SCIENTIFIC REPORTS
nature research

OPEN

Pharmacogenetics of amfepramone in healthy Mexican subjects reveals potential markers for tailoring pharmacotherapy of obesity: results of a randomised trial

Magdalena Gómez-Silva[1,2], Everardo Piñeyro-Garza[3], Rigoberto Vargas-Zapata[4], María Elena Gamino-Peña[5], Armando León-García[6], Mario Bermúdez de León[7], Adrián Llerena[8] & Rafael B. R. León-Cachón[9*]

Amfepramone (AFP) is an appetite-suppressant drug used in the treatment of obesity. Nonetheless, studies on interindividual pharmacokinetic variability and its association with genetic variants are limited. We employed a pharmacokinetic and pharmacogenetic approach to determine possible metabolic phenotypes of AFP and identify genetic markers that could affect the pharmacokinetic variability in a Mexican population. A controlled, randomized, crossover, single-blind, two-treatment, two-period, and two sequence clinical study of AFP (a single 75 mg dose) was conducted in 36 healthy Mexican volunteers who fulfilled the study requirements. Amfepramone plasma levels were measured using high-performance liquid chromatography mass spectrometry. Genotyping was performed using real-time PCR with TaqMan probes. Four AFP metabolizer phenotypes were found in our population: slow, normal, intermediate, and fast. Additionally, two gene polymorphisms, *ABCB1*-rs1045642 and *CYP3A4*-rs2242480, had a significant effect on AFP pharmacokinetics ($P < 0.05$) and were the predictor factors in a log-linear regression model. The *ABCB1* and *CYP3A4* gene polymorphisms were associated with a fast metabolizer phenotype. These results suggest that metabolism of AFP in the Mexican population is variable. In addition, the genetic variants *ABCB1*-rs1045642 and *CYP3A4*-rs2242480 may partially explain the AFP pharmacokinetic variability.

Currently, obesity is a major health problem worldwide. According to the organization for economic co-operation and development (OECD), in 2017, Mexico was among the countries with the highest obesity rates worldwide[1]. Today, 36.3% of adolescents aged 12–19 years, and 72.5% of adults are overweight or obese[2]. Strictly, obesity is abnormal fat accumulation that can compromise health and be associated with other clinical conditions such as cardiovascular diseases, diabetes, musculoskeletal disorders, and cancer[3].

Obesity is mainly caused by an energy surplus between calories consumed and calories expended[3]. Therefore, most treatments are focused on calorie-restricted diets and an increase in physical activity. Only when aforementioned treatments yield unsatisfactory results, pharmacological treatment is recommended[4,5]. Anti-obesity

[1]Forensic Medicine Service, School of Medicine, Autonomous University of Nuevo Leon, Monterrey, Nuevo Leon, Mexico. [2]Analytical Department of the Research Institute for Clinical and Experimental Pharmacology, Ipharma S.A, Monterrey, Nuevo Leon, Mexico. [3]Clinical Department of the Research Institute for Clinical and Experimental Pharmacology, Ipharma S.A, Monterrey, Nuevo Leon, Mexico. [4]Quality Assurance Department of the Research Institute for Clinical and Experimental Pharmacology, Ipharma S.A, Monterrey, Nuevo Leon, Mexico. [5]Statistical Department of the Research Institute for Clinical and Experimental Pharmacology, Ipharma S.A, Monterrey, Nuevo Leon, Mexico. [6]Pharmaceutical Research S.A, Mexico City, CDMX, Mexico. [7]Department of Molecular Biology, Center for Biomedical Research of the Northeast, Mexican Institute of Social Security, Monterrey, Nuevo Leon, Mexico. [8]Clinical Research Center of Health Area, Hospital and Medical School of Extremadura University, Badajoz, Spain. [9]Center of Molecular Diagnostics and Personalized Medicine, Department of Basic Sciences, Division of Health Sciences, University of Monterrey, San Pedro Garza Garcia, Nuevo Leon, Mexico. *email: rafael.reyesleon@udem.edu

