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Prediction models for voriconazole pharmacokinetics based on pharmacogenetics: AN exploratory study in a Spanish population ☆

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ABSTRACT

Individualisation of the therapeutic strategy for the oral antifungal agent voriconazole (VCZ) is extremely important for treatment optimisation. To date, regulatory agencies include *CYP2C19* as the only major pharmacogenetic (PGx) biomarker in their dosing guidelines; however, the effect of other genes might be important for VCZ dosing prediction. We developed an exploratory PGx study to identify new biomarkers related to VCZ pharmacokinetics. We first designed a 'clinical practice VCZ-AUC prediction model' based on *CYP2C19* to be used as a reference model in this study. We then designed a multifactorial polygenic prediction model and found that genetic variability in *FMO3, NR1I2, POR, CYP2C9* and *CYP3A4* partially contributes to VCZ total area under the concentration–time curve ($AUC_{0-\infty}$) interindividual variability, and its inclusion in VCZ $AUC_{0-\infty}$ prediction algorithms improves model precision. To our knowledge, there are no PGx studies specifically relating *POR, FMO3* and *NR1I2* polymorphisms to VCZ pharmacokinetic variability. Further research is needed in order to test the model proposed here.

1. Introduction

Voriconazole (VCZ) is a second-generation triazole antifungal agent generally accepted as the first-line treatment for invasive aspergillosis and indicated for the treatment and prophylaxis of a variety of other fungal infections. However, its use is sometimes limited by its narrow therapeutic range and its wide interpatient variability in serum concentrations, which are directly related both to VCZ efficacy and the occurrence of adverse drug reactions [1].

In this context, individualisation of the VCZ therapeutic strategy is extremely important for treatment optimisation. VCZ pharmacokinetic (PK) variability depends on many clinical covariates such as age, hepatic function, concomitant medications, inflammation and genetic factors [1,2]. Therefore, implementation of a multidisciplinary approach in clinical practice combining both therapeutic drug monitoring [3,4] and pre-emptive pharmacogenetic (PGx) studies shows great potential for improving drug efficacy and reducing toxicity.

VCZ exhibits extensive hepatic metabolism, with <2% of the original dose excreted in an unchanged form. The main circulating metabolite is voriconazole *N*-oxide, which has minimal antifungal activity [5]. It is estimated that approximately 70–75% of total VCZ metabolism is mediated through cytochrome P450 (CYP) enzymes, mostly CYP3A4 and CYP2C19, with the flavin-containing monooxygenase (FMO) family mediating the remaining 25–30% [5–8]. VCZ also has the potential to be both a substrate and an inhibitor of the CYP2C19, CYP3A4 and CYP2C9 enzymes [9]. Therefore, genetic heterogeneity in the genes encoding these enzymes appears to have a great influence on VCZ PK interindividual variability.

The association between *CYP2C19* genotype and VCZ PK variability is well described. In fact, to date VCZ dosing guidelines based on pharmacogenetics are monogenic and rely exclusively

☆ The authors confirm that the Principal Investigator for this paper is Alberto M. Borobia and that he had direct clinical responsibility for the patients.

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on *CYP2C19* as a relevant biomarker for VCZ PK prediction and therapeutic recommendations [1,10]. There is significant evidence suggesting that single nucleotide polymorphisms (SNPs) in the *CYP2C19* gene explain 50–55% of VCZ metabolism [6,8,11]. Up to 35 variant star (*) alleles along the *CYP2C19* gene have been described by the Human Cytochrome P450 Allele Nomenclature Committee (https://www.pharmvar.org/) related to absent, reduced or increased enzymatic CYP2C19 activities. Supplementary Table S1 shows some of the most relevant variants related to CYP2C19 enzymatic activity in the White population.

In vivo and in vitro studies suggest that 20–30% of human VCZ metabolism can be catalysed by CYP3A4 [6,8,11]; thus, *CYP3A4* preemptive genotyping could be also useful before VCZ administration [12]. In addition, the effect of CYP3A4 on VCZ metabolism appears to be more relevant among those with the CYP2C19 poor metaboliser phenotype [8]. Several SNPs have been reported in association with CYP3A4 activity [13–15] (Supplementary Table S1). Studies on the influence of CYP3A5 on VCZ pharmacokinetics have yielded contradictory findings; however, an in vitro study suggested that interindividual differences in drug clearance and drug interactions of triazole antifungal drugs, including VCZ, are appreciably affected by *CYP3A5* genotypes [16]. The most relevant variants among Whites are shown in Supplementary Table S1.

