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ARTICLE

*CYP2C8**3 and *4 define CYP2C8 phenotype: An approach with the substrate cinitapride

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Abstract

Cinitapride is a gastrointestinal prokinetic drug, prescribed for the treatment of functional dyspepsia, and as an adjuvant therapy for gastroesophageal reflux disease. In this study, we aimed to explore the impact of relevant variants in *CYP3A4* and *CYP2C8* and other pharmacogenes, along with demographic characteristics, on cinitapride pharmacokinetics and safety; and to evaluate the impact of *CYP2C8* alleles on the enzyme's function. Twenty-five healthy volunteers participating in a bioequivalence clinical trial consented to participate in the study. Participants were genotyped for 56 variants in 19 genes, including cytochrome P450 (CYP) enzymes (e.g., CYP2C8 or CYP3A4) or transporters (e.g., SLC or ABC), among others. *CYP2C8**3 carriers showed a reduction in AUC of 42% and C_{max} of 35% compared to *1/*1 subjects ($p = 0.003$ and $p = 0.011$, respectively). *4 allele carriers showed a 45% increase in AUC and 63% in C_{max} compared to *1/*1 subjects, although these differences did not reach statistical significance. *CYP2C8**3 and *4 alleles may be used to infer the following pharmacogenetic phenotypes: ultrarapid (UM) (*3/*3), rapid (RM) (*1/*3), normal (NM) (*1/*1), intermediate (IM) (*1/*4), and poor (PM) metabolizers (*4/*4). In this study, we properly characterized RMs, NMs, and IMs; however, additional studies are required to properly characterize UMs and PMs. These findings should be relevant with respect to cinitapride, but also to numerous CYP2C8 substrates such as imatinib, loperamide, montelukast, ibuprofen, paclitaxel, pioglitazone, repaglinide, or rosiglitazone.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

CYP2C8 is a poorly described gene from a pharmacogenetic perspective, traditionally assumed to have a minor impact on pharmacotherapy with numerous substrates.

Diana María Campodónico and Pablo Zubiaur contributed equally to this work.

WHAT QUESTION DID THIS STUDY ADDRESS?

Characterizing the functional impact of highly prevalent alleles on the enzymatic activity of CYP2C8 with the substrate cinitapride.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The pharmacogenetic phenotype of CYP2C8 can be established by *3 and *4 alleles. It significantly affected the metabolism of cinitapride, and may also affect the metabolism of other relevant CYP2C8 substrates such as paclitaxel, statins, etc.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

CYP2C8 may play a clinically relevant role in the use of numerous drug substrates; therefore, it may eventually be used as a biomarker for prescription personalization. We urge efforts to characterize the impact of its variants on the pharmacokinetics, safety, and efficacy of its drug substrates.

INTRODUCTION

Functional dyspepsia (FD) and gastroesophageal reflux disease (GORD) are associated with significantly increased healthcare costs related to doctor visits, diagnostic examinations, and therapeutic procedures, and a reduced quality of life.[1,2] The prevalence of GORD worldwide is 13.3%, and in Europe is from 15% to 19.1%,[3] whereas the global prevalence of uninvestigated FD is from 6.9% to 17.6%.[4] In Spain, 9.8% of the population manifests typical symptoms of GORD more than once a week, as well as dyspeptic symptoms (39% of patients present it at some time throughout their life).[5,6]

Cinitapride (4-amino-N-[1-(3-cyclohexen-1-yl-methyl)-4-piperidinyl]-2-ethoxy-5 nitrobenzamide) is a gastrointestinal prokinetic drug, developed and commercialized in Spain in 1989. It is prescribed for the treatment of FD, and as an adjuvant therapy for GORD.[7] Due to its procholinergic/serotoninergic activity, cinitapride increases the tone of the lower esophageal sphincter with a potent gastrokinetic effect, generating significant evacuation of the bowel. It is also a D2 receptor antagonist which can contribute to the prokinetic effect.[8] The frequencies of some adverse drug reactions (ADRs) are not entirely known, being similar among benzamides. Consistent with its mechanism of action, neurological disorders related to extrapyramidal symptoms (neck, tongue, and face muscle spasm), somnolence (with a prevalence of 0.1% to 1%), gynecomastia, galactorrhea, skin and subcutaneous tissue disorders like rash, pruritus, and angioedema, are described in the drug label.[7,9] Cinitapride is marketed in India, Pakistan, Peru, Argentina, Paraguay, Uruguay, Mexico, Costa Rica, Guatemala, Honduras, Nicaragua, Panama, and El Salvador.[10]

Cinitapride undergoes significant hepatic first-pass metabolism. More than 70% of an oral dose is rapidly absorbed and metabolized by the isoforms of the cytochrome P450 (CYP), CYP3A4 and CYP2C8.[7] After 1 mg single oral dose, cinitapride's area under the curve from 0 to 24 h (AUC_{0-t}) was from 1580 to 3464 pg*h/ml, while C_{max} ranged between 330 and 1398 pg/ml.[8,11,12]

For cinitapride, the lack of articles describing its pharmacogenetics and the absence of clinical guidelines indicates the importance of further research in this area. Likewise, *CYP2C8* (the gene coding for CYP2C8) is poorly characterized; Indeed, the impact of *CYP2C8* alleles on the enzyme's function is not clearly known.[13–15] Therefore, the assessment of their function on a well-known substrate such as cinitapride represents a valuable model which may be extended to other substrates.