	All subjects	Males	Females
n	36	19	17
Age (years)	24.25 ± 6.35	25.74 ± 8.25	22.59 ± 2.48
BMI (kg/m^2)	23.81 ± 2.16	24.19 ± 1.96	23.39 ± 2.36
Height (m)	1.67 ± 0.10	1.74 ± 0.08[a]	1.59 ± 0.06
Weight (kg)	66.57 ± 11.23	73.12 ± 9.58[b]	59.25 ± 8.05
C_{max} (ng/mL)	7.04 ± 2.86	7.40 ± 3.46	6.64 ± 2.01
$AUC_{0\text{-}t}$ (ng/mL/h)	29.87 ± 12.40	30.91 ± 15.57	28.72 ± 7.79
$AUC_{0\text{-}\infty}$ (ng/mL/h)	36.59 ± 13.66	38.38 ± 17.40	34.55 ± 7.71
K_e	0.1923 ± 0.0539	0.2056 ± 0.0637	0.1774 ± 0.0365
$T_{1/2}$ (h)	3.95 ± 1.45	3.84 ± 1.83	4.07 ± 0.91
Cl (L/h/kg)	2.32 ± 0.90	2.37 ± 1.16	2.27 ± 0.51
V_d (L/kg)	12.92 ± 5.47	12.47 ± 6.38	13.42 ± 4.38

Table 1. Demographic data and pharmacokinetic descriptive statistics of volunteers. Data shown as mean ± standard deviation. [a]$P = 5.24 \times 10^{-6}$; [b]$P = 6.97 \times 10^{-5}$. *BMI*, body mass index; C_{max}, maximum plasma concentration; *AUC*, area under the plasma concentration-time curve; $AUC_{0\text{-}t}$, AUC from time 0 to the time of last measurement; $AUC_{0\text{-}\infty}$, AUC from time 0 extrapolated to infinity; K_e, elimination rate constant in the terminal drug phase; $T_{1/2}$, half-life drug; V_d, volume of distribution; *Cl*, total drug clearance.

pharmacotherapy is limited to a few options that either suppress appetite or increase energy expenditure[4]. At present, the Food and Drug Administration (FDA) has approved the short-term use of appetite suppressants such as amfepramone (AFP) and phentermine (PHE) to treat obesity[5–7]; however, adverse drug reactions have been reported for some of them[4,8,9].

The drug AFP, is classified as a schedule IV controlled substance in the United States and Canada because it acts on the central nervous system[10], where it not only induces the release of the neurotransmitters noradrenaline (NA) and dopamine (DA) but also suppresses their reuptake. This mechanism of action results in less appetite[11,12]. However, owing to the psychostimulant effects of AFP, cautions should be taken regarding the dose and in patients with a psychiatric or cardiovascular history[13–15]. Specifically, in a Mexican population, AFP has been proven to be effective and safe for long-term treatment[16]. Nonetheless, some mild to moderate adverse events have been associated with AFP use, such as dry mouth, insomnia, anxiety, irritability, constipation, headache, dizziness, and polydipsia[12,13,16].

The wide range of pharmacological effects of drugs, from being safe and effective to the risk of adverse reactions, may involve genetic factors that control absorption, distribution, metabolism, and excretion (ADME) processes[17,18] and, therefore, pharmacokinetic parameters[19]. For example, recent studies in a Mexican population have shown that genetic variation may explain 30–90% of the pharmacokinetic variability[20,21] of Statins. Here, the most common genetic variation responsible for these pharmacokinetic discrepancies are polymorphisms in genes encoding metabolizing enzymes and transporters[19–21]. Nevertheless, no pharmacogenetic data is available for AFP.