In vitro studies using human liver microsomes showed that VCZ is also metabolised to a lesser extent by CYP2C9 [9,11]. The most common SNPs related to CYP2C9 activity are shown in Supplementary Table S1, both of which lead to a poor metaboliser phenotype [17,18].

CYP enzymes are regulated by a variety of other regulatory proteins; therefore, the activity of these proteins appears to have an additive effect on the pharmacokinetics of some CYP-metabolised drugs. Cytochrome P450 oxidoreductase (POR) enables the activity of P450 enzymes and is essential for CYP-mediated drug oxidation. Polymorphisms in the *POR* gene are related to alterations in POR modulation of CYP3A enzyme activity [19–22] (Supplementary Table S1). To our knowledge, there are no PGx studies specifically relating *POR* genetic variability to VCZ PK interindividual heterogeneity. However, recent studies have reported that POR concentrations significantly contribute to interindividual variability in CYP2C19 activity and CYP2C19-catalysed drug metabolism, such as VCZ metabolism [23].

On the other hand, nuclear receptor subfamily 1 group I member 2 (*NR1I2*), which encodes the human pregnane X receptor (PXR), is responsible for upstream regulation of CYP3A enzymes. Therefore, variants in the *NR1I2* gene are associated with alterations in CYP metabolic activity, which in turn might alter the pharmacokinetics of CYP-metabolised drugs [24].

FMOs represent the second-most important group of human phase I drug-metabolising enzymes, after CYPs. An important role of FMOs in VCZ metabolism has been reported in the literature, especially among children. An in vitro study suggested a contribution of approximately 20% of FMOs, mainly the FMO3 enzyme, in VCZ *N*-oxidation [8,25]. To our knowledge, the association between *FMO3* SNPs and VCZ pharmacokinetics has not yet been well described; however, a study performed on liver transplant patients treated with tacrolimus reported an association between *FMO3* SNPs and both FMO3 activity and rapid tacrolimus elimination [26]. Therefore, these SNPs might also be associated with VCZ PK variability (Supplementary Table S1).

Finally, P-glycoprotein [P-gp; also known as multidrug resistance protein 1 (MDR1)], encoded by the *ABCB1* gene, is the most important drug efflux transporter and has been associated with VCZ pharmacokinetics [27]. Several SNPs have been reported in *ABCB1* related to altered transport function of P-gp and leading to interindividual variability in the pharmacokinetics of its substrates [27,28] (Supplementary Table S1).

In conclusion, VCZ efficacy and toxicity show great variability both in adult and paediatric populations. It has been reported that *CYP2C19* genotyping can be a robust tool for guiding VCZ therapy. However, part of this variability might also be explained by genetic variability in other genes (such as *ABCB1, CYP3A4, CYP3A5, CYP2C9, POR, NR1I2* and *FMO3*). We therefore performed an observational study nested to three bioequivalence clinical trials of two VCZ formulations in 106 Spanish patients. The studies were performed at La Paz University Hospital and La Princesa University Hospital in Madrid (EUDRA-CT: 2012-004029-26; 2014-001964-36 and 2014-005342-22), with the aim of: (i) evaluating the additional contribution of PGx variability in other biomarkers, different to *CYP2C19*, to the VCZ PK profile; and (ii) studying whether the creation of polygenic prediction algorithms may help to improve VCZ area under the concentration–time curve (AUC) prediction rates.

2. Methods

2.1. Patients

The present study was performed within three randomised crossover clinical trials to evaluate the bioequivalence of two 200 mg VCZ formulations. A total of 106 Spanish healthy volunteers were included. All of the participants provided written consent before study initiation and after reception of written and oral information related to the objectives, characteristics, procedures, risks and rights of participation in the study. Bioequivalence of the two formulations was demonstrated for AUC in all three trials, following the criteria accepted by the current European Medicines Agency (EMA) regulations. Therefore, both formulations were included in the analysis as described in Section 2.4.