The aim of this candidate gene study was to describe relevant pharmacogenetic biomarkers for cinitapride prescription. In this regard, we aimed to confirm the impact of relevant variants in *CYP3A4* and *CYP2C8* (i.e., the principal candidate genes) along with demographic characteristics on cinitapride's pharmacokinetics and safety and to evaluate the impact of *CYP2C8* alleles on the enzyme's function. Furthermore, we aimed to evaluate in an exploratory manner other variants in other relevant pharmacogenes (i.e., secondary candidates). The reason for including single nucleotide polymorphisms (SNPs) in genes apparently unrelated to cinitapride was the scarcity of information included in its drug label and previous literature. This work is promoted by La Princesa University Hospital Multidisciplinary Initiative for the Implementation of Pharmacogenetics, PriME-PGx.[16]

MATERIALS AND METHODS

Study design and population

The information for this candidate gene pharmacogenetic study was obtained from a bioequivalence clinical trial comprising 36 healthy volunteers, performed at the Clinical Trials

Unit of Hospital Universitario de La Princesa (UECHUP) (EUDRA-CT: 2018–002444-90). It was a randomized, open-label, one-center, single-dose, crossover bioequivalence clinical trial of two cinitapride 1 mg tablet formulations, under fasting conditions, with a wash-out period of at least 7 days between the administration of both drugs. The reference formulation (R) was Cidine® 1 mg tablets (cinitapride marketed by Almirall, S.A.). In each period, volunteers were randomly assigned to receive one formulation, and in the following period they received the other one. The bioequivalence clinical trial was approved by the Independent Ethics Committee on Clinical Research (IECCR) of the Hospital de La Princesa and the Spanish Drug's Agency (AEMPS), conducted in accordance with Spanish legislation and following the International Conference on Harmonization-Good Clinical Practice (ICH-GCP) guidelines and the Revised Declaration of Helsinki.[17,18] Inclusion criteria were as follows: man or woman, aged 18–55 years, with no physical or psychiatric pathology, and normal laboratory tests. Exclusion criteria included: having received prescribed drugs in the previous 15 days, or any kind of medication in the 48 h prior to dosing, except for contraception, a body mass index (BMI) outside the 18.5–30 kg/m^2 range, history of sensitivity to any drug, lactose intolerance, positive drug screening or alcohol poisoning in the last week before hospitalization, smoking, having donated blood in the last month before hospitalization, pregnant or breastfeeding women, participation in another study in the previous 3 months, inability to collaborate during the study, and history of swallowing difficulties.

Study design and procedures

Twenty-five volunteers gave their informed consent for participation in the pharmacogenetic study. During hospitalization at UECHUP and subsequent controls, 17 samples were obtained for pharmacokinetic profiling, between baseline to 72 h post-dose. Subsequently, EDTA-K2 tubes were centrifuged and plasma was stored at −80°C until drug plasma determinations, which was outsourced to an external analytical laboratory. A high-performance liquid chromatography triple quadrupole mass spectrometer (HPLC-MS/MS) instrument was used for the determinations, using a method fully validated according to Spanish current legislation (i.e., the European Medicine's Agency's guideline on bioanalytical method validation[19]). The lower limit of quantification (LLOQ) of the method was 0.5 pg/ml.

Pharmacokinetic analysis

Cinitapride pharmacokinetic data were analyzed with WinNonLin Professional Edition Version 8 (Pharsight Corporation). The following pharmacokinetic parameters were collected directly from the plasma time–concentration curves: cinitapride's maximum concentrations (C_{max}) and time to reach that concentration (t_{max}). The AUC from baseline to *t*, '*t*' being the last time-point (i.e., 72 h) (AUC_{0-t}), was calculated using the linear trapezoidal rule. The drug clearance adjusted for bioavailability (Cl/*F*) was calculated as dose (D) divided by AUC; the volume of distribution adjusted for bioavailability (Vd/*F*) was calculated by dividing Cl/*F* by ke, ke being the apparent terminal elimination rate. The test (T) and R formulations were demonstrated to be bioequivalent; therefore, for each pharmacokinetic parameter, the mean of both formulations was calculated to reduce intraindividual variability.

Genotyping, haplotyping, and phenotyping

DNA was extracted from 500 µl of thawed peripheral blood stored in EDTA-K2 tubes in a Maxwell® RSC Instrument (Promega Biotech Ibérica S.L.). Genotyping was performed by real-time quantitative polymerase chain reaction (RT-qPCR) with TaqMan® hydrolysis probes. A QuantStudio 12 K Flex qPCR instrument (Applied Biosystems, ThermoFisher) was used along with an OpenArray® thermal block and a customized array.

