Microsampling with dried blood spots and mass spectrometry enables PK/PD profiling of responses to praziquantel in a *Schistosoma haematobium*–exposed Zimbabwean population

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Introduction **Praziquantel (PZQ) is commonly used to treat schistosomiasis; however, there is considerable interindividual variability in its efficacy, partly because of genetic variation. Data on this relationship is scarce across Africa – where schistosomiasis is prevalent. This study aimed to investigate the pharmacokinetic/pharmacodynamic and pharmacogenetic relationship between PZQ and its metabolites in a Zimbabwean population infected with *Schistosoma haematobium* by leveraging dried blood spots (DBS) and mass spectrometry (MS).**

Methods **DBS were obtained from 38 Zimbabwean participants on PZQ treatment at four-time points (0.5, 1.5, 2.5, and 4 h). We compared two extraction methods for recovering PZQ and its metabolites from the DBS cards and performed MS analysis to determine the concentrations. A random forest model was used to determine whether *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5* known variants were predictive of PZQ efficacy. The relationships between PZQ/metabolite concentration, metabolite ratio, and drug exposure with genotype were determined using a one-way analysis of variance.**

Results **An acetonitrile and water (4 : 1) mixture was determined to be optimal for recovering PZQ and its metabolites from the DBS cards. Subsequent MS analysis identified PZQ and six metabolite compounds – including phase 1 metabolites (–2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ. Pooled MS sampling was comparable to individual MS sampling for determining pharmacokinetic profiles at the 2.5 and 4-h time points. The (–2H)-O-PZQ and O2-PZQ metabolites had significantly higher concentrations in participants with *CYP2C9*1/*9* and **9/*9* versus those with *CYP2C9*1/*1*. *CYP1A2* rs2069514-A (formerly **1C*) and rs762551-A (*CYP1A2*30*; formerly **1F*) were observed to alter PZQ pharmacokinetic profiles; however, differences in analyte concentrations across the corresponding genotypes were NS.**

Conclusion **We show that low-cost microsampling using DBS and MS is feasible for detecting and quantifying PZQ and its metabolites. Furthermore, our pharmacogenetics analysis elucidates the impact of known cytochrome P450 variants on PZQ drug response in an African setting.** ***Pharmacogenetics and Genomics*** **35: 197–213**

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Introduction

Schistosomiasis (bilharzia) is an acute and chronic disease caused by parasitic trematode worms of the genus *Schistosoma* [1]. It is highly prevalent in Africa, where both the intestinal (predominantly caused by *Schistosoma mansoni* and other species) and urogenital (caused by *Schistosoma haematobium*) forms of the disease occur. Praziquantel (PZQ)-based mass drug administration is the main approach for controlling schistosomiasis in Zimbabwe and other endemic areas in Africa [1,2]; however, there is considerable interindividual variability in the efficacy of PZQ regarding clearing *S. haematobium* infections. While this could be because of decreased PZQ sensitivity or resistance

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in the schistosomes, multiple studies have also attributed cases of variable PZQ efficacy to variations in the host – focusing on factors such as high pretreatment egg intensities, poor PZQ absorption, age, and altered PZQ metabolism (reviewed previously by Zdesenko and Mutapi) [3]. Regarding PZQ metabolism, pharmacogenetic (PGx) variations in cytochrome P450 (*CYP*) genes are likely major determinants of the efficacy of PZQ treatment as they can influence concentrations of the drug or its metabolite(s) via altering the functioning of CYP enzymes. However, there is a scarcity of PGx studies conducting pharmacokinetic/pharmacodynamic (PK/PD) analyses of PZQ in African populations. This could be linked to several factors such as the lack of appropriate tools for sample collection and processing in low-resource field settings, not to mention the limited accessibility to genomic sequencing. To obtain a pharmacokinetic profile, sequential blood samples must be collected from the participants – which is a highly invasive, inconvenient, and time-consuming procedure that is currently impractical in resource-limited settings [4]. Second, ascertaining the pharmacodynamics outcome and measuring PZQ efficacy requires resources to conduct parasitological diagnosis at follow-up. Moreover, the mass spectrometry (MS) analysis required to analyse the *in vivo* drug concentrations is costly [5,6]. Therefore, there is a need for better sample collection methods and lower-cost MS processing linked with cost-effective genomic sequencing. This study therefore aimed to investigate the PK/PD and PGx relationship between PZQ, and its metabolites, in a Zimbabwean population infected with *S. haematobium*, using a fieldwork-applicable microsampling technique. A considerable body of research supports the use of dried blood spots (DBS) for estimating concentrations of pharmacokinetic analytes [7,8]. As DBS is a relatively convenient, less invasive, and microvolume approach, we hypothesised that it would be a practical and feasible approach for facilitating the identification of PZQ analytes in a rural Zimbabwean setting.

Methods

Ethical approval

Ethical approval for this study was granted by the Medical Research Council of Zimbabwe (MRCZ/A/2435) and the University of Zimbabwe Institutional Review Board. The Provincial Medical Director provided permission for the research to be conducted in the Mashonaland Central Province of Zimbabwe, and local permission was obtained from community leaders and representatives. The aims and procedures of the study were explained to each participant or their parent/guardian in their local language, Shona, before enrolment. Written consent was then obtained upon enrolment from the participant or their parent/guardian.

Table 1 Demographics of the Zimbabwean study population

Variable	*N* (%)
Age category	
SAC (6–17 years)	28 (73.7)
Adults (≥18 years)	10 (26.3)
Sex	
Male	24 (63.2)
Female	14 (36.8)
Baseline *Schistosoma haematobium* intensity	
Light (<50 eggs/10 ml)	31 (81.6)
Heavy (≥50 eggs/10 ml)	7 (18.4)
Infection status at efficacy check	
Cleared (100% ERR)	36 (94)
Not cleared (<100% ERR)	2 (5.3)

ERR, egg reduction rate; SAC, school-aged children.

Study population

This study involved 38 Zimbabwean participants (Table 1) on PZQ treatment for *S. haematobium* infection. All the participants were recruited from the Mupfure clinic in the Shamva district, one of the seven districts in the Mashonaland Central Province of Zimbabwe.

The Zimbabwean study population was selected using the following criteria: (a) positive for *S. haematobium*, (b) treated with PZQ, (c) gave a baseline venous blood sample for PGx analysis, (d) had a DBS sample for each of the 0.5-, 1.5-, 2.5-, and 4-h timepoints post-PZQ administration, and (e) had a 3-week parasitological diagnosis to determine the efficacy of PZQ. The efficacy of PZQ treatment was the measurable pharmacodynamic outcome utilised in this study and was determined by the egg reduction rate (ERR), which is determined by the reduction in the mean number of eggs excreted in urine from pre-PZQ to post-PZQ treatment [9]. At the 3-week efficacy check, the subjects were divided into either: (a) negative for schistosome eggs and have cleared infection (n = 36, 100% ERR), indicative of a successful treatment, or (b) still positive for schistosome eggs and did not clear infection (n = 2, <100% ERR), indicative of an unsuccessful treatment. Age, sex, and initial infection intensity were the confounder variables accounted for in the study as they are known to affect PZQ efficacy [10]. Supplementary Table S1, Supplemental digital content 1, *https://links.lww.com/FPC/B516* summarises the case-matching based on these criteria. To ensure treatment compliance was not the cause of reduced PZQ efficacy, each participant was checked by a health worker to confirm they had swallowed the PZQ tablet.

Collection of dried blood spots

To obtain capillary blood (±0.1 ml) for the DBS, a standard lancet was used to perform a finger prick. DBSs were obtained from the study participants at 0.5, 1.5, 2.5, and 4-h timepoints after treatment with PZQ in Zimbabwe. Each DBS corresponding to these timepoints comprised five drops of blood transferred onto the DBS card (Whatman 903 Protein Saver Card), dried for

approximately 1 h, then stored in sealed plastic bags with desiccant. DBS cards were transferred to a –80 °C freezer at the University of Zimbabwe until their shipment on dry ice to the University of Edinburgh for analysis (i.e. extraction of PZQ and its metabolites).

Optimisation of the extraction method

For this part of the study, we tested samples provided by two male participants (in the 6–17 years age category) with a mean infection intensity of about 18.83 eggs/10 ml from the same Zimbabwean population who were treated with PZQ for *S. haematobium* infection but did not possess a 3-week efficacy check. These samples were also collected from the Mupfure clinic but did not fulfil the eligibility criteria for further inclusion and therefore were determined to be valuable samples for method development to ensure good analyte recovery. For each timepoint, two extraction methods were tested to determine the best solvent conditions to recover PZQ and its metabolites from the DBS cards; extraction method A (EMA) involving acetonitrile (ACN) and water (4 : 1), and extraction method C (EMC) involving chloroform, methanol, and water (1 : 3 : 1). Each DBS card was defrosted for 30 min before beginning each extraction. Then, three circular DBS (12.5 mm in diameter) were punched from the DBS filter card for both the 0.5- and 4-h timepoints and were added to the separate Eppendorf's along with 600 µl of either EMA or EMC reagents. Three DBS spots were selected to allow for technical replicates of each timepoint to check the reproducibility between each spot. The samples were thermos-mixed for 5 min at 1200 rpm and sonicated in an ultrasonic bath for 1 h at room temperature. The samples were then centrifuged at 2250 rpm for 10 min at 25 °C. The DBS extracts were then placed in separate autosampler vials for MS analysis.

Extraction method selection

Both the EMA and EMC approaches recovered measurable quantities of PZQ and its metabolites from the DBS. The EMA approach had higher intensity of parent PZQ (active drug) compared with EMC at the 0.5-h timepoint (Fig. 1a). Similarly, EMA had higher intensity (implying higher recovery) for the oxidised form of PZQ (metabolite) compared with EMC at the 4-h timepoint. Overall, EMA was selected as the optimal extraction method and these solvent conditions were used for the sample preparation of the main Zimbabwean study population to recover PZQ and its metabolites from the DBS card.