2.2. Pharmacokinetic study

Venous blood samples (3 mL) were collected and placed in tubes containing ethylene diamine tetra-acetic acid K_2 (K_2-EDTA) as anticoagulant at baseline and at 0.33, 0.67, 1, 1.33, 1.67, 2, 2.33, 2.67, 3, 4, 5, 6, 8, 10, 12, 24 (±1) and 32 (±1) h (or 36 h depending on the study) following drug administration. VCZ and internal standard were measured by reversed-phase high-performance liquid chromatography coupled to a tandem mass spectrometry detector (LC-MS/MS) by a certified laboratory compliant with EMA regulation for bioavailability and bioequivalence studies. The lower limit of quantification was 5.03 ng/mL. The PK analysis was performed using WinNonlin 6.3 software (Pharsight Corp., Cary, NC) by means of a non-compartmental analysis.

The maximum VCZ concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from the plasma concentration results. The total AUC ($AUC_{0-\infty}$ in ng/mL·h) was calculated from the addition of two partial AUCs: (i) AUC_{last}, area between the dosage time and the last time with detectable concentration, calculated by the trapezoidal rule; and (ii) $AUC_{t-\infty}$, calculated as the ratio C/k, in which C is the last detectable concentration and k is the slope obtained in the lineal regression calculated from the points corresponding to the elimination phase of the drug. PK data were log-transformed; C_{max} and AUC were adjusted to dosage/weight.

2.3. Patient genotyping

A molecular analysis was performed on all 106 patients for the selected SNPs (Supplementary Table S1) using the custom SNP array platform PharmArray®: rs4244285, rs4986893, rs12248560 and rs28399504 in *CYP2C19*; rs2032582 and rs1045642 in *ABCB1*; rs55785340, rs4646438, rs2740574 and rs35599367 in *CYP3A4*; rs776746, rs55965422, rs10264272, rs41303343 and rs41279854 in *CYP3A5*; rs1799853 and rs1057910 in CYP2C9; rs1057868 and

rs2868177 in *POR*; rs3814055 in *NR1I2*; and rs1800822, rs2266782 and rs909530 in *FMO3*. For those SNPs that were not included in the array design (rs2740574, rs35599367, rs3814055 and *FMO3*), Sanger sequencing was performed. Diplotypes were codified to the star allele nomenclature (*) using the Haplotype Set IDs provided by PharmGKB [7] and PhamVar [29]: *CYP3A5* (PA165980507); *CYP3A4* (PA165980506); *CYP2C19* (PA166128323); and *CYP2C9*. CYP2C19 phenotypes were inferred using the Clinical Pharmacogenetics Implementation Consortium (CPIC) standardised allele definition and functionality tables (PA166124411) as well as specific clinical guidelines [1].

2.4. Statistical analysis

Descriptive statistics of continuous variables are presented as minimum, maximum, standard deviation and mean. Effects of factors in these variables were evaluated with a non-parametric test. Qualitative variables and factors are presented in terms of frequency and contingency tables. Fisher's exact test was used to determine the association between factors. Statistical analyses were focused on $AUC_{0-\infty}$, transformed using a decimal logarithm after correction for dosage/weight.

According to previous knowledge, we adjusted a linear repeated measures model [30], where individuals are considered as random, under minimum Akaike information criterion (AIC) [31], performing an automatic backward elimination of all effects [age, sex, height, weight, body mass index (BMI), CYP2C19 phenotype] of a linear mixed-effects model, which includes the CYP2C19 phenotype as a covariate. We refer to this model as the *CYP2C19* model and we used it as the gold standard for comparison with the proposed polygenic model herein.

For each model, the coefficient of determination R^2 (or Rsq) was calculated taking into account that we are fitting linear mixed models, according to the approaches of Edwards et al. and Jaeger et al. [32,33]. The value for the overall model is provided. In order to know the contribution of each fixed effect, a partial R^2 was also calculated. This partial R^2 should not be considered equivalent to the partial R^2 in a linear model; these values show a hierarchy of importance of the fixed effect in the model. Larger R^2 values indicate higher importance in the pharmacological process described by the model. We considered as acceptable a percentage R^2 increase of more than 2.5%, according to other previous publications [34].

We also calculated the root mean square error of prediction (RMSEP) [35] as the prediction ability measure of the models:

$$RMSEP = \sqrt[2]{\frac{\sum_1^n \left(y_i - \hat{y}_i\right)^2}{n}}$$

where y_i is the observed value, $\hat{y}_i$ is the predicted value, and n is the number of observations. We considered as acceptable a percent RMSEP decrease of more than 5%.