The analyzed genes and variants are summarized in Table 1. Alleles were selected based on their prevalence and functional impact. For instance, for *CYP2C8*, the three most relevant alleles in Madrid's population are *2, *3, and *4; *2 has a 19% prevalence in Africans and 1.2% in Latin Americans (ethnic groups representing 19% and 62% of the immigrant population of Madrid, respectively) and *3 and *4 have a prevalence of 7%–15% in Iberians.[20] Alleles located in genes with available Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines were selected based on CPIC's allele function and frequency tables. The exploratory analysis of variants in genes apparently unrelated to cinitapride pharmacokinetics follows the same rationale as for previous publications[21–23] to describe and discover new associations and clarify pharmacokinetic processes. *CYP3A5*, *CYP2C19*, *SLCO1B1*, *CYP2B6*, *CYP2D6* (including a copy number variation assay targeting exon 9), and *UGT1A1* variants were used to infer the pharmacogenetic phenotype according to the CPIC guidelines.[24–30] *CYP3A4* alleles were used to infer the enzyme's phenotype according to the Dutch Pharmacogenetics Working Group (DPWG) indications.[31] The impact of CYP and UGT enzyme or ABC and SLC transporter genotypes or phenotypes on the pharmacokinetics and safety of cinitapride was evaluated; similarly, receptor genes (i.e., *ADRA2A*, *HTR2A*, *HTR2C*, *DRD2*, *DRD3*) were analyzed for their potential relationship with ADR incidence.

TABLE 1 Variants/alleles genotyped for this study

Gene	Allele (variant)	Gene	Allele (variant)
CYP1A2	*1C (rs2069514)	*CYP3A4*	*22 (rs35599367)
	*1F (rs762551)		*2 (rs55785340)
	*1B (rs2470890)		*6 (rs4646438)
CYP2B6	*5 (rs3211371)	*ABCB1*	C3435T (rs1045642)
	*9 (rs3745274)		C1236T (rs1128503)
	*4 (rs2279343)		G2677T (rs2032582)
CYP2C8	*2 (rs11572103)	*SLCO1B1*	*1B (rs2306283)
	*3 (rs10509681)		*5 (rs4149056)
	*4 (rs1058930)		rs4149015
CYP2C9	*2 (rs1799853)		*4 rs11045819
	*3 (rs1057910)	*SLC22A1*	*2 (rs72552763)
CYP2C19	*2 (rs4244285)		*3 (rs12208357)
	*3 (rs4986893)		*5 (rs34059508)
	*4 (rs28399504)	*COMT*	rs13306278
	*17 (rs12248560)		rs4680
CYP2D6	*3 (rs35742686)	*ADRA2A*	rs1800544
	*4 (rs3892097)	*HTR2A*	rs6313
	*6 (rs5030655)		rs6314
	*7 (rs5030867)		rs7997012
	*8 (rs5030865)	*HTR2C*	rs1414334
	*9 (rs5030656)		rs3813929
	*10 (rs1065852)		rs518147
	*14 (rs5030865)	*DRD2*	rs1799732
	*17 (rs28371706)		rs1800497
	*41 (rs28371725)		rs6277
CYP3A5	*3 (rs776746)	*DRD3*	rs6280
	*6 (rs10264272)	*ABCC2*	rs2273697
UGT1A1	*80 (rs887829)		rs717620

Statistical analysis

Statistical analysis was conducted with SPSS Statistics Version 21.0. (IBM Corp.). AUC_{0-t} and C_{max} were divided by the dose/weight ratio (DW), and Cl/*F* and Vd/*F* were divided by weight to correct the impact of subject weight on pharmacokinetic variability, which may be variable according to sex or race. All pharmacokinetic data were logarithmically transformed to normalize distributions. Initially, an univariate analysis was conducted, whereby pharmacokinetic parameters were compared according to categorical variables (e.g., sex, race, genotypes, phenotypes) by means of an ANOVA test (for variables with three or more categories) or a *t*-test (variables with two categories). A Bonferroni post-hoc test was applied after ANOVA tests. Afterwards, all pharmacokinetic parameters were individually analyzed with a multivariate analysis, using linear regression. Only variables with $p < 0.05$ in the univariate analysis were included as independent variables, transformed into dummy variables when necessary. Moreover, due to the high number of tests and the subsequent high risk for type-1 error, the Bonferroni correction for multiple comparisons was used to adjust the level of significance in the multivariate analysis. The incidence of ADRs could not be analyzed because none of the adverse events reported in the clinical bioequivalence trial showed a causal relationship with drug administration. The Spanish Pharmacovigilance System Algorithm was used for the determination of causality.[32]

RESULTS

Demographic characteristics

Twelve men and 13 women were enrolled in this study. Weight and height were significantly superior in men compared to women ($p = 0.018$ and $p < 0.001$, respectively) but there was no difference in BMI. Moreover, there were no significant differences in age, weight, height, or BMI according to race (Table 2).

Pharmacokinetics

Mean AUC_{0-t} was lower for men (total = 3447.35 ± 1261.56 h*pg/ml, 2764.08 ± 1264.76 h*pg/ml for men, 4078.06 ± 904.94 h*pg/ml for women; $p = 0.005$) and similar for Caucasians and Latin Americans (3505.33 ± 1387.17 h*pg/ml and 3401.79 ± 1205.37 h*pg/ml, respectively; $p = 0.829$). Mean C_{max} was 980.80 ± 388.93 h*pg/ml lower for men than women (858.90 ± 463.96 h*pg/ml vs. 1093.33 ± 276.84 h*pg/ml, respectively; $p = 0.042$) and similar for Caucasians and Latin Americans (1010.05 ± 357.83 h*pg/ml Caucasians vs. 957.82 ± 423.65 h*pg/ml Latin Americans; $p = 0.578$). After DW correction, no significant differences were found for either AUC/DW or C_{max}/DW and other pharmacokinetic parameters concerning sex and race (Table 3).