Final dried blood spot preparation and internal standard preparation

One circular DBS (12.5 mm in diameter) collected at the 0.5-h timepoint was punched from each participant's DBS filter card and was added into a separate Eppendorf's along with 600 µl of ACN : H_2O (4 : 1) containing 600 ng/ml of the internal standard d11-PZQ (Cayman Chemical). The samples were thermos-mixed for 5 min at 1200 rpm and sonicated in an ultrasonic bath for 1 h at room temperature. The samples were then centrifuged at 2250 rpm for 10 min at 25 °C. The DBS extracts were then placed in separate autosampler vials for MS analysis. This process was repeated for the 1.5-, 2.5-, and 4-h timepoints, to produce individual samples for each participant at every timepoint. Furthermore, following these extractions, each of the 0.5-h timepoints was pooled into one autosampler vial to allow for the pooled MS sampling of the entire Zimbabwean study population at this timepoint. This was repeated for the 1.5- and 2.5-h using DBS in this study.

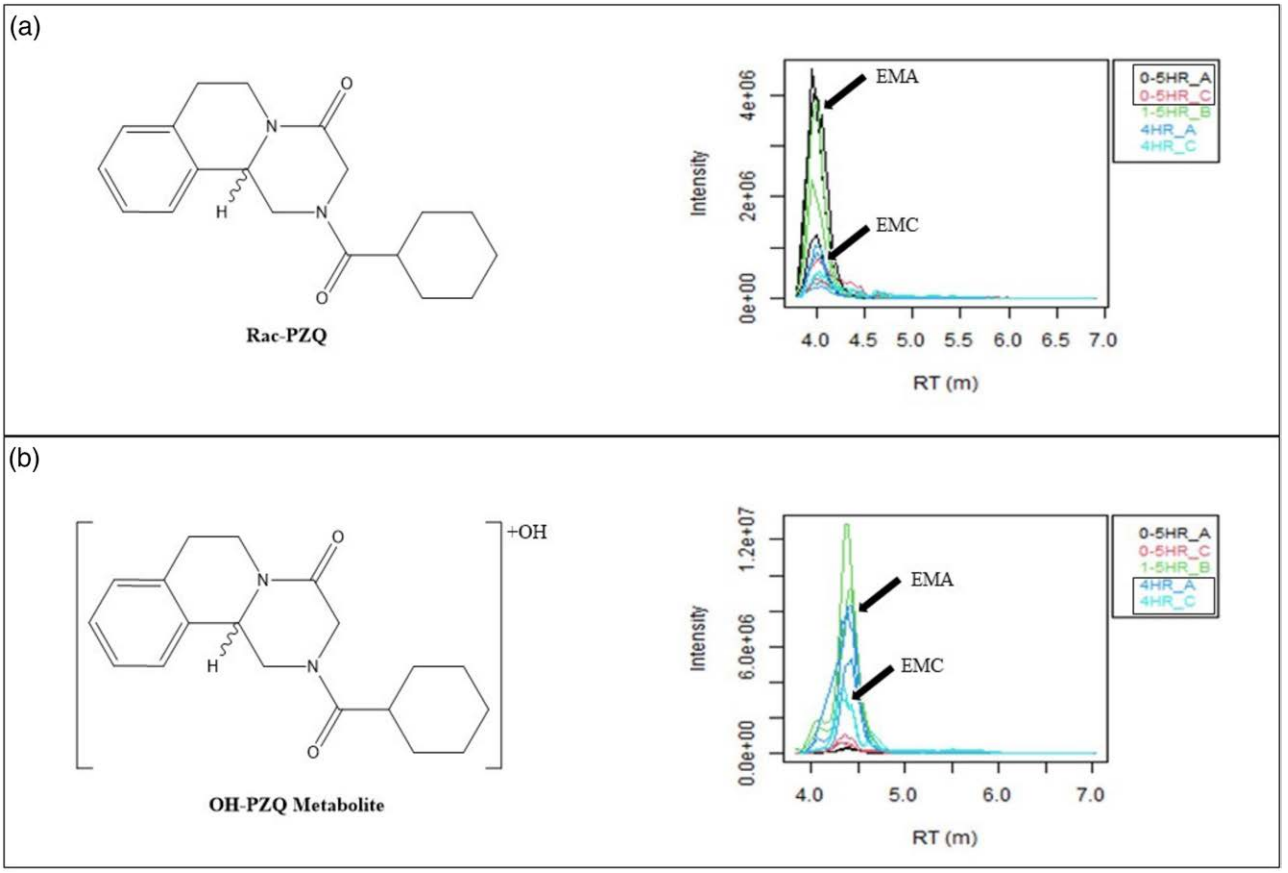
Mass spectrometry

Liquid chromatography MS was performed to separate and detect PZQ and its metabolites (Supplementary Tables S2 and S3, Supplemental digital content 1, *https://links.lww.com/FPC/B516*). We used analyte identifiers (Supplementary Table S4, Supplemental digital content 1, *https://links.lww.com/FPC/B516*) previously determined by Wang *et al.* [11] and Meister *et al.* [12] to identify PZQ and its metabolites during MS in this study. To allow comparisons to other pharmacokinetic studies on PZQ, the concentration of each analyte was converted into units of ng/ml. Details on the quality control of the detected MS peaks are provided in Supplementary Table S5, Supplemental digital content 1, *https://links.lww.com/FPC/B516*.

The individual MS sampling method was implemented to investigate each participant's DBS sample at the 2.5- and 4-h timepoints (DBS samples at the 0.5- and 1.5-h timepoints not investigated because of costs) and, thereafter, compared this approach to a pooled MS sampling method – investigating the concentration of the analytes at the 0.5-, 1.5-, 2.5- and 4-h timepoints via pooling of all the participant samples. For the individual MS sampling method, we assumed that the C_{max} had not been obtained before 2.5 h. The pharmacokinetic profile of each analyte in the individual MS sampling method was plotted from 0-, 2.5- and 4-h data using the 'loess' function in the R environment v3.6.1, and the Data Thief III software then extrapolated the 0.5- and 1.5-h timepoints for each individual for further analysis.

Noncompartmental analysis (NCA) was also conducted to derive the pharmacokinetic parameters of the analytes and to evaluate the relationship between PZQ exposure and efficacy. NCA calculated the C_{max}, T_{max} (time of maximum observed concentration) and area under the curve (AUC) parameters of racemic PZQ and its metabolites. The AUC is also commonly used to assess exposure to the drug and will hence be referred to as such throughout this study [13]. Specifically, in this study, the AUC (0-last) was reported which measures the AUC from dosing to the time of the last measured concentration,

Fig. 1



Preliminary analysis of the DBS extractions and the intensity of the response detected. (a) Detection of PZQ at 0.5 h using EMA involving acetonitrile and water (4 : 1), and EMC involving chloroform, methanol, and water (1 : 3 : 1). (b) Detection of a mono-oxidised metabolite of PZQ at 4 h using EMA and EMC. Peak colours key: black = 0.5-h timepoint sample using EMA, red = 0.5-h timepoint sample using EMC, dark blue = 4-h timepoint sample using EMA, light blue = 4-h timepoint sample using EMC, green = 1.5-h timepoint sample. DBS, dried blood spot; EMA, extraction method A; EMC, extraction method C; PZQ, praziquantel.

which is calculated using the linear-up log-down method. This calculates the AUC (0–4) from the initial time of dosing ($t = 0$ h) to the time of the last sampling timepoint ($t = 4$ h). The linear trapezoidal rule was used for the ascending part of the pharmacokinetic curve, while the log trapezoidal rule was used for the descending part of the pharmacokinetic curve [14]. NCA was conducted using the NonCompart and ncar packages in R v3.6.1 (https://www.r-project.org/). The NCA results obtained using these packages were found to be comparable to the commonly used commercial software WinNonlin [15].

DNA sequencing and bioinformatics analysis

The sample processing done in the study is detailed in Supplementary Methods, Supplemental digital content 1, *https://links.lww.com/FPC/B516*. Briefly, genomic DNA was extracted from each participant's whole blood sample using QIAamp DNA MicroKit (Qiagen GmbH, Germany). Paired-end whole exome sequencing – including custom target capture of *CYP1A2* regions covering rs2069514 and rs762551 – was performed for all participants using the Illumina HiSeq 4000 platform (Illumina, San Diego, California, USA) at the Beijing Genomics Institute (BGI, Shenzhen, China), generating 150 bp read sequences in FASTQ format. Read mapping to the GRCh38.p14 reference sequence was done using Burrows-Wheeler Aligner (BWA-MEM algorithm) [16]. Joint variant calling and filtering was performed using BCFtools (Li, 2011) and varFilter (vcfutils.pl), respectively, focusing on *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5* genomic regions as these genes encode key enzymes involved in the metabolism of PZQ [11,17]. The final variant set was annotated using the Ensembl Variant Effect Predictor [18].

Pharmacogenetics and statistical analysis

A random forest model (described in our previous work [19]), based on the R package *randomForest*, was used to infer whether the detected CYP variants were predictive of PZQ efficacy. Briefly, in the random forest model,

various subsets of the study participants are randomly sampled to form an 'out-of-bag dataset', from which the model randomly selects and searches for the best variant predictive of a PZQ treatment outcome. The optimal variant becomes the first node in the classification tree. The random forest model continues to randomly select a subset of variants at each node while partitioning the data until a full tree is grown. The PGx analysis was focused on known variants, that is, *CYP1A2* rs2069514-A and rs762551-A; *CYP2C9* rs2256871-A; *CYP2D6* rs28371706-A, rs16947-A, rs1135840-G, rs3892097-T; *CYP3A5* rs776746-C, rs10264272-T, and rs41303343-insA prioritised according to literature and our previous findings in this study region [19]. For this study, we implemented a dominant genetic model, that is assessing whether the presence of at least one minor allele (a/a versus A/a+A/A) was associated with an individual clearing or not clearing infection (Goldstein *et al.*, 2011) [20] . This allowed for a higher-powered model compared to a genotypic model (a/a versus A/a vs A/A). For the random forest run, ntree (number of trees to grow) was set to 1000; and defaults were used for all other parameters, including nodesize (1) and maxnodes (null; this allows decision trees in the random forest to be grown to the maximum possible). Given the small sample size in our study, no correction was made regarding imbalanced data.

Univariate association of CYP genotype to PZQ efficacy was performed using PLINK [21] to visualise and interpret results, using chi-squared tests to assess each genetic model. Each variant's minor allele (a) and major allele (A) was represented as a contingency table of PZQ treatment outcome by either genotype count (aa, Aa, AA) or dominant model count [a/a, (A/a+A/A)] according to corresponding genetic models [22]. The remaining statistical analyses were computed using IBM SPSS Statistics v.27 (https://www.ibm.com/uk-en/analytics/spss-statistics-software) unless otherwise stated. Fisher's exact test was used to assess significant differences between the allele frequency of the CYP variants compared with African and European populations in the 1000 Genomes Project [23], and the Zimbabwean population based on previous studies in African populations [24–28].