The *CYP2C19* model was cross validated in two sets, where the four ultrarapid metaboliser subjects were assigned randomly in two sets, and 100 replicates were done using both sets, first to fit the model, and second to predict $\log_{10}$ $AUC_{0-\infty}$. Poor metaboliser subjects were excluded since only two individuals presented this phenotype.

To detect possible variables of interest for VCZ $AUC_{0-\infty}$ prediction, we used a model based on all genotyped SNPs and other demographic variables instead of CYP2C19 phenotype only after variable selection under minimum AIC, called the 'SNP model'. R^2 was calculated for the overall model as well as the partial contribution of each variable. RMSEP was also obtained.

Statistical computations were performed using R [36] and RStudio [37]. R package lme4 [38] was used when the repeated measures models approach holds, and Genetics [39] for Hardy–Weinberg disequilibrium testing. A P-value of <0.05 was considered statistically significant. For the false discovery rate (FDR), a P-value of <0.0316 was considered as significant applying the method proposed by Benjamini and Hochberg [40].

3. Results

3.1. Study population characteristics

Table 1 shows the demographic features as well as SNP and haplotype frequencies found in the study population. The population consisted of 106 individuals with the following *CYP2C19* genotypes: 4 ultrarapid metabolisers (UM); 34 rapid metabolisers (RM); 38 normal metabolisers (NM); 28 intermediate metabolisers (IM); and 2 poor metabolisers (PM).

3.2. CYP2C19 reference predictive model

We first designed a monogenic reference predictive model of VCZ $AUC_{0-\infty}$ based only on *CYP2C19* as a PGx biomarker by creating a linear repeated measures model under minimum AIC, including the CYP2C19 phenotype as a covariate. The resulting predictive model based on the CYP2C19 phenotype is shown in Table 2 and Fig. 1. The global R^2 for this model was 0.438 and the RMSEP was 0.18 (Table 3). This model was cross-validated in two groups (A and B) as there were only four UM patients. We performed 100 simulations for validation. The *CYP2C19* predictive model will be used later as a reference model for comparison with the proposed polygenic predictive model, including additional biomarkers related to VCZ $AUC_{0-\infty}$ revealed in the exploratory study.

3.3. Identification of additional candidate biomarkers related to voriconazole pharmacokinetics: design of a multifactorial predictive model for VCZ $AUC_{0-\infty}$

We performed an exploratory study for the identification of additional candidate biomarkers that could be related to VCZ pharmacokinetics in this study cohort. Table 4 shows the best model estimated under minimum AIC following the inclusion of all biomarkers genotyped for this study. For this analysis, we included demographic data and SNP information of all biomarkers genotyped in the study population. SNP results for *CYP3A5, CYP2C19* and *CYP2C9* were codified into haplotypes using the star allele nomenclature. The best model obtained selects SNPs among the *CYP2C9, CYP3A4, POR, NR1I2* and *FMO3* genes (in addition to the *CYP2C19* gene) as variables of interest for VCZ $AUC_{0-\infty}$ prediction (Table 4). BMI and sex were also selected as relevant covariates. The inclusion of additional biomarkers in the *CYP2C19* predictive model increased R^2 values and decreased RMSEP calculation ($R^2 = 0.587$, RMSEP = 0.165; Table 3).

When evaluating the contribution and significance of each of the selected variables, we saw that *POR* accounts for ca. 18% of the R^2 of the model, followed by *CYP2C9* (ca. 8%), BMI (ca. 10%), *NR1I2* (ca. 4%), *FMO3* (ca. 4%), *CYP3A4* (ca. 3%) and sex (ca. 2%), in addition to *CYP2C19* (ca. 39%) that remains the main factor explaining interindividual variability (Table 4).