Table 4 shows the significant relationships observed between genotyped variants or phenotypes and cinitapride pharmacokinetic parameters. Subjects with *CYP2C8* *1A/*3 and *3/*3 genotypes showed significantly lower cinitapride AUC/DW and C_{max}/DW compared to subjects with the *1A/*1A genotype (decrease of 42% and 35% and $p = 0.003$, $p = 0.011$, respectively) and with the *1A/*4 genotype carriers ($p = 0.004$, $p = 0.002$, respectively). Likewise, *1A/*4 genotype showed a tendency towards higher AUC/DW and C_{max}/DW compared to *1A/*1A genotype ($p = 0.422$ and $p = 0.097$). Conversely, significantly higher values of Vd/F_W and Cl/F_W were observed

TABLE 2 Demographic characteristics of the healthy volunteers who participated in the study

		Age (years)		Weight (kg)		Height (cm)		BMI (kg/m^2)	
	N	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Sex									
Male	12	29.58	8.32	75.29*	9.92	174.83*	5.33	24.60	2.76
Female	13	32.54	8.21	65.38	9.59	162.61	6.26	24.64	2.66
Total	25	31.12	8.25	70.14	10.80	168.48	8.45	24.62	2.65
Race									
Caucasian	11	29.36	7.47	74.22	10.49	170.72	8.42	25.47	3.10
Latin-American	14	32.50	8.84	66.92	10.27	166.71	8.35	23.96	2.11
Total	25	31.12	8.25	70.14	10.80	168.48	8.45	24.62	2.65

Abbreviations: BMI, body mass index; SD, standard deviation.

*$p < 0.05$ after *t*-test compared to the other category.

in subjects with *CYP2C8* *1A/*3 and *3/*3 genotypes compared to those with *1A/*1A ($p < 0.001$, $p = 0.003$, respectively) and *1A/*4 diplotypes ($p = 0.001$, $p = 0.004$, respectively). In the case of the comparison between *1/*1 and *1A/*4, AUC/DW and C_{max}/DW values were 45% and 63% higher in *1A/*4, although these differences did not reach statistical significance ($p = 0.422$ and $p = 0.097$, respectively). Furthermore, CYP2C9 normal metabolizers (NMs) showed significantly higher AUC/DW, C_{max}, and t_{max} and significantly lower Vd/*F* and Cl/*F* compared to CYP2C9 intermediate (IM) and poor (PM) metabolizers ($p = 0.002$, $p = 0.003$, $p = 0.038$, $p < 0.001$, and $p = 0.002$, respectively). No significantly differences were found between *CYP3A4* normal (NMs, $n = 23$) and intermediate (IMs, $n = 2$) metabolizers either in AUC/DW and C_{max}/DW ($p = 0.719$ and $p = 0.523$, respectively) or in the rest of the pharmacokinetics parameters.

Additionally, volunteers carrying the *CYP1A2* *1/*1 diplotype were significantly associated with a higher $t_{1/2}$ than volunteers with the*1B/*1B diplotype ($p = 0.030$). Individuals carrying the *ABCB1* C1236T T/T genotype presented significantly higher values of $t_{1/2}$ and Vd/F_W than those carrying the C/C diplotype ($p = 0.020$, $p = 0.031$), while there were no differences in other SNPs of the *ABCB1* gene. *COMT* rs13306278 C/C genotype carriers showed lower $t_{1/2}$ ($p < 0.040$) and Vd/*F* ($p < 0.01$) in comparison with C/T subjects. *SLC22A1**1/*1 individuals presented higher AUC/DW and lower Cl/F than *1/*3 ($p = 0.027$, $p = 0.026$, respectively). Finally, individuals with *ABCC2* rs2273697 G/G genotype exhibited a lower $t_{1/2}$ compared with those with G/A and A/A genotypes ($p < 0.02$). No significant results were observed for variants in the remaining genes.

Regarding multivariate analysis, *CYP2C8* *3 genotype and *SLC22A1* *3 were significantly related to AUC/DW variability (Table 5); *CYP2C8* genotype to C_{max}/DW variability; *CYP2C8* genotype, CYP2C9 phenotype, and *ABCB1* C1236T to Vd/F_W; and *ABCB1* C1236T to $t_{1/2}$ variability; and *CYP2C8* genotype and *SLC22A1**3 to Cl/*F* variability. No variables were related to t_{max} variability (Table 5). After Bonferroni correction for multiple comparisons, only *CYP2C8* associations with AUC/DW, C_{max}/DW, Vd/*F*, and Cl/*F* variability and *ABCB1* C1236T with Vd/*F* variability remained significant (i.e., $p < 0.006$).

No adverse event was causally related to cinitapride intake; therefore, no ADR was noted. Therefore, the effect of *ADRA2A*, *HTR2A*, *HTR2C*, *DRD2*, and *DRD3* (genes potentially involved in cinitapride pharmacodynamics) polymorphism could not be evaluated concerning cinitapride's tolerability nor the impact of the remaining variants located in genes affecting cinitapride pharmacokinetics.