The assessment of the PGx changes in the concentration of PZQ and its metabolites, metabolite ratio, and AUC with genotype was performed using a one-way analysis of variance (ANOVA). Where required, Tukey multiple comparisons of means of the ANOVA results were performed to determine which genotype drove the significance of the ANOVA results. A binary logistic regression was also performed to determine if PZQ exposure (AUC and C_{max}) contributed to an individual clearing *S. haematobium* infection. Principal component analysis (PCA) was performed to test whether within-group dispersion of the concentration of parent PZQ and the three detected metabolites could be attributed to sample-related metadata or genotypic changes. All statistical significance was set at a P value less than 0.05.

Results

Metabolite identification

After extraction from DBS, MS analysis successfully identified the parent drug (PZQ) and six likely metabolite signals – including phase 1 metabolites (−2H)-O-PZQ and O2-PZQ, whose pathways have not been reported in the literature, and the well-characterised 4-OH-PZQ. All seven analytes (Table 2) were identified using their mass and retention time and were allocated a metabolic description based on previous PZQ metabolite studies [11]. As this study was primarily focused on CYP-mediated phase I drug metabolism, glucuronide metabolites formed via phase II glucuronidation biotransformation were excluded from further analysis while 4-OH-PZQ, (−2H)-O-PZQ, and O2-PZQ (Supplementary Figure S1, Supplemental digital content 1, *https://links.lww.com/FPC/B517*) were retained.

Comparison of mass spectrometry sampling methods

From the NCA analysis, the C_{max} of PZQ, (−2H)-O-PZQ, O2-PZQ and 4-OH-PZQ using the pooled MS sampling method were 82.2, 38.9, 53.8, and 258.2 ng/ml, respectively, at a T_{max} of 1.5 h (Fig. 2). In comparison, the C_{max} ± SD of PZQ, (−2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ using the individual MS sampling method was 33.9 ± 21.1, 32.3 ± 14.8, 39.8 ± 17.5, and 210.1 ± 80.7 ng/ml, respectively, at a T_{max} of 2.5 h. Second, the AUC of PZQ, (−2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ using the pooled MS sampling method was 165.3, 108.3, 156.7, and 772.9 ng/ml, respectively. In comparison, the mean AUC ± SD of PZQ, (−2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ using the individual MS sampling method was 91.2 ± 47.1, 88.6 ± 36.8, 109.5 ± 43, and 567.1 ± 195.6 ng/ml, respectively. The concentrations of the pooled MS samples at both the 2.5- and 4-h timepoint were within the SD of the mean concentration of the individual MS samples (Fig. 2 and Supplementary Tables S6 and S7,

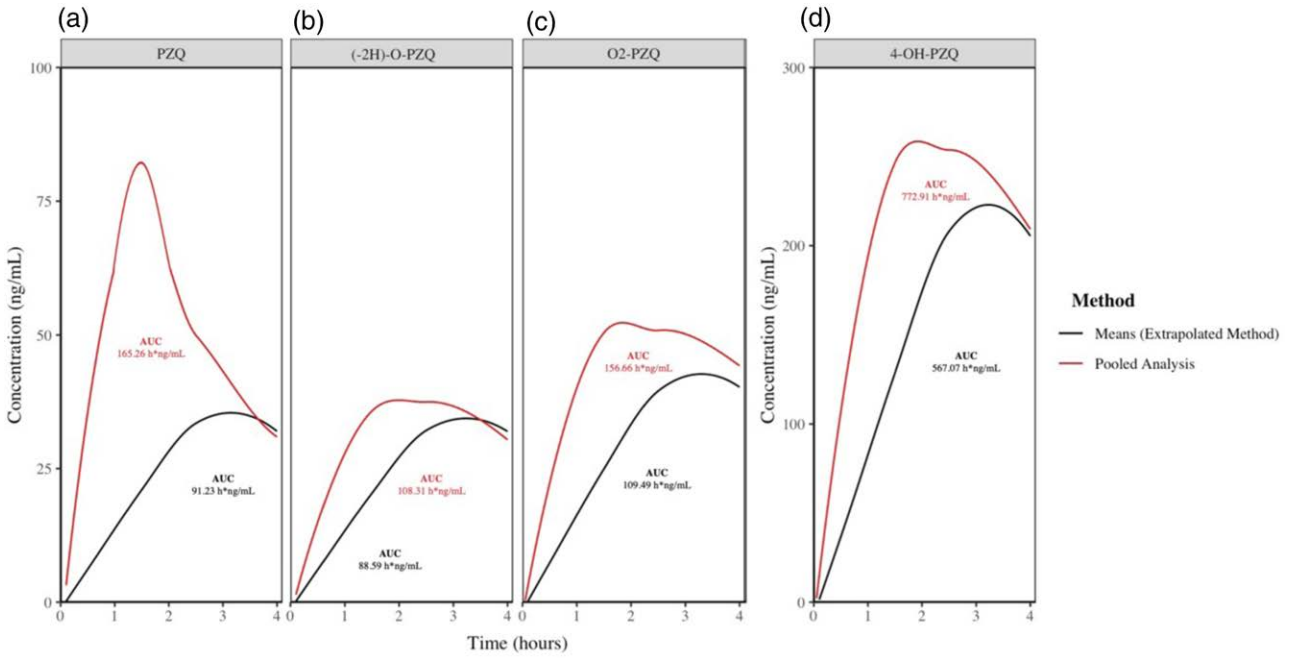
Table 2 The analytes detected using dried blood spots collected from a *Schistosoma haematobium*–infected Zimbabwean population following a praziquantel treatment

ID	Metabolic description	Precursor ion (*m/z*)	Retention time (min)
PZQ	Parent	313.1930	3.71
4-OH-PZQ	Phase I metabolite	329.1860	4.17
(−2H)-O-PZQ	Phase I metabolite	327.1710	4.41
O2-PZQ	Phase I metabolite	345.1820	4.46
O-PZQ-glucuronide	Phase II metabolite	505.2130	4.90
(−2H)-O-PZQ-glucuronide	Phase II metabolite	503.1800	5.41
O2-PZQ-glucuronide	Phase II metabolite	521.1900	6.60

The structure of each of the three phase I metabolites and their reported cytochrome P450 pathways are displayed in Supplementary Figure S1, Supplemental digital content 1, *https://links.lww.com/FPC/B517*

4-OH, 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; *m/z*, mass-to-charge ratio; O2, dioxidised; PZQ, praziquantel.

Fig. 2



Comparison of the pharmacokinetic profile of the Zimbabwean study population between the pooled and individual analyses. The pooled analysis curve displays results from the pooled mass spectrometry samples for each timepoint. The means curve is derived from the mean of each participant's 2.5-h sample and 4-h sample, with the 0.5- and 1.5-h extrapolated from this data. The concentration of PZQ and three PZQ metabolites at each timepoint were obtained using DBS cards. 4-OH, 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; DBS, dried blood spot; O2, dioxidised; PZQ, praziquantel.

Supplemental digital content 1, *https://links.lww.com/FPC/B517*), indicating that the pooled method was a good representation of the Zimbabwean study population's pharmacokinetic profiles at these timepoints.

Mean analyte concentrations of the study population

The 2.5-h timepoint was selected to capture the effect of PGx. The concentration of parent PZQ was skewed to the right with the mean higher than the median for the Zimbabwean population (Fig. 3). The mean concentration at the C_{max} of the three PZQ metabolites in this Zimbabwean population deviated from the median, with a slight skew from the symmetrical distribution to the right for (−2H)-O-PZQ, O2-PZQ and the left for 4-OH-PZQ.

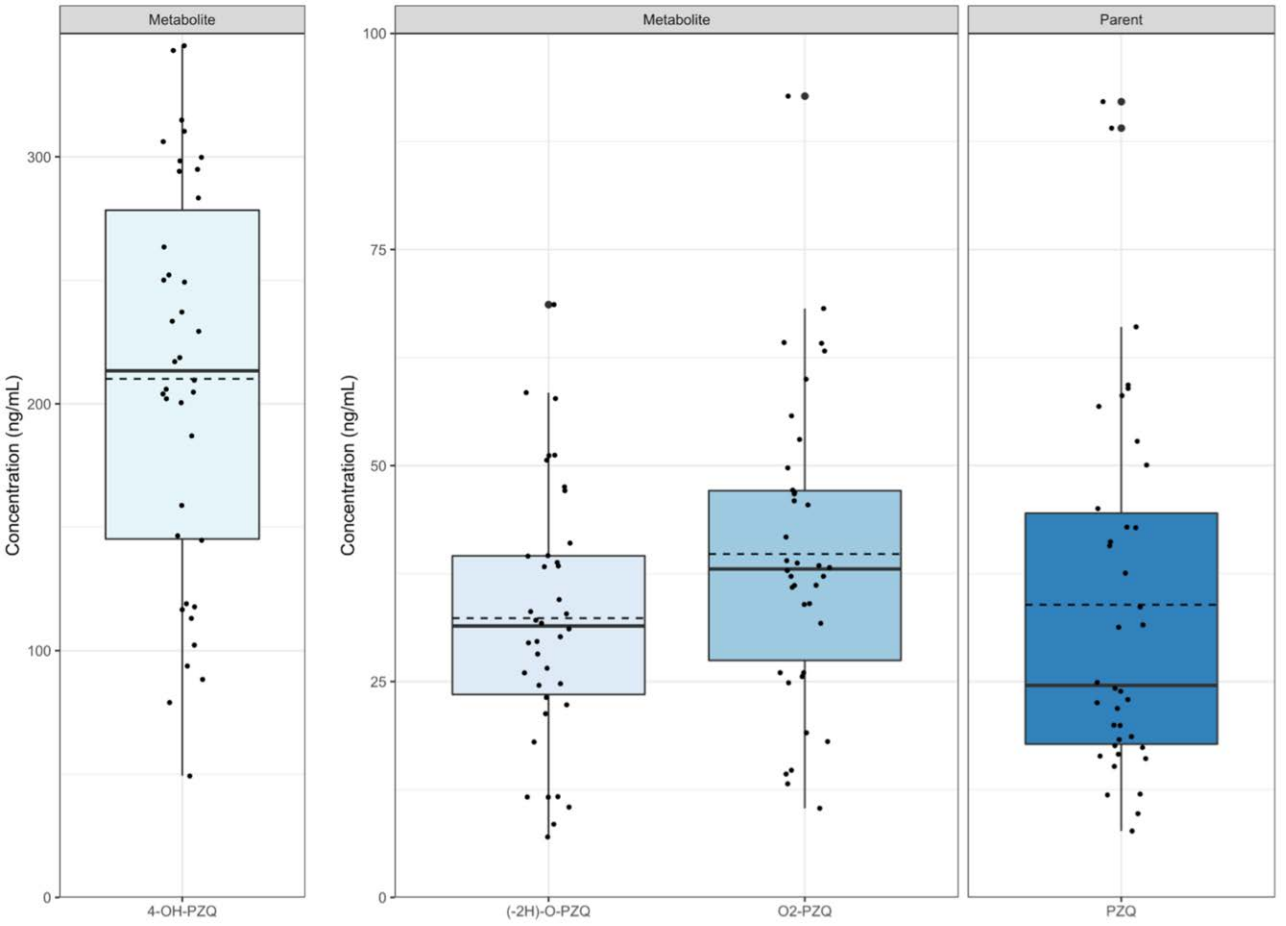
From the PCA performed to assess whether the age category, sex, or baseline infection intensity had any influence on analyte concentrations after control matching across the study population, the concentration profiles did not appear to cluster separately based on the tested metadata (Supplementary Figure S2, Supplemental digital content 1, *https://links.lww.com/FPC/B517*).