Therefore, to date, although after oral administration, AFP is extensively biotransformed to secondary metabolites; until now, the responsible metabolic enzymes are unknown[22–24]. Two metabolic pathways seem to be involved: 1) N-dealkylation and reduction[22,23] and 2) N-deethylation[24]. The enzymes encoded by *CYP3A4* and *CYP3A5* are the most important because they metabolize a significant percentage of drugs. These enzyme isoforms have similar substrate selectivity, and therefore metabolize the same compounds[25]. Previous studies reported that CYP3A4 performs N-dealkylation and N-deethylation[26,27]. Therefore, CYP3A4 and CYP3A5 may have an important role in AFP metabolism. However, other CYP isoforms[25] and other enzymes of phase II metabolism, such as glutathione S-transferases (GSTs), may also be involved[28].

Amfepramone and its metabolites are excreted via the kidney[22–24]. Transporter proteins known to be involved in drug removal, such as the ones encoded by *ABCB1*, *ABCG2*, and *SLCO1B1*, may affect AFP plasma levels in the Mexican population[20,21].

Because variations in genes involved in the ADME processes may affect the pharmacokinetic profile and, therefore, the effectivity of a drug, the aims of this study were: (1) to classify the AFP metabolism, (2) to evaluate the impact of genetic polymorphism related to drug metabolism in the Mexican population on AFP pharmacokinetics, and (3) to assess a possible association between genotypes and metabolizer phenotypes.

Results

Study population. A total of 36 unrelated healthy volunteers, aged between 18 and 51 years, of either sex and of Mexican origin, were enrolled and treated with AFP. Demographical, clinical, and pharmacokinetic data are presented in Table 1. Despite the fact that height and weight values were significantly higher in males than in females ($P \leq 6.97 \times 10^{-5}$); the Body Mass Index (BMI) was not significantly different. No other features displayed significant variability (Table 1).

Amfepramone pharmacokinetics. The mean ± SD values for the pharmacokinetic parameters for all subjects were as follows: maximum plasma concentration (C_{max}) = 7.04 ± 2.86 ng/mL, area under the plasma concentration-time curve from 0 to the time of last measurement ($AUC_{0\text{-}t}$) = 29.87 ± 12.39 ng/mL/h, AUC from time 0 extrapolated to infinity ($AUC_{0\text{-}\infty}$) = 36.59 ± 13.66 ng/mL/h, elimination rate constant in the terminal

Pharmacokinetic parameters	Metabolizer phenotypes for all subjects			
	Slow	Intermediate	Normal	Fast
N	7	9	12	8
C_{max} (ng/mL)	11.07 ± 3.49^{a}	7.21 ± 0.91^{a}	6.51 ± 1.16^{a}	4.12 ± 0.89^{a}
$AUC_{0\text{-}t}$ (ng/mL/h)	45.84 ± 14.69^{b}	34.98 ± 2.13^{b}	26.44 ± 2.87^{b}	15.30 ± 3.74^{b}
$AUC_{0\text{-}\infty}$ (ng/mL/h)	51.61 ± 15.59^{c}	43.17 ± 10.58^{c}	32.87 ± 2.96^{c}	21.62 ± 4.67^{c}
K_e	0.2013 ± 0.0431	0.2063 ± 0.0708	0.1794 ± 0.0452	0.1880 ± 0.0577
$T_{1/2}$ (h)	3.58 ± 0.80	4.01 ± 1.15	4.11 ± 1.15	3.96 ± 1.08
Cl (L/h/kg)	1.53 ± 0.32^{d}	1.80 ± 0.31^{d}	2.30 ± 0.20^{d}	3.64 ± 0.91^{d}
V_d (L/kg)	7.89 ± 2.13^{e}	9.59 ± 2.87^{e}	13.68 ± 4.08^{e}	19.90 ± 3.75^{e}