DISCUSSION

GORD and FD are highly prevalent in the world's population; consequently, the prescription of drugs for their treatment, including cinitapride, is very frequent and usually prolonged in time.[2] To date, few studies have properly characterized cinitapride's pharmacokinetic profile. It is known as a substrate of CYP2C8 and CYP3A4,[7] but the clinical relevance of genetic polymorphisms in these genes on cinitapride's exposure remains unknown. Moreover, no pharmacogenetic guideline has been published to date reporting a clinically relevant phenotype of CYP2C8 that can be inferred based on allele genotyping. Table 6 shows a list of relevant CYP2C8 substrates which may be affected by the presence of gene polymorphisms. Further research should be conducted evaluating the impact of *CYP2C8* polymorphisms on the effectiveness, safety, and pharmacokinetics of these drugs. Similarly, only recently a pharmacogenetic guideline on CYP3A4 has been published for the substrate quetiapine.[31,33,34] Hence, the present work is a convenient model to interrogate the effects of *CYP3A4*

TABLE 3 Cinitapride pharmacokinetic parameters based on sex and race

	N	AUC/DW (kg*h*pg/ml*mg)		C_{max}/DW (kg*pg/ml*mg)		t_{max} (h)		$t_{1/2}$ (h)		Vd/*F* (L/kg)		Cl/*F* (L/h*kg)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sex[a]													
Male	12	211,346.15	114,436.22	64,158.91	34,207.46	0.78	0.11	57.92	33.78	505.52	373.76	6.12	3.08
Female	13	270,792.58	86,889.98	71,585.16	22,121.53	0.84	0.10	50.66	9.95	302.42	131.11	4.15	1.57
Total	25	242,258.29	103,421.06	68,020.56	28,201.75	0.81	0.10	54.15	24.21	399.91	288.70	5.10	2.56
Race[a]													
Caucasian	11	266,267.96	126,378.82	75,783.44	30,633.93	0.81	0.07	61.45	34.89	430.94	368.04	4.72	2.31
Latin American	14	223,393.56	81,175.26	61,921.16	25,600.52	0.82	0.13	48.41	8.06	375.53	219.59	5.39	2.80
Total	25	242,258.29	103,421.06	68,020.56	28,201.75	0.81	0.10	54.15	24.21	399.91	288.70	5.10	2.56

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; Cl/*F*, drug clearance adjusted for bioavailability; DW, dose/weight ratio; SD, standard deviation; $t_{1/2}$, time to reach half maximum concentration; t_{max}, time to reach maximum concentration; Vd/*F*, volume of distribution adjusted for bioavailability.

[a]No significant relationships were observed after univariate and multivariate analysis.

and *CYP2C8* variants or phenotypes on the enzyme's performance in a controlled environment.

The pharmacokinetic parameters here observed generally resembled those previously reported in the literature. For instance, in Chinese, Mexican and German populations, AUC_{0-t} and C_{max} after 1 mg cinitapride administration to young and healthy volunteers were 1580–3464 pg*h/ml and 330–1398 pg/ml,[8,11,12] while in the present study, an AUC_{0-t} of 3447 ± 1261 pg*h/ml and a C_{max} of 980 ± 388 pg*h/ml were observed, with significant differences between males and females. Nevertheless, a high pharmacokinetic interindividual variability is observed among studies. Although some of this variability can be explained due to the study design (e.g., depending on fasting or fed condition), a non-negligible part of variability remains unexplained. When AUC_{0-t} and C_{max} were divided by the DW ratio to correct the impact of weight on pharmacokinetic variability, the abovementioned significant sex differences disappeared. It can therefore be concluded that sex does not influence cinitapride's exposure but weight does.

Concerning pharmacogenetics, *CYP2C8* diplotypes had a clear effect on cinitapride exposure variability. *CYP2C8**3 carriers showed a reduction in AUC of 42% and C_{max} of 35% compared to *1/*1 subjects. *4 allele carriers showed a 2.47-fold increase in AUC and a 2.53-fold increase in C_{max} compared to *3 allele carriers. Moreover, volunteers with the *1/*4 diplotype showed a 1.45-fold and a 1.63-fold increase in AUC/DW and C_{max}/DW compared to *1/*1 volunteers, but these association did not reach the level of significance due to the low sample size (i.e., only two *1/*4 individuals were identified). Accordingly, the following pharmacogenetic phenotypes can be proposed: *CYP2C8* UMs (*3/*3), RMs (*1/*3), NMs (*1/*1), IMs (*1/*4), and PMs (*4/*4). To our knowledge, this is the first study to report a pharmacogenetic phenotype for CYP2C8. Since UMs and PMs were not sufficiently represented they could not be properly characterized. Additional studies are warranted to characterize UM and PM phenotypes. Not only are our findings relevant with respect to cinitapride, but also to numerous additional CYP2C8 substrates such as imatinib, loperamide, montelukast, ibuprofen, paclitaxel, pioglitazone, repaglinide, or rosiglitazone. Potentially, CYP2C8 phenotype may become a clinically relevant pharmacogenetic biomarker to individualize pharmacotherapy with various drugs.