Population PK/PD analysis

To compare the pharmacokinetic experimental data with PZQ efficacy, the infection status at 3 weeks was compared with the in-vivo concentrations of PZQ, 4-OH-PZQ, (−2H)-O-PZQ, and O2-PZQ. The comparison of the mean concentration of PZQ and the three detected metabolites at each timepoint between the participants who cleared infection versus those who did not clear infection is displayed in Supplementary Table S8, Supplemental digital content 1, *https://links.lww.com/FPC/B517* and visualised in Fig. 4. There were no significant differences between the two groups for the PZQ, 4-OH-PZQ, and O2-PZQ mean concentrations; however, there was a significant difference in the concentration of the (−2H)-O-PZQ metabolite between the participants who cleared infection versus those who did not clear infection at 2.5 h. Generally, the participants who had cleared infection had higher concentrations of the three metabolites, 4-OH-PZQ, (−2H)-O-PZQ and O2-PZQ, for both timepoints analysed by the individual MS sampling (2.5/4 h); however, the participants who had not cleared infection had higher PZQ concentrations at 2.5 h compared to those who cleared the infection, but a lower PZQ concentration at 4 h. Those who did not clear infection did not sustain PZQ concentrations like those who were confirmed to have cleared the infection, with a decreased mean PZQ concentration of 29.5 ± 34.9 ng/ml compared with those who had cleared infection at 34.4 ± 20.6 ng/ml at 4 h.

In addition, there were no significant differences in the mean AUC between the two groups, and the NCA

Fig. 3



Concentration of analytes (praziquantel and the respective metabolites) detected at the 2.5-h timepoint across the Zimbabwean study population. This distribution is based on pooled mass spectrometry sampling. PZQ, praziquantel; 4-OH: 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; O2, dioxidised; PZQ, praziquantel.

parameters (Supplementary Table S9, Supplemental digital content 1, *https://links.lww.com/FPC/B517*). A binary logistic regression was also performed to further characterise PK/PD associations, with no relationship between PZQ exposure (for both AUC and C_{max}) and the probability of an individual clearing infection detected ($P = 0.587$). PCA also indicated that the concentration profiles did not appear to cluster separately based on 3-week infection status (Supplementary Figure S3, Supplemental digital content 1, *https://links.lww.com/FPC/B517*), indicating that the concentrations of the samples are similar to each other regardless of 3-week infection status.

Pharmacogenetic variation in the study cohort

Of the variants detected across *CYP* genes, ten were considered for PGx characterisation with regard to PZQ metabolism in this Zimbabwean population (Table 3). All variants were in Hardy–Weinberg equilibrium, except rs2069514 and rs28371706, likely because of sampling from the same rural setting (Table 3).

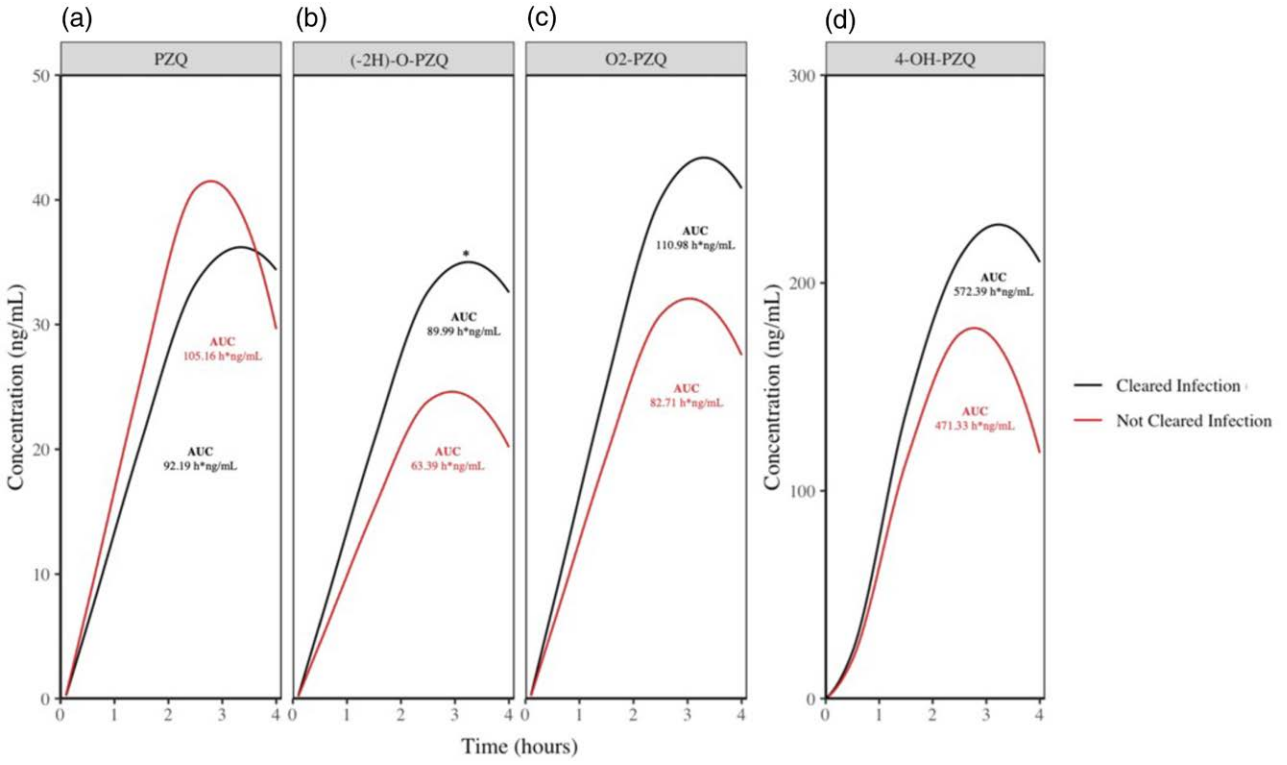
Effect of cytochrome P450 genotypes on the in-vivo analyte concentration

To investigate whether the analyte concentrations of the Zimbabwean study population were skewed because of PGx changes, the in-vivo concentration of PZQ (pharmacokinetic outcome) and its metabolites at 2.5 h were analysed with the participants corresponding PGx data. The NCA parameters were also obtained for PZQ and each metabolite, as well as the metabolic ratio between PZQ and its metabolites.

Dominant model

Given the relatively small sample size in this study, the dominant genotypic model was utilised to increase power, for example, with aA+AA genotypes grouped, to investigate the impact of the CYP genotype on the

Fig. 4



Comparison of the mean concentrations of the participants who cleared infection versus the participants who did not clear infection for (a) PZQ, (b) (−2H)-O-PZQ, (c) O2-PZQ, and (d) 4-OH-PZQ. The area under the curve values are presented under the relevant curves. * Represents a significant difference ($P < 0.05$) in concentration between the groups using one-way ANOVA. 4-OH, 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; ANOVA, analysis of variance; AUC, area under the curve; O2, dioxidised; PZQ, praziquantel.

Table 3 Cytochrome P450 genetic variants investigated across the Zimbabwean study population

				Allele frequency (%)		
Enzyme	Variant	Star allele	Reported enzyme activity/impact	ZIM	AFR	EUR
CYP1A2	rs2069514-A	*Formerly CYP1A2*1C*	Decreased function[a]	29.39[b]	31.32[b]	1.99[b]
	rs762551-A	*CYP1A2*30*	Increased function[c]	57	56.2	67.99[d]
CYP2C9	rs2256871-A (His251Arg)	*CYP2C9*9*	Decreased function[e]	13	8.17	0.1[b]
CYP2D6	rs28371706-A (Thr107Ile)	*CYP2D6*17*	Decreased function[e]	20[b]	21.80[b]	0.2[b]
	rs3892097-T (splice defect)	*CYP2D6*4*	No function[e]	2	6.05	18.59[d]
	rs1135840-G (Ser486Thr)	Part of *2 and other alleles	Normal function[e]	65.8	62.28	56.74
	rs16947-A (Arg296Cys)	Part of *2 and other alleles	Normal function[e]	31.6	33.98	31.82
CYP3A5	rs776746-C (splice defect)	*CYP3A5*3*	No function[e]	22.4	18	94.33[d]
	rs10264272-T (splice defect)	*CYP3A5*6*	No function[e]	22	15.43	0.3[b]
	rs41303343-insA (frameshift)	*CYP3A5*7*	No function[c]	19	11.8	0[b]

AFR, African populations; *CYP*, cytochrome P450; EUR, European populations; ZIM, Zimbabwean population in this study.
[a]Nakajima *et al.* [29].
[b]Significantly higher difference in the allele frequency in this Zimbabwean study population compared with the reported allele frequency (AF) in the African populations and the European populations in the 1000 Genomes Project using Fisher's exact test.
[c]Lu *et al.* [30].
[d]Significantly lower difference in the allele frequency in this Zimbabwean study population compared with the reported MAF in the African populations and the European populations in the 1000 Genomes Project using Fisher's exact test.
[e]Clinical Pharmacogenetics Implementation Consortium (CPIC; https://cpicpgx.org/).

concentration of PZQ and its three detected metabolites. Comparison of the mean concentrations of PZQ, 4-OH-PZQ, (−2H)-O-PZQ, and O2-PZQ and the metabolic ratio (metabolite/PZQ) between the different CYP genotypes are summarised in Table 4. In this analysis, there were only three variants of interest – *CYP1A2* rs2069514 (formerly **1C*), *CYP1A2* rs762551 (**30*), and *CYP2C9* rs2256871 (**9*) – for which analyte

concentration varied with genotype (Supplementary Figure S4, Supplemental digital content 1, *https://links.lww.com/FPC/B517*). The frequency of these variants by genotype in this Zimbabwean study population is presented in Fig. 5.

