CYP2A6* −48A→C was independently associated with a higher CSF 8-hydroxy-efavirenz:efavirenz ratio, which may suggest that in *CYP2B6* slow metabolizers, efavirenz may be metabolized to 8-hydroxy-efavirenz in the CNS by the accessory pathway *CYP2A6*. However, we did not find a statistically significant association with *CYP2A6* −48A→C and CSF 8-hydroxy-efavirenz or plasma 8-hydroxy-efavirenz.

We explored pharmacokinetic–pharmacodynamic relationships of efavirenz and neurocognition. Although CSF efavirenz and 8-hydroxy-efavirenz concentrations were above the *in vitro* CSF toxicity threshold, in 15.2% and 29.8% of participants, respectively, we did not find a relationship between GDS performance and plasma or CSF efavirenz concentrations or CSF 8-hydroxy-efavirenz concentrations. Participants with detectable CSF 8-hydroxy-efavirenz scored worse on the Colour Trails Test ($P = 0.04$) and had a higher GDS (median 1.39 compared with median 1.0), which was not statistically significant. It is possible that *CYP2A6* −48A→C predisposes *CYP2B6* slow metabolizers to higher CSF 8-hydroxy-efavirenz concentrations and worse neurocognitive performance. Various cellular mechanisms for efavirenz toxicity have been proposed.[46] Higher efavirenz concentrations, which are associated with *CYP2B6* slow-metabolizer status, are associated with neurological symptoms, which may include serious presentations such as encephalopathy.[18,47] In a CSF substudy of the ENCORE1 trial, 8-hydroxy-efavirenz exposure correlated with adverse neuropsychology outcomes.[19]

Our study has limitations. We had limited power, with 47 participants, to detect genetic associations between infrequent genotypes with small effect sizes (increase in plasma or CSF concentrations). The *CYP2A6* −48A→C polymorphism has been associated with increased plasma efavirenz concentrations in *CYP2B6* slow metabolizers, but we found no association as only three participants with *CYP2B6* slow-metabolizer genotype carried a single *CYP2A6* −48A→C allele.[36] This may have also limited our ability to detect associations between *CYP2B6* 15582C→T and plasma efavirenz concentrations, as 15582CT heterozygosity has been associated with increased plasma efavirenz exposure, and there were no 15582TT homozygotes in our study.[7] We were not able to detect pharmacokinetic–pharmacodynamic associations due to limited power to detect smaller differences in cognitive impairment. However, to our knowledge this is the largest sample size examining pharmacogenetic, pharmacokinetic and pharmacodynamic associations with CSF efavirenz. Our study was cross-sectional. Neurocognitive changes would have been better assessed longitudinally. We did not measure unbound concentrations of efavirenz, and protein-free CSF:plasma concentrations of efavirenz in particular may have more accurately reflected the pharmacodynamically active concentrations.

In summary, we identified novel genetic associations with plasma efavirenz, plasma 7-hydroxy-efavirenz, plasma 7-hydroxy-efavirenz:efavirenz ratio, plasma 8-hydroxy-efavirenz:efavirenz ratio, CSF efavirenz and CSF 8-hydroxy-efavirenz:efavirenz ratio. No polymorphisms were associated with: CSF:plasma ratios of efavirenz; plasma or CSF 8-hydroxy-efavirenz concentrations; or neurocognitive performance.

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Transparency declarations

None to declare.

Author contributions

E. H. D.: study concept and design, data acquisition, data analysis and interpretation of data, drafting and revising the manuscript for content, study supervision and obtaining funding. P. Z. S.: data analysis, interpretation of data and revising the manuscript for content. G. U. v. Z.: sample analysis, interpretation of data and revising the manuscript for content. L. W.: sample analysis and revising the manuscript for content. S. K.: sample analysis and revising the manuscript for content. J. A. J.: study concept and design, revising the manuscript for content and study supervision. D. W. H.: sample analysis, data analysis, interpretation of data and revising the manuscript for content. G. M.: study concept and design and revising the manuscript for content.

Supplementary data

Figures S1 to S8 and Tables S1 to S3 are available as Supplementary data at *JAC* Online.

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