Notwithstanding the current findings, the effect of *CYP2C8* alleles should be described further in depth. CYP2C8 is involved in the metabolism of a diverse number of drugs, the prototypical CYP2C8 substrate being paclitaxel.[37,38] *CYP2C8**2 and *3 alleles were associated with decreased metabolism of paclitaxel and arachidonic acid,[37,39] and *CYP2C8**3 with increased metabolism of

TABLE 4 Cinitapride pharmacokinetic parameters based on genotypes or phenotypes showing statistically significant variability in the univariate analysis

Genotype or phenotype		*N*	AUC/DW (kg*h*pg/ml*mg)		C_{max}/DW (kg*pg/ml*mg)		t_{max} (h)		$t_{1/2}$ (h)		Vd/*F* (L/kg)		Cl/*F* (L/h*kg)	
			Median	SD	Median	SD	Median	SD	Median	SD	Median	SD	Median	SD
CYP2C8[a]	*1A/*1A	12	280,762.57	85,231.95	75,673.43	23,803.30	0.85	0.11	48.62	10.43	261.62	62.80	3.91	1.13
	*1/*3 + *3/*3	10	162,863.15*[1]	63,250.72	48,951.85*[1]	14,131.46	0.76	0.07	63.68	35.18	627.35*[1]	344.41	7.13*[1]	2.79
	*1/*4	2	403,033.83	40,280.38	124,334.12	6660.84	0.75	0.00	42.40	6.77	151.90	42.41	2.51	0.23
CYP2C9[b]	NM	14	293,193.04*	93,972.24	81,663.87*	29,152.95	0.85*	0.11	47.45	10.35	249.25*	73.57	3.78*	1.19
	IM + PM	11	177,432.25	77,041.78	50,656.35	14,549.46	0.76	0.07	62.66	33.54	591.66	347.53	6.76	2.91
*CYP1A2**1B	*1/*1	2	214,491.16	65,685.21	55,649.07	7714.45	0.75	0.00	101.71*[2]	79.59	804.22	777.99	4.94	1.53
	*1/*1B	12	270,460.10	132,861.33	67,668.36	33,308.76	0.80	0.10	53.60	12.32	393.54	279.82	5.13	3.45
	*1B/*1B	11	216,541.25	63,129.07	70,654.14	25,378.17	0.83	0.12	46.10	9.08	333.34	125.55	5.08	1.57
ABCB1 C1236T rs1128503	C/C	10	202,527.12	92,016.32	64,764.72	29,073.89	0.80	0.11	46.57	11.10	395.32	207.06	5.75	2.06
	C/T	12	287,405.91	97,873.86	75,587.37	28,222.68	0.84	0.11	51.39	11.55	287.55*[4a]	120.07	4.14	2.40
	T/T	3	194,105.06	118,163.39	48,606.12	19,936.73	0.75	0.00	90.41*[3]	58.67	864.63	572.84	6.74	4.04
ABCC2 rs2273697	G/G	17	232,045.46	96,946.17	66,551.96	27,286.33	0.81	0.11	47.38*	10.07	346.06	177.17	5.21	2.49
	G/A + A/A	8	263,960.57	119,990.45	71,141.34	31,759.91	0.81	0.10	68.53	37.82	514.34	438.18	4.85	2.87
COMT rs13306278	C/C	17	263,826.22	101,380.57	72,865.07	30,798.21	0.82	0.12	47.77*	10.47	306.75*	170.37	4.59	2.43
	C/T	8	196,426.45	98,228.01	57,725.99	19,511.35	0.80	0.07	67.70	38.08	597.87	391.86	6.17	2.66
*SLC22A1*3* rs12208357	*1/*1	22	255,124.99*	99,082.99	69,141.44	24,925.98	0.83	0.10	55.16	25.41	371.61	272.52	4.59*	1.89
	*1/*3	3	147,902.53	100,460.33	59,800.78	53,899.87	0.71	0.07	46.69	12.71	607.42	383.32	8.78	4.27
Total		25	242,258.29	103,421.06	68,020.56	28,201.75	0.81	0.10	54.15	24.21	399.91	288.70	5.10	2.56

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; CI/*F*, drug clearance adjusted for bioavailability; DW, dose/weight ratio; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; SD, standard deviation; $t_{1/2}$, time to reach half maximum concentration; t_{max}, time to reach maximum concentration; Vd/*F*, volume of distribution adjusted for bioavailability.

[a]For the *CYP2C8* analysis, one *3/*4 subject was excluded.

[b]CYP2C9 NM phenotype included *1/*1 diplotype; IM phenotype included *1/*2, *2/*2, and *1/*3 diplotypes; only one volunteer showed the *2/*3 diplotype (PM phenotype).

*$p < 0.05$ after ANOVA or *t*-test; *1 $p < 0.05$ vs. *1/*1 and *1/*4, after ANOVA and Bonferroni post-hoc test; * 2 $p < 0.05$ vs. *1B/1*B after ANOVA and Bonferroni post-hoc test; *3 $p < 0.05$ vs. *1/*1; *4 $p < 0.05$ vs. T/T.