For *CYP2C9* rs2256871 (**9*), the parent PZQ concentrations did not vary between the reference (AA) and *CYP2C9*9* (AG & GG) genotypes ($P = 0.991$), with mean PZQ concentrations of 33.8 ± 22.4 and 34.1 ± 17.6 ng/ml, respectively. The mean concentration of the main metabolite 4-OH-PZQ was higher (but NS; $P = 0.098$) in *CYP2C9*9* (AG & GG) genotype carriers at 244.4 ± 71.9 ng/ml compared with the wild-type (AA) genotype at 199.4 ± 81.4 ng/ml; however, for both the (−2H)-O-PZQ and O2-PZQ metabolites, participants with *CYP2C9*9* (AG & GG) genotypes had significantly higher concentrations ($P \leq 0.05$) than the participants with *CYP2C9*9/*9* (Fig. 6c). For *CYP1A2* rs762551 (**30*), participants with rs762551 (CC) had a higher (but NS; $P = 0.074$) mean PZQ concentration (44.8 ± 24.1 ng/ml) than the mean concentration (30.5 ± 19.3 ng/ml) in participants with *CYP1A2*30* (rs762551 C/A and A/A) genotypes (Fig. 6a). For *CYP1A2* rs2069514, although there were no significant findings between the reference genotype (CC) and the alternate genotypes (G/A and A/A), the differences in the pharmacokinetic profiles (Supplementary Figure S4, Supplemental digital content 1, *https://links.lww.com/FPC/B517*) and the mean concentrations of each analyte (Table 4) do suggest a difference in CYP1A2 activity between the genotypes. Of note, there was no distinct clustering of concentration profiles by genotype (Supplementary Figure S5, Supplemental digital content 1, *https://links.lww.com/FPC/B517*).

Genotypic model

Comparison of the mean concentrations of PZQ, 4-OH-PZQ, (−2H)-O-PZQ, and O2-PZQ and the metabolic ratio (metabolite/PZQ) between the separate CYP genotypes using the genotypic model are summarised in Supplementary Table S10, Supplemental digital content 1, *https://links.lww.com/FPC/B517*. Only three CYP variants, that is, *CYP1A2* rs762551, *CYP2C9* rs2256871 (**9*), and *CYP2D6* rs1135840 (S486T) (see Fig. 5b for frequency data), required genotypic analysis as they did not correlate with the dominant model with regard to changes in PZQ concentration. Comparisons of the three rs762551 (*CYP1A2*30*) genotypes with analytes concentrations found no significant associations, although there was a lower mean PZQ concentration for both the participants with *CYP1A2*1/*30* (CA genotype) and participants with *CYP1A2*30/*30* (AA genotype) (Fig. 7a), corresponding with dominant model results. On the other hand, *CYP2C9*9* genotypes had significantly different mean concentrations of the (−2H)-O-PZQ and O2-PZQ metabolites (Fig. 7b). The genotypes of *CYP2D6* rs1135840 did not have any significant relationship with the concentration of any detected analyte. Regarding the separation of the analytes concentration profiles by CYP variant genotype using PCA, there was no distinct clustering by genotype (Supplementary Figure S6, Supplemental digital content 1, *https://links.lww.com/FPC/B517*).

Effect of cytochrome P450 variant genotypes on metabolite ratio

Dominant model

On the basis of the dominant model, the metabolic ratio (PZQ/metabolite) was significantly higher in participants with *CYP1A2*1/*30* and those with *CYP1A2*30/*30* compared participants with *CYP1A2*1/*1* for all three metabolites, 4-OH-PZQ/PZQ, (−2H)-O-PZQ/PZQ and O2-PZQ/PZQ (Fig. 6b and Table 4). The remaining CYP variants did not have any significant relationship between the CYP genotype and metabolic ratio.

Genotypic model

There were no metabolic ratios (PZQ concentration/metabolite concentration; see Supplementary Table S10, Supplemental digital content 1, *https://links.lww.com/FPC/B517*) that were found to be significantly different between the CYP genotypes; however, the ratios for 4-OH-PZQ/PZQ in participants with *CYP1A2*1/*30* and those with *CYP1A2*30/*30* were 8.5 and 7.3, respectively, and were higher than those with *CYP1A2*1/*1* at 5.5. This correlated with the general trend of the dominant model results above although, unlike the dominant model, it was NS via the genotypic model ($P = 0.076$).

Effect of cytochrome P450 variant genotypes on analyte exposure

Dominant model

The NCA parameters of PZQ and the three detected metabolites using the dominant model are displayed in Supplementary Table S11, Supplemental digital content 1, *https://links.lww.com/FPC/B517*. Regarding PZQ exposure, the mean AUC of the *CYP1A2*1/*1* genotype was found to be significantly higher than the *CYP1A2*1/*30* and *CYP1A2*30/*30* genotypes ($P < 0.05$) (Fig. 8a). For PZQ and all three of its detected metabolites, the *CYP1A2 rs2069514* reference genotype (G/G) had a higher mean AUC compared with the corresponding alternate genotypes (G/A and A/A), but not significantly different for any analyte. For *CYP2C9*9*, the mean AUC of both (−2H)-O-PZQ and O2-PZQ were found to be significantly higher ($P < 0.05$) in participants with *CYP2C9*1/*9* and those with *CYP2C9*9/*9* compared with participants with *CYP2C9*1/*1* (Fig. 8a).

Genotypic model

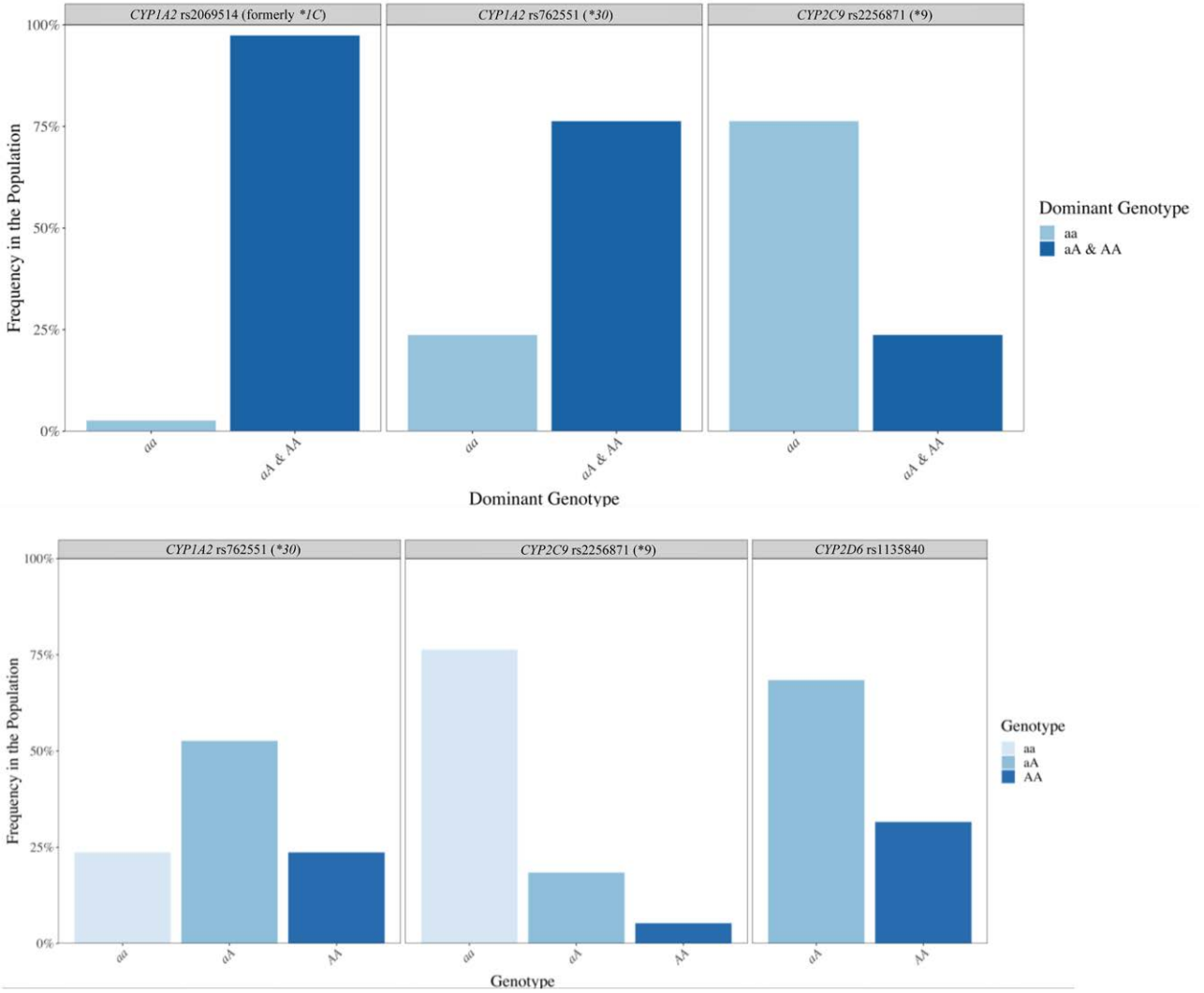
The NCA parameters of PZQ and the three detected metabolites using the genotypic model are displayed in

Table 4 Comparison of the means ± SD of praziquantel, 4-OH-PZQ, (−2H)-O-PZQ, and O2-PZQ concentrations (ng/ml) and the metabolic ratio (metabolite/ praziquantel) between the detected cytochrome P450 variant genotypes using one-way analysis of varaince