4. Discussion

We performed an observational study nested to three bioequivalence clinical trials of two VCZ formulations in 106 Spanish patients with the aim of evaluating whether the incorporation of additional PGx biomarkers to the *CYP2C19*-based VCZ dosing

Table 1
Patient characterisation ($n = 106$)

Patient characteristics					
Sex (no. male/female)					57/49
Age (years) (mean ± S.D.)					23.8 ± 4.3
Weight (kg) (mean ± S.D.)					67.9 ± 12.2
Height (cm) (mean ± S.D.)					170.2 ± 9.6
BMI (kg/m^2) (mean ± S.D.)					23.3 ± 2.9
Genetic information					
Gene	Codified genotype	Inferred phenotype	Study N	Study frequency ($n = 106$)	Population frequency (European)
CYP2C19	*2/*2	PM	2	0.02	*1
	*1/*1	NM	38	0.36	HapFreq = 0.621
	*1/*2	IM	28	0.26	*2
	*1/*4a				HapFreq = 0.146
	*1/*17	RM	34	0.32	*4A
	*17/*17	UM	4	0.04	HapFreq = 0.003
					*17
					HapFreq = 0.213
CYP3A5	*3/*3	PM	90	0.85	*1
	*3/*6				HapFreq = 0.078
	*1/*3	IM	16	0.15	*3
					HapFreq = 0.921
					*6
					HapFreq = 0.100
CYP2C9	*2/*2	PM	3	0.03	*1
	*2/*3				HapFreq = 0.80
	*1/*2	IM	37	0.35	*2
	*1/*3				HapFreq = 0.12
	*1/*1	NM	66	0.62	*3
					HapFreq = 0.07
CYP3A4	*1/*1		91	0.86	*1
	*1/*1B		7	0.07	HapFreq = 0.92
	*1/*22		8	0.08	*1B
					HapFreq = 0.02
					*22
					HapFreq = 0.04
FMO3	rs1800822				MAF = 0.60 (T)
	CC		97	0.92	
	CT		8	0.08	
	TT		1	0.01	
	rs2266782				MAF = 0.37 (A)
	GG		48	0.45	
	GA		43	0.41	
	AA		15	0.14	
	rs909530				MAF = 0.24 (T)
	CC		73	0.69	
	CT		28	0.26	
	TT		5	0.05	
POR	rs1057868				MAF = 0.30 (T)
	CC		59	0.56	
	CT		39	0.37	
	TT		8	0.08	
	rs2868177				MAF = 0.34 (G)
	AA		54	0.51	
	AG		38	0.36	
	GG		14	0.13	
NR1I2	rs3814055				MAF = 0.37 (T)
	CC		34	0.32	
	CT		54	0.51	
	TT		18	0.17	
ABCB1	rs2032582				MAF = 0.41 (A)/0.02 (T)
	CC		41	0.39	
	CA		56	0.53	
	AA		6	0.06	
	CT		2	0.02	
	TA		1	0.01	
	rs1045642				MAF = 0.52 (A)
	GG		26	0.25	
	GA		68	0.64	
	AA		12	0.11	

S.D., standard deviation; BMI, body mass index; PM, poor metaboliser; NM, normal metaboliser; IM, intermediate metaboliser; RM, rapid metaboliser; UM, ultrarapid metaboliser; HapFreq, haplotype frequency; MAF, mutation annotation format.