TABLE 5 Coefficients and significance deriving from the multivariate analysis of pharmacokinetic parameters

Factor		β	R^2	Significance (p)
AUC/DW				
CYP2C8[#]	*1A/*3 + *3/*3 vs. *1A/*1A + *1A/*4	0.57	0.557	<0.001
*SLC22A1*3	*1/*1 vs. *1/*3	−0.44		0.041
C_{max}/DW				
CYP2C8[#]	1A/*3 + *3/*3 vs. *1A/*1A + *1A/*4	0.51	0.376	0.001
t_{max}				
No variables related.				
$t_{1/2}$				
ABCB1 C1236T	C/C vs. C/T + T/T	0.507	0.266	0.010
Vd/*F*				
CYP2C8[#]	1A/*3 + *3/*3 vs. *1A/*1A + *1A/*4	−1.600	0.71	0.001
ABCB1 C1236T	C/C vs. C/T + T/T	0.933		0.002
CYP2C9	NM vs. IM + PM	−0.936		0.047
Cl/*F*				
CYP2C8[#]	1A/*3 + *3/*3 vs. *1A/*1A + *1A/*4	−0.571	0.556	<0.001
*SLC22A1*3	*1/*1 vs. *1/*3	0.45		0.041

Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; Cl/*F*, drug clearance adjusted for bioavailability; DW, dose/weight ratio; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; $t_{1/2}$, time to reach half maximum concentration; t_{max}, time to reach maximum concentration; Vd/*F*, volume of distribution adjusted for bioavailability.

[#]$p < 0.006$ after Bonferroni correction for multiple comparisons in multivariate analysis.

rosiglitazone, pioglitazone, and repaglinide.[40,41] These discrepancies encountered concerning the impact of *CYP2C8**3 on the enzyme's activity may be explained in two ways. First, as a result of the linkage disequilibrium (LD) between *CYP2C8**3 and *CYP2C9**2. The variants encoding for these alleles (2130G > A (rs11572080) and 30411A > G (rs10509681) for *CYP2C8**3 and 3608C > T (rs1799853) for *CYP2C9**2) are located in a region of the *CYP2C19*, *CYP2C8*, and *CYP2C9* locus in close proximity on chromosome 10, and are inherited together very frequently (i.e., 96% of *CYP2C8**3 allele carriers also carry the *CYP2C9**2 allele, and approximately 85% of *CYP2C9**2 allele carriers also carry a *CYP2C8**3 allele.[42,43] Here, we observed an apparent effect of CYP2C9 phenotype on cinitapride exposure variability; however, this association is not consistent as, even if cinitapride was metabolized via CYP2C9 (which to our knowledge has not been reported to date), IM and PMs should accumulate the drug in plasma compared to NMs, and the opposite effect was observed. Therefore, we confidently conclude that these effects are due to *CYP2C8**3. Consistently, it should be emphasized that in the multivariate analysis, the association of *CYP2C9* disappeared, supporting the above hypotheses. Second, the effect of *CYP2C8**3 allele could be substrate-specific. This effect is well described for other genes and drugs, such as *CYP2D6**17, a well-known decreased-function allele, which is related to higher debrisoquine clearance.[44–46] Further studies should clarify the effect of *CYP2C8**3 allele on cinitapride's or other substrates' pharmacokinetics, but we suggest that the LD with *CYP2C9**2 is a clear confusing factor that may have led to biased conclusions in previous pharmacogenetic observational studies. In fact, the only subject with *CYP2C9* *1/*3 and *CYP2C8* *1/*1 diplotypes exhibited a normal value of AUC/DW and C_{max}/DW (data not shown), which confirms that *CYP2C9* polymorphism does not impact the pharmacokinetics of cinitapride. In contrast, *CYP2C8* *4 allele is generally assumed to cause a reduction in the enzyme's function for drugs such as montelukast[47] and paclitaxel,[48] which is consistent with the effects observed with cinitapride in the present study. Moreover, in a previous study conducted by our group, *CYP2C8**3 allele was related to a higher clearance of ibuprofen as compared with individuals with *CYP2C9* *1/*1 and *CYP2C8* *1/*1 diplotypes,[49] which is consistent with the findings reported here.

One additional variant, *ABCB1* C1236T, was related to cinitapride's exposure variability. *ABCB1* gene variants determine the activity of the P-glycoprotein, an efflux transporter localized in cell membranes throughout the entire body, which participates in the pharmacokinetics of several substrates.[50,51] The effect of C1236T variant on the transporter's performance is currently still unknown,[52,53] therefore further studies are warranted to confirm this association as no good comparators are available in the literature.[54–56] Concerning *CYP3A4*, we failed to demonstrate a significant effect of