				PZQ		4-OH-PZQ				(−2H)-O-PZQ				O2-PZQ			
Enzyme	Variant	Genotype	*N*	Mean concentration (ng/ml) ± SD	*P* value	Mean concentration (ng/ml) ± SD	*P* value	Ratio ± SD	*P* value	Mean concentration (ng/ml) ± SD	*P* value	Ratio ± SD	*P* value	Mean concentration (ng/ml) ± SD	*P* value	Ratio ± SD	*P* value
CYP1A2	*rs2069514 (formerly *1C)*	*GG*	1	≈58.1	0.25	≈306.3	0.232	≈5.4	0.506	≈39.6	0.627	≈0.7	0.473	≈45.4	0.747	≈0.8	0.434
		GA & AA	37	33.2 ± 21		207.5 ± 80.2		7.6 ± 3.4		32.1 ± 15		1.2 ± 0.7		39.6 ± 17.7		1.5 ± 0.9	
	*CYP1A2*30 (formerly *1F)*	*CC*	9	44.8 ± 24.1	0.074	208.4 ± 73.6	0.944	5.5 ± 2.8	0.035*	28.8 ± 11.4	0.423	0.8 ± 0.4	0.031*	33.9 ± 11.3	0.25	0.9 ± 0.6	0.03*
		CA & AA	29	30.5 ± 19.3		210.6 ± 84		8.1 ± 3.3		33.4 ± 15.8		1.3 ± 0.7		41.6 ± 18.7		1.7 ± 1	
CYP2C9	rs2256871 *(*9)*	*AA*	29	33.8 ± 22.4	0.991	199.4 ± 81.4	0.098	7.1 ± 3.1	0.244	29.3 ± 12.9	0.009*	1.1 ± 0.6	0.134	36 ± 14.1	0.008*	1.4 ± 0.8	0.157
		AG & GG	9	34.1 ± 17.6		244.4 ± 71.9		8.6 ± 3.9		42.1 ± 17.2		1.5 ± 0.9		52 ± 22.2		1.9 ± 1.2	
CYP2D6	rs3892097 *(*4)*	*CC*	37	33.2 ± 21	0.25	207.5 ± 80.2	0.232	7.6 ± 3.4	0.506	32.1 ± 15	0.627	0.2 ± 0.7	0.473	39.6 ± 17.7	0.747	1.5 ± 0.9	0.434
		CT & TT	1	≈58.1		≈306.3		≈5.3		≈39.6		≈0.7		≈45.4		≈0.8	
	rs28371706 *(*17)*	*GA & AA*	38	33.9 ± 21.1	–	210.1 ± 80.7	–	7.5 ± 3.3	–	32.3 ± 14.8	–	1.2 ± 0.7	–	39.8 ± 17.5	–	1.5 ± 0.9	–
	rs1135840 (*2 and others)	*CG & GG*	38	33.9 ± 21.1	–	210.1 ± 80.7	–	7.5 ± 3.3	–	32.3 ± 14.8	–	1.2 ± 0.7	–	39.8 ± 17.5	–	1.5 ± 0.9	–
	rs16947 (*2 and others)	*GG*	14	36.9 ± 21.1	0.541	196.4 ± 69.5	0.56	7.4 ± 3.6	0.876	34.8 ± 17.2	0.332	1.3 ± 0.8	0.533	41.6 ± 16.1	0.659	1.7 ± 1.2	0.496
		GA & AA	24	32.5 ± 21		210.3 ± 71		7.6 ± 3.3		30.2 ± 11.7		1.1 ± 0.6		39.4 ± 13.9		1.4 ± 0.8	
CYP3A5	rs776746 *(*3)*	*TT*	30	33.9 ± 21.6	0.992	211.1 ± 88.5	0.879	7.3 ± 3	0.39	33.3 ± 16	0.439	1.2 ± 0.7	0.778	40.7 ± 18.7	0.524	1.5 ± 1	0.964
		TC & CC	8	33.9 ± 20.7		206.2 ± 43.9		8.4 ± 4.6		28.6 ± 9.5		1.1 ± 0.6		36.2 ± 11.8		1.5 ± 1	
	rs10264272 *(*6)*	*CC*	22	30.6 ± 20.5	0.273	193.5 ± 77	0.139	7.6 ± 3.4	0.771	31.4 ± 15.3	0.662	1.3 ± 0.7	0.444	39.7 ± 18.7	0.965	1.7 ± 1	0.295
		CT & TT	16	38.3 ± 21.7		232.9 ± 82.5		7.3 ± 3.3		33.6 ± 14.7		1.1 ± 0.6		39.9 ± 16.1		1.3 ± 0.8	
	rs41303343 *(*7)*	*A*	29	34.7 ± 22.2	0.668	208.2 ± 81.3	0.795	7.3 ± 3.3	0.474	32.4 ± 15.1	0.926	1.2 ± 0.7	0.872	40 ± 18	0.898	1.5 ± 1	0.917
		AA	9	31.2 ± 18.1		216.3 ± 83		8.2 ± 3.7		31.9 ± 14.8		1.2 ± 0.7		39.1 ± 16.5		1.6 ± 0.9	

One-way analysis of variance was performed using the dominant genotypic model. * indicates statistically significant differences.
4-OH, 4-monohydroxy; (–2H)-O, dehydro-mono-oxidised; *CYP*, cytochrome P450; O2, dioxidised; PZQ, praziquantel.

Fig. 5



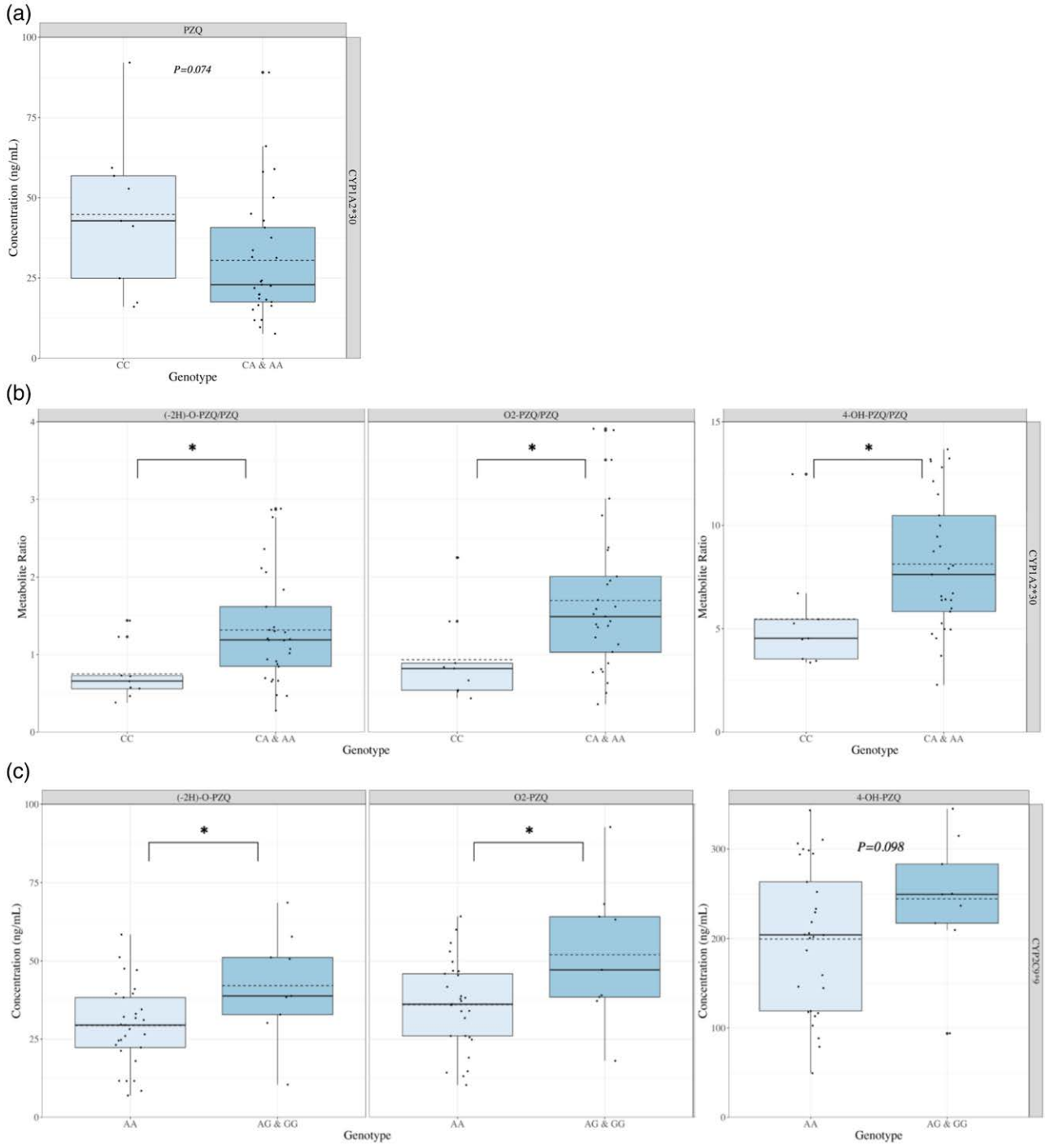
Frequency of CYP variant genotypes tested for effect on analyte concentrations in the Zimbabwean study population. (a) Frequency of genotypes comprising *CYP1A2* rs2069514 (formerly *1C), *CYP1A2* rs762551 (*30) and *CYP2C9* rs2256871 (*9) as used for the dominant model. (b) Frequency of genotypes comprising *CYP1A2* rs762551 (*30)*, *CYP2C9* rs2256871 (*9)*, and *CYP2D6* rs1135840 as used for the genotypic model. *aa* represents the reference genotype, *aA* represents the heterozygous alternate genotype, and *AA* represents the homozygous alternate genotype. *CYP*, cytochrome P450.

Supplementary Table S12, Supplemental digital content 1, *https://links.lww.com/FPC/B517*. According to this analysis, *CYP1A2*30* had no significant association with analyte exposure, although there was a lower mean PZQ AUC (Fig. 8b) for both the *CYP1A2*1/*30* genotype and the *CYP1A2 rs2069514* (A/A) genotype which correlated with the dominant results above. For *CYP2C9*9*, the mean AUC of both the (−2H)-O-PZQ and O2-PZQ metabolites was significantly higher ($P < 0.05$) in participants with *CYP2C9*1/*9* compared with participants with *CYP2C9*1/*1* and those with *CYP2C9*9/*9* (Fig. 8b). On the basis of Tukey multiple comparisons of means of the ANOVA results, the mean AUC was significantly higher ($P < 0.05$) in participants with *CYP2C9*9* (A/G). According to the genotypic model, *CYP2D6* rs1135840 did not have any significant association with the exposure of the analytes.