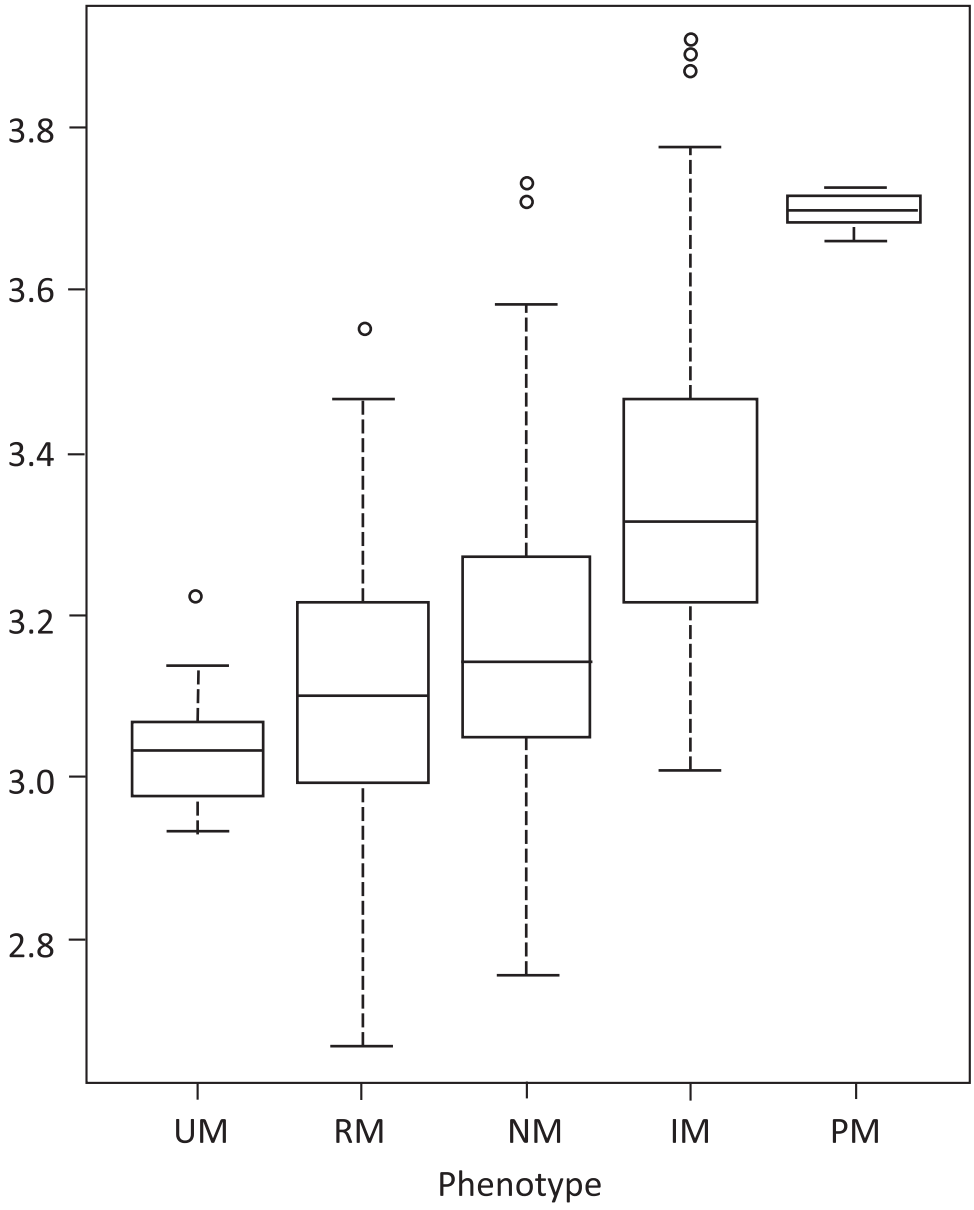

Fig. 1. Effect of CYP2C19 phenotype on voriconazole $AUC_{0-\infty}$ after administration of a single 200 mg dose. $AUC_{0-\infty}$, total area under the concentration–time curve. UM, ultrarapid metaboliser; RM, rapid metaboliser; NM, normal metaboliser; IM, intermediate metaboliser; PM, poor metaboliser.

Table 2
Predictive model based on the CYP2C19 phenotype: reference model

	Estimate	Std. Error	Pr(> |t|)	R^2
(Intercept)	3.038	0.089	0.000	
CYP2C19 RM	0.074	0.095	0.437	0.010
CYP2C19 NM	0.129	0.094	0.172	0.030
CYP2C19 IM	0.325	0.096	0.001	0.157
CYP2C19 PM	0.657	0.156	0.000	0.203

RM, rapid metaboliser; NM, normal metaboliser; IM, intermediate metaboliser; PM, poor metaboliser.
The ultrarapid metaboliser (UM) group was assigned an estimate value of 0. The global R^2 for the proposed model was 0.438 and the root mean square error of prediction (RMSEP) was 0.180.

algorithms could improve VCZ $AUC_{0-\infty}$ prediction rates. This is highly challenging owing to VCZ non-linear pharmacokinetics and its inherent variability [41].

As previously reported and as stated in clinical guidelines [1,2,10], CYP2C19 has a major effect on VCZ metabolism and is therefore the main factor explaining $AUC_{0-\infty}$ heterogeneity in the study cohort (Fig. 1). In this context, our first step was the development of a monogenic reference predictive model, based on the existing clinical algorithms for VCZ treatment optimisation, relying exclusively on CYP2C19 phenotype and referred to as the reference *CYP2C19* predictive model (Table 2). The *CYP2C19* genotype divides the study population into five *CYP2C19* phenotypic subgroups: UM ($n=4$); RM ($n=34$); NM ($n=38$); IM ($n=28$); and PM ($n=2$). The calculated R^2 for this model was 0.438 and the RMSEP was 0.180.