TABLE 6 Examples of CYP2C8 substrates

Almotriptan	Elagolix	Mavacamten	Roxadustat
Aminophenazone	Enasidenib	Meloxicam	Samidorphan
Amiodarone[b]	Enzalutamide	Mephenytoin	Selegiline
Amitriptyline[a]	Erlotinib	Mestranol	Selexipag
Amodiaquine[a,b]	Estradiol	Methadone	Selumetinib
Anastrozole	Eszopiclone	Mitapivat	Simvastatin[a]
Antipyrine	Ethinylestradiol	Montelukast[b]	Sitagliptin
Apalutamide	Febuxostat	Morphine[a]	Sorafenib
Apixaban	Finerenone	Mycophenolate[a]	Sulfadiazine
Apomorphine	Fluorouracil	Nabilone	Tazarotene[b]
Atorvastatin[a]	Fluvastatin[a]	Naproxen	Tegafur
Azelastine	Fosphenytoin	Nicardipine	Temazepam
Azilsartan medoxomil	Glasdegib	Nicotine	Tepotinib
Belumosudil	Halofantrine	Olodaterol	Terbinafine
Brigatinib	Hydroxychloroquine	Ombitasvir	Testosterone
Buprenorphine	Ibuprofen[a]	Omeprazole	Theophylline
Cabazitaxel	Ifosfamide[a]	Ozanimod	Tirbanibulin
Caffeine	Imatinib[a]	Paclitaxel[ab]	Tolbutamide
Cannabidiol	Irbesartan	Palovarotene	Torasemide
Carbamazepine[a]	Ixazomib	Pazopanib	Treprostinil
Celecoxib	Ketamine	Perphenazine	Tretinoin
Cerivastatin[a,b]	Ketorolac	Phenprocoumon[a]	Trifarotene
Chloroquine[b]	Lansoprazole	Phenytoin[a]	Trimethadione
Cisapride	Lapatinib	Pioglitazone[b]	Trimethoprim
Clozapine	Levomilnacipran	Piroxicam	Tucatinib
Cyclophosphamide[a]	Lidocaine	Pitavastatin[a]	Velpatasvir
Dabrafenib	Lonafarnib	Ponatinib[a]	Verapamil[a]
Dapsone	Loperamide	Propofol	Vortioxetine[a]
Dasabuvir	Loratadine	Quinine	Voxilaprevir
Dexibuprofen	Lorlatinib	Relugolix	Warfarin[a]
Diazepam	Lovastatin[a]	Remdesivir	Zafirlukast
Diclofenac[a]	Lumateperone	Repaglinide[a]	Zidovudine
Diltiazem	Macitentan	Rosiglitazone[a,b]	Zopiclone
Eltrombopag			

Note: List of substrates obtained from DrugBank.[35]

[a]PharmGKB pathway available describing CYP2C8–drug interaction.

[b]Substrates majorly metabolized by CYP2C8.[36]

its phenotype on cinitapride exposure variability due to the low sample size, as only two *1/*22 (IMs) subjects were identified.[25,39]

Finally, cinitapride is marketed in a few countries and it is indicated for highly prevalent pathologies. Therefore, patients diagnosed with GORD or FD under treatment with cinitapride may be able to avoid a certain percentage of ADRs and even lack of efficacy. For instance, we speculate that CYP2C8 UMs could require a daily dose increase of 50%–100%, while PMs may require dose reductions to avoid ADRs. Alternatively, another drug may be used. However, the relevance of this work extends beyond the association with cinitapride, as other more relevant substrates could be affected by these phenotypes (e.g., chemotherapy agents like paclitaxel). Having characterized with such clarity the effect of *CYP2C8* polymorphisms and identified the methodological problem of LD with *CYP2C9* in the literature is a major finding. This opens the door to properly evaluate the impact of *CYP2C8* phenotype on the exposure and

safety of other relevant substrates of *CYP2C8* such as ibuprofen, paclitaxel, and pioglitazone. Potentially, this will contribute to advancing precision pharmacotherapy with *CYP2C8* substrates.

Limitations

The most important limitation of our study is the sample size, which prevented the finding of genotypes of interest (e.g., *CYP3A4**22) among the participating population. Moreover, some potentially relevant alleles could be present in our study population but were not genotyped (e.g., *CYP3A4**20). In addition, these results are from a single-dose phase I clinical trial, in which healthy volunteers were recruited, therefore we were unable to draw any conclusion about cinitapride's effectiveness. Moreover, the observed relationships regarding pharmacokinetics may not be extrapolable to patients, whose gastric motility may be affected, and therefore the process of absorption may significantly differ. Likewise, these results may not apply to patients outside the BMI range implemented in the inclusion criteria (e.g., obese patients). Moreover, no ADRs were noted and therefore we could not conclude as to cinitapride's tolerability. In contrast, this study was performed under strictly controlled conditions, thus it is a good model to address the effects of genetic polymorphism on drug pharmacokinetics without the interference of smoking or other confounding factors.

CONCLUSIONS

*CYP2C8**3 and *4 alleles may be used to infer the PM, IM, NM, and UM phenotypes. Not only is this relevant with respect to cinitapride, but also with respect to numerous additional substrates such as imatinib, loperamide, montelukast, ibuprofen, paclitaxel, pioglitazone, repaglinide, or rosiglitazone. Further studies are needed to validate the utility of this phenotype with cinitapride (particularly the impact of UM and PM phenotypes) and other substrates.

AUTHOR CONTRIBUTIONS

D.M.C., P.Z., and F.A.-S. wrote the manuscript. D.M.C., P.Z., and F.A.-S. designed the research. D.M.C., P.Z., P.S.-C., A.C., G.V.-G., M.N.-G., A.G.-F., R.P.G., G.M.-A., M.R., S.M.-V., D.O., and F.A.-S. performed the research. D.M.C., P.Z., and F.A.-S. analyzed the data.

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CONFLICT OF INTEREST

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