Effect of cytochrome P450 genotype on praziquantel efficacy

The efficacy of PZQ treatment, which was designated as whether the individual had cleared or not cleared the infection, was the designated pharmacodynamic outcome of this study. Overall, 94.8% (36/38) of the treated Zimbabwean study population had cleared infection at 3 weeks posttreatment. Fisher's exact tests were performed between each CYP genotype and the 3-week outcome (pharmacodynamic–PGx analysis),

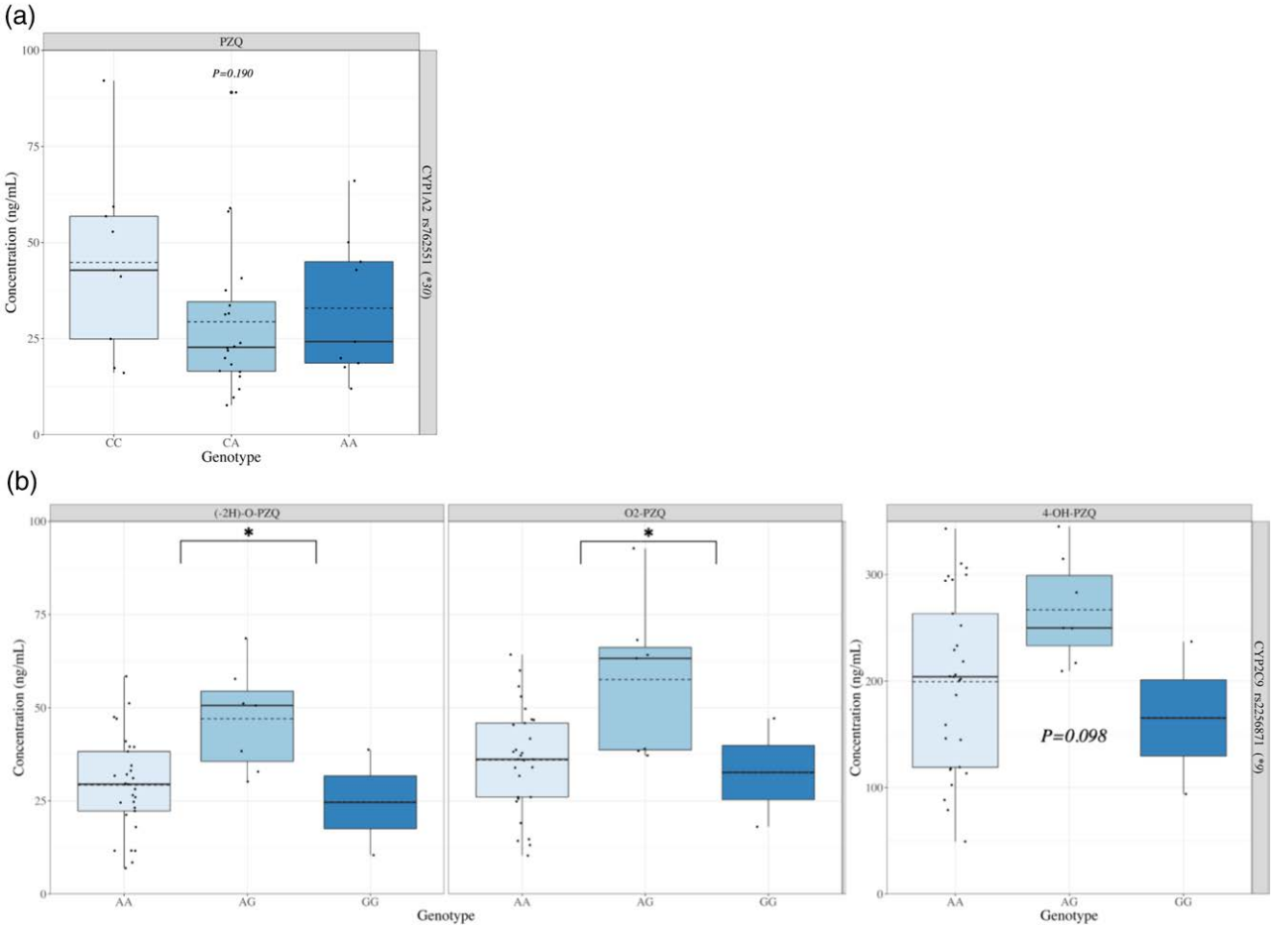
Fig. 6



Analyte concentrations in the Zimbabwean study population with respect to genotype in the dominant model. (a) Distribution of PZQ concentration, grouped by *CYP1A2* rs762551 (**30*) genotype. (b) Distribution of the metabolite ratio of (−2H)-O-PZQ, O2-PZQ and 4-OH-PZQ, grouped by *CYP1A2* rs762551 genotype. (c) Concentrations of (−2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ in the Zimbabwean study population, grouped by *CYP2C9* rs2256871 (**9*) genotype. 4-OH, 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; *CYP*, cytochrome P450; O2, dioxidised; PZQ, praziquantel.

with no significant associations found for any CYP variant using the dominant (Supplementary Table S13, Supplemental digital content 1, *https://links.lww.com/FPC/B517*) or the genotypic (Supplementary Table S14, Supplemental digital content 1, *https://links.lww.com/FPC/B517*) model.

Fig. 7



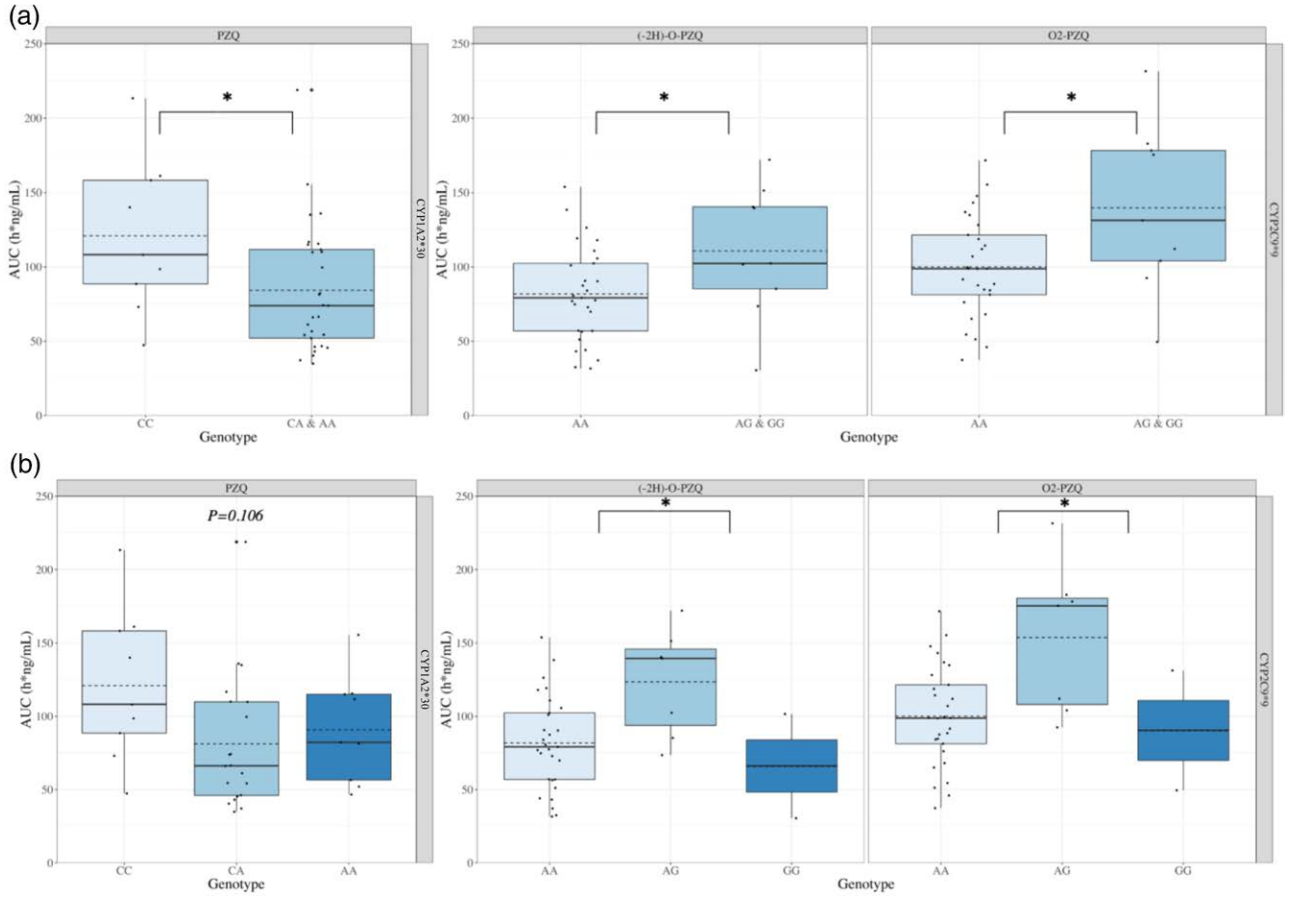
Analyte concentrations in the Zimbabwean study population with respect to genotype in the genotypic model. (a) Boxplots of the distribution of the concentrations of PZQ grouped by *CYP1A2* rs762551 (**30*; formerly **1F*) genotype. Boxplots of the distribution of the (−2H)-O-PZQ, O2-PZQ, and 4-OH-PZQ concentrations, grouped by *CYP2C9* rs2256871 (**9*) genotype. On the basis of Tukey multiple comparisons of the means of the ANOVA results, the concentrations were significantly higher ($P \leq 0.05$) in participants with *CYP2C9*1/*9* (A/G genotype) compared with *CYP2C9*1/*1* (A/A genotype) and *CYP2C9*9/*9* (G/G genotype). The mean concentration of the main metabolite, 4-OH-PZQ, was also higher in participants with *CYP2C9*1/*9* (A/G genotype) compared with the *CYP2C9*1/*1* (A/A genotype) and *CYP2C9*9/*9* (G/G genotype), although it was not statistically significant. 4-OH, 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; ANOVA, analysis of variance; *CYP*, cytochrome P450; O2, dioxidised; PZQ, praziquantel.