To date, most of the dosing algorithms based on pharmacogenetics implemented in clinical practice are monogenic, and that is the case for VCZ [7]. However, when studying the VCZ metabolic pathway, one can realise that there are additional biomarkers involved in VCZ pharmacokinetics that could help in the explanation of VCZ residual variability in plasma concentrations. We then developed an exploratory statistical analysis in order to evaluate the additional contribution of other biomarkers, different to *CYP2C19*, to VCZ metabolism and to investigate whether the

Table 3
Root mean square error of prediction (RMSEP) comparison between the *CYP2C19* reference model and the proposed multifactorial predictive model

RMSEP			R^2		
CYP2C19 reference model	Proposed model	% change	*CYP2C19* reference model	Proposed model	% change
0.180	0.165	−8.33	0.438	0.587	34.02

Table 4
Variables of importance for $AUC_{0-\infty}$ in the polygenic model based on single nucleotide polymorphisms (SNPs)

	Variable	Estimate	Std. Error	Pr(> |t|)	R^2/% of total R^2 explained by each biomarker	
Associated gene	(Intercept)	2.687	0.093	0.000		
	GenderM	−0.045	0.020	0.024	0.018 (ca. 2%)	
	BMI	0.023	0.004	0.000	0.103 (ca. 10%)	
CYP2C19	*1/*1	−0.026	0.032	0.429	0.002	ca. 39%
	*1/*17	−0.0136	0.034	0.000	0.058	
	*1/*2	0.234	0.035	0.000	0.150	
	*17/*17	−0.170	0.058	0.004	0.031	
	*2/*17	−0.064	0.076	0.404	0.002	
	*2/*2	0.547	0.077	0.000	0.142	
CYP2C9	*1/*2	0.097	0.025	0.000	0.052	ca. 8%
	*1/*3	0.037	0.028	0.188	0.006	
	*2/*2	0.137	0.102	0.182	0.007	
	*2/*3	0.137	0.071	0.055	0.014	
FMO3	rs1800822CT	−0.087	0.041	0.033	0.018	ca. 4%
	rs1800822TT	0.248	0.089	0.006	0.023	
NR1I2	rs3814055CT	−0.010	0.022	0.661	0.001	ca. 4%
	rs3814055TT	−0.090	0.026	0.001	0.037	
POR	rs1057868CT	−0.062	0.027	0.021	0.023	ca. 18%
	rs1057868TT	0.222	0.046	0.000	0.114	
	rs2868177AG	0.045	0.024	0.060	0.015	
	rs2868177GG	−0.078	0.033	0.017	0.023	
CYP3A4	rs2740574AG	−0.128	0.045	0.005	0.032	ca. 3%

$AUC_{0-\infty}$, total area under the concentration–time curve; BMI, body mas index.
The global R^2 for the best proposed model is 0.587 and the root mean square error of prediction (RMSEP) is 0.165.

incorporation of polygenic prediction algorithms may improve VCZ $AUC_{0-\infty}$ prediction rates based only on *CYP2C19* genotype.

To this aim, we decided to create a model including all previously selected biomarkers as variables of interest for this study, as well as other demographic data. This model was referred to as the 'multifactorial predictive model for VCZ $AUC_{0-\infty}$' (Table 3). The aim of this model was to detect all variables that could be involved in VCZ $AUC_{0-\infty}$ interindividual variability. For this analysis, haplotypes were inferred for the major metabolising enzymes (*CYP3A4, CYP3A5, CYP2C9* and *CYP2C19*) and diplotypes were codified using the star allele nomenclature to detect the overall effect of the protein instead of the individual SNPs. The best predictive model obtained confirmed the major metabolic effect of *CYP2C19* in this study cohort of a Spanish population (with a contribution of ca. 39% to global R^2 of the model). In addition, other biomarkers were revealed as possible variables of interest for VCZ $AUC_{0-\infty}$ prediction: rs1800822 in *FMO3* (ca. 4%); rs3814055 in *NR1I2* (ca. 4%); rs1057868 and rs2868177 in *POR* (ca. 18%); and CYP3A4 (ca. 3%) and *CYP2C9* genotype (ca. 8%), as well as sex (ca. 2%) and BMI (ca. 10%). Incorporation of all of these biomarkers and demographic data increased the global R^2 of the reference model ($R^2_{\text{Multifactorial model}} = 0.587 > R^2_{\text{Reference model}} = 0.438$) as it decreased the global RMSEP of the model ($\text{RMSEP}_{\text{Multifactorial model}} = 0.165 < \text{RMSEP}_{\text{Reference model}} = 0.180$), indicating an increase in precision of VCZ $AUC_{0-\infty}$ predictions compared with the *CYP2C19* reference model (Table 3).

In this study, we identified several biomarkers, related to the VCZ metabolic pathway in the literature, that partially explained VCZ $AUC_{0-\infty}$ residual variability in the study population. In this context, inclusion of these biomarkers into existing algorithms based only on *CYP2C19* as a PGx biomarker for guiding VCZ treatment appears to improve VCZ $AUC_{0-\infty}$ predictions (Table 3). To our knowledge, there are no PGx studies specifically linking *POR, FMO3* and *NR1I2* genetic variability to VCZ PK interindividual heterogeneity, however the implication of these proteins in the VCZ metabolic pathway has been well described.

Owing to a strong influence of CYP2C19 on VCZ metabolism, these biomarkers do not individually explain a great percentage of VCZ AUC interindividual variability (Table 3). However, overall they contribute to the explanation of ca. 60% of the residual variability that is not explained by *CYP2C19* genotype. In addition, we hypothesise that the reported biomarkers might become even more relevant in patients with a CYP2C19 PM phenotype (e.g. CYP2C19*2/*2 patients), where in the absence of a functional CYP2C19 enzyme the effect of other metabolising enzymes may increase. This has previously been described for other drugs such as tacrolimus where complementary biomarkers such as *POR* and *ABCB1* become more relevant in *CYP3A5*-defective genotypes [42]. Owing to the small number of PMs found in the study cohort ($n = 2$), we were not able to design prediction models for these patients and the specific effect of these biomarkers in CYP2C19 PMs should be tested in future studies.

However, after the evaluation of the two models proposed here based on R^2 and RMSEP coefficients, we can see that the most suitable model for VCZ $AUC_{0-\infty}$ prediction in this population is the polygenic predictive model (Table 3). Taking into account these results and previous literature, we propose that *FMO3, NR1I2, POR, CYP3A4* and *CYP2C9* genotypes might represent a useful tool for explaining VCZ PK variability and that their incorporation to the 'clinical practice *CYP2C19*' prediction algorithms could improve VCZ $AUC_{0-\infty}$ prediction rates. Although it could be considered that the global predictive ability of this model is moderate, we think that the ca. 15% decrease in the non-controlled variability

in the predicted VCZ concentration could be relevant in the significant proportion of patients who are usually below the target concentration with the standard dose [4].

In any case, further research is needed in larger cohorts with a higher representation of rare diplotypes and in different populations to test the model proposed herein. In addition, this study was performed in healthy volunteers with a single dose of VCZ and therefore further research in patient cohorts where multiple doses are administered is necessary to validate the biomarkers proposed here for its clinical use.

To achieve a cost-efficient implementation of polygenic models into clinical practice it is necessary to rely on genotyping platforms that allow simultaneous genotyping of multiple biomarkers in the same run. In this context, as the costs and analysis time of next-generation sequencing decrease, there is a general tendency towards the application of genotyping panels and exomes in order to have available a great amount of molecular information to be extracted for different purposes. However, to date the authors believe the application of SNP arrays constitutes the best solution for the implementation of polygenic models in clinical practice in terms of costs and response time [43], mostly because whole exome sequencing does not cover many SNPs that map to non-coding genomic regions.

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Competing interests

FA-S and DO have been consultants or investigators in clinical trials sponsored by Abbott, Alter, Bristol-Myers, Chemo, Cinfa, FAES Farma, Farmalíder, Ferrer, GlaxoSmithKline, Galenicum, Gilead, Italfarmaco, Janssen-Cilag, Kern Pharma, Normon, Novartis, Servier, Silverpharma, Teva and Zambon. All other authors declare no competing interests.

Ethical approval

Ethical approval was obtained from the Clinical Research Ethics Committee of La Paz University Hospital (Madrid, Spain).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.06.026.

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