Discussion

PZQ is widely used in national schistosomiasis control programmes in Africa; however, there is considerable variability in interindividual responses to the medication which is partly explained by genetic differences. PK/PD research and the identification of genetic variants that can impact response to essential drugs such as PZQ is critical for precision public health programmes and individualised therapy with a view towards improving healthcare in Africa (and globally). Furthermore, these studies could inform future drug development efforts; however, African populations are understudied in PK/PD and PGx research to date. This is partly because of the lack of access to methodologies applicable in low-resource settings. Moreover, obtaining the relevant samples to perform *in vivo* PK/PD analysis tends to be invasive, time-consuming, and requires specialised clinical settings [4]. This is especially sensitive in the context of combating *Schistosoma* infections as school-age children tend to be most affected [31]. Therefore, this study aimed to investigate the PK/PD and PGx relationship between PZQ and its metabolites in a Zimbabwean population infected with *S. haematobium* using a fieldwork-applicable microsampling technique.

To begin with, we demonstrate that microsampling using DBS and MS is a feasible method to detect and quantify PZQ and its metabolites. The use of DBS sampling for therapeutic drug monitoring is well-established [7,8,32]. Advantages of DBS, including the relatively simple and minimally invasive sampling, use of small blood volumes, stability of analytes, convenient storage, and

Fig. 8



Distribution of the AUC of PZQ, (−2H)-O-PZQ, and O2-PZQ in the Zimbabwean study population, separated by CYP variant and then genotype in the dominant model (a) and the genotypic model (b). The concentrations of each analyte were calculated using the 2.5-h timepoint. The dotted lines represent the means for the sample population. *P* values less than 0.05 from the one-way ANOVA calculation were considered significant. ANOVA, analysis of variance; AUC, area under the curve; 4-OH, 4-monohydroxy; (−2H)-O, dehydro-mono-oxidised; *CYP*, cytochrome P450; PZQ, praziquantel.

transportation can facilitate the feasibility of more PK/PD and PGx studies in Africa, especially in locations where resources are limited such as the rural areas of Zimbabwe where this study was performed. In this study, DBS sampling enabled the identification of 4-OH-PZQ (main PZQ metabolite), (−2H)-O-PZQ, and O2-PZQ. The 4-OH-PZQ metabolite and its pathway (involving CYP1A2, CYP2C9, and CYP2C19) have been well-characterised in the literature [11,17,33–35]. Conversely, the evaluation of the metabolic pathway and the PK/PD analysis of (−2H)-O-PZQ and O2-PZQ have not yet been performed in humans. The O2-PZQ metabolite has been previously detected in the urine/faeces of mice and healthy human volunteers, but the (−2H)-O-PZQ had only been detected in mice [11,33]. Despite the vague knowledge about (−2H)-O-PZQ, it is postulated to be a further product of the 4-OH-PZQ metabolite based on the MS/MS spectra [11]. Thus, the CYP-mediated pathway for this metabolite may be similar to that of the major PZQ metabolite. The recently identified X-OH-PZQ [36], which is metabolised by the CYP3A4/5 pathways, was not identified in this study. Overall, the detection of 4-OH-PZQ, (−2H)-O-PZQ and O2-PZQ, in addition to the parent drug (and three glucuronide phase II metabolites), shows the viability of the DBS-sampling approach in this study for collection of metabolite data in low-resource settings and for sensitive study populations such as school-age children.

Regarding characterising the pharmacokinetic profile of the Zimbabwean population, the concentration–time curves of 4-OH-PZQ, (−2H)-O-PZQ, and O2-PZQ were similar between two MS sampling methods tested in this study (pooled sampling at 0.5, 1.5, 2.5, and 4-h timepoints versus individual sampling of each participant at the 2.5- and 4-h timepoints). Hence, the pooled MS samples were a good representation of the in-vivo analyte concentrations of the Zimbabwean study population at

these timepoints and could be a good way of obtaining a population pharmacokinetic profile using a smaller number of samples. We note that pooling of MS data at certain time points might have affected the accuracy of pharmacokinetic profiling, potentially masking interindividual variability. Assessing the granularity of potential biases introduced by pooled MS was out of the scope of this study; however, as the choice of the 2.5-h timepoint for the pooled MS data used for PGx was supported by the similarity to the mean concentration of the individual MS samples, we are confident that the effect of other biases was likely minimal for this cohort.

At the time of this study, the only published research on the impact of PGx variants on PZQ and its metabolite concentrations in African populations was by Mnkugwe *et al.* [35]; however, their study only focused on the main 4-OH-PZQ metabolite and was conducted in *S. mansoni* infected individuals, not *S. haematobium*. In our study, although differences in the analyte concentrations across rs762551 (*CYP1A2*30*) genotypes were not statistically significant, the 4-OH-PZQ, (−2H)-O-PZQ and O2-PZQ metabolic ratios were found to be significantly higher for individuals with rs762551 C/A and A/A compared to those with the reference C/C genotype. This is in line with previous reports on *CYP1A2*30* (formerly *CYP1A2*1F*) in the literature with regard to increase in the activity of CYP1A2 [30]. Patients with *CYP1A2*30* who are taking the standard dose of PZQ are therefore likely to have decreased active (R)-PZQ, consequently decreasing the efficacy of PZQ treatment [37,38]. For *CYP1A2* rs2069514 (previously *CYP1A2*1C*), although the mean AUC was not significantly different between the G/G reference genotype and the alternate genotypes (G/A and A/A), our findings were suggestive of the A allele being associated with slower metabolism, similar to observations in previous PGx investigations [29]. Of note, rs2069514 is not being used in recently-curated *CYP1A2* major allele definitions by PharmVar (https://www.pharmvar.org/gene/CYP1A2; accessed 8 April 2025). Another key finding was the altered metabolite concentrations – particularly higher mean concentrations and AUC for (−2H)-O-PZQ and O2-PZQ – in participants with *CYP2C9*9* (rs2256871 A/G and G/G genotypes) compared with the reference rs2256871 A/A genotype. This would suggest that *CYP2C9*9* (normal function allele according to Clinical Pharmacogenetic Implementation Consortium) potentially increases CYP2C9's activity to metabolise PZQ; however, given that no significant changes were observed with the major 4-OH-PZQ metabolite, the low sample size in this study, and the fact that the involvement of (−2H)-O-PZQ and O2-PZQ in the human PZQ pathway are not well understood [11], it is difficult to definitively conclude that *CYP2C9*9* is partly responsible for altered PZQ metabolism. Nonetheless, as *CYP2C9*9* is common across African-ancestry populations (frequency of ~12%) [39], Africa-based PGx studies with larger sample sizes are warranted to better characterise its impact on PZQ response.

There were some limitations to this study. Because of participant recruitment challenges, the study is based on a relatively small sample size, which limited the statistical power to detect strong associations between genetic variation in the cohort and PZQ efficacy. The *P* values derived from the analysis should therefore be interpreted with caution. Caution should also be taken regarding generalising the PGx findings in this study given the small sample size and the sampling of participants from one rural area in Zimbabwe. To increase the statistical power for the genetic associations, we implemented the dominant model, which involved pooling homozygous and heterozygous allele carriers to compare against the wild-type. While the dominant model enabled the identification of the reported trending associations (Figs. 6 and 8a), the genotypic model revealed apparent mismatches in the observed versus expected concentration differences between heterozygous carriers of effect alleles and homozygous carriers (Figs. 7b and 8b). For example, *CYP2C9*9* (rs2256871 G/G) carriers would be expected to have higher mean concentrations and AUC for (−2H)-O-PZQ and O2-PZQ compared to participants with rs2256871 A/G (and those with A/A); however, only two participants had rs2256871 G/G (Supplementary Table S11, Supplemental digital content 1, *https://links.lww.com/FPC/B517*), compared to seven with rs2256871 A/G, leading to a skew from the expected trend. It is therefore important to carry out studies with better representation across the genotypes to obtain more concrete variant associations. Another limitation we met is that the concentrations of analytes in DBS are not necessarily equal to the concentrations in venous blood [40]. For instance, the recovered concentrations of PZQ and 4-OH-PZQ from the DBS in this study were only approximately 10% of the plasma concentrations reported by Mnkugwe *et al.* [35] As there were no observed recovery failures during quality control (based on recovery of the d11-PZQ standard), the lower recovered concentrations could be because of the relatively poorer extraction approach of the analytes from the DBS cards and potential analyte degradation. Another well-known issue with the DBS technique is hematocrit bias [12], which can alter the recovered concentrations of the analytes because of its influence on blood viscosity and the spread of the blood spot before drying. In general, the concentrations of PZQ analytes recovered from DBS in this study were in a similar range to the Meister *et al.* [12] study, where hematocrit bias was found to have minimal influence on PZQ concentrations. While the in-vivo concentrations obtained during this study allowed for PZQ PGx comparisons, the lack of completely representative plasma concentrations hindered the evaluation of sustained lethal schistosome concentrations to relate to PZQ efficacy. Future work comparing observations based on DBS versus other

microsampling approaches is warranted to obtain further insights into the extraction efficiency and the potential degradation of analytes for each approach.

In conclusion, this is the first study investigating the impact of CYP PGx variations in *S. haematobium*–infected individuals using in-vivo concentrations of PZQ and its metabolites [including 4-OH-PZQ, (–2H)-O-PZQ and O2-PZQ], in relation to PZQ efficacy, in an African setting. Our research shows that the low-cost microsampling using DBS and MS is a potentially feasible method to detect and quantify these analytes. Identifying drivers of variable drug response is critical in schistosomiasis control programmes as this can inform how the Ministry of Health responds to infection hotspots, that is, by targeting both parasite-related factors and host-related factors. More broadly, studies generating PK/PD and PGx data from African populations will increasingly drive precision public health in Africa which could in turn prevent the waste of medical resources, treatment failure, and adverse drug reactions.

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G.Z. and F.M. designed the study. G.Z. and T.M. led the sample collection in the study population. G.B. and R.B. contributed to mass spectrometry analysis. G.Z. led the data analysis. G.Z. (and afterward D.T.) led the data interpretation and drafting of the manuscript. D.T., T.M., G.B., R.B., and F.M contributed to guiding the analysis, data interpretation, and extensively editing the manuscript.

Exome sequences of the study participants are available on the NCBI Sequence Read Archive, under project number PRJNA897802.

Conflicts of interest

There are no conflicts of interest.

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