Table 2. Pharmacokinetic parameters according to metabolizer phenotype. Data shown as mean ± standard deviation. $^{a}P \leq 9.37 \times 10^{-3}$; $^{b}P \leq 0.001$; $^{c}P \leq 0.023$; $^{d}P \leq 0.026$; $^{e}P \leq 0.002$; $^{c}P \leq 9.79 \times 10^{-6}$. C_{max}, maximum plasma concentration; *AUC*, area under the plasma concentration-time curve; $AUC_{0\text{-}t}$, AUC from time 0 to the time of last measurement; $AUC_{0\text{-}\infty}$, AUC from time 0 extrapolated to infinity; K_e, elimination rate constant in the terminal drug phase; $T_{1/2}$, half-life drug; V_d, volume of distribution; *Cl*, total drug clearance.

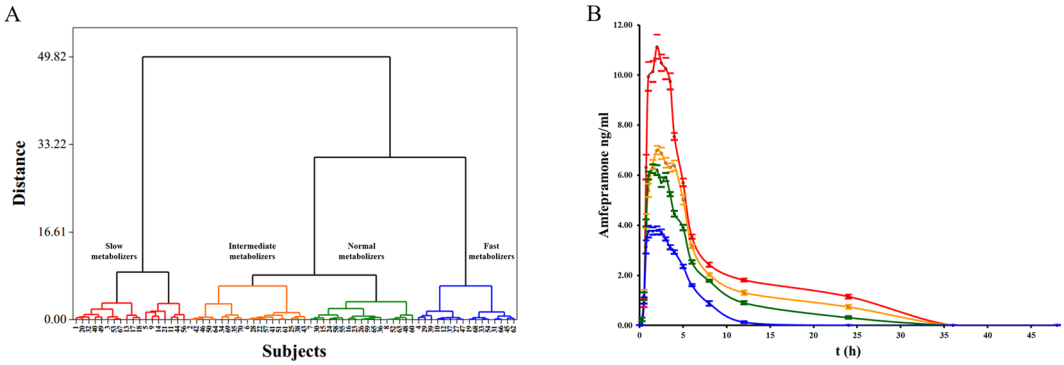

Figure 1. Classification of amfepramone (AFP) metabolic phenotypes. (**A**) Dendrogram generated with Manhattan distance and Ward's linkage method. (**B**) Pharmacokinetic profiles of different metabolic phenotypes. Mean peak plasma AFP concentration-time curves after single 75 mg dose of AFP. Data shown are mean ± standard error (SE) concentrations. For both (**A**,**B**): slow metabolizers (*red*), intermediate metabolizers (*orange*), normal metabolizers (*green*), and fast metabolizers (*blue*).

drug phase (Ke) = 0.1923 ± 0.0539, half-life ($T_{1/2}$) = 7.04 ± 2.86 h, total drug clearance (Cl) = 2.32 ± 0.90 L/h/kg, volume of distribution (Vd) = 7.04 ± 2.86 L/kg. No sex differences were found in pharmacokinetic parameters (Table 1). Despite the lack of significant differences in the demographic and clinical features (Table 1), there was a remarkable pharmacokinetic variability (Table 2).

Metabolic phenotype classification. Based on the multivariate analysis[20,29–31] of the parameters C_{max} and $AUC_{0\text{-}t}$, we identified four AFP phenotypes–slow metabolizers (n = 7), intermediate metabolizers (n = 9), normal metabolizers (n = 12), and fast metabolizers (n = 8)–as shown in Fig. 1A. The means of most pharmacokinetic parameters were significantly different among the four metabolizer phenotypes ($P \leq 0.026$, Table 2). We used $AUC_{0\text{-}t}$ values to validate the pharmacokinetic variability. The regression analysis suggests that the four metabolizer phenotypes offer a better prediction of the variability of $AUC_{0\text{-}t}$ (R = 0.888, $R^2 = 0.788$, adjusted R = 0.781, $P = 5.50 \times 10^{-13}$; AICC = 144.02; and ASE = 7.15). There was a > 6-fold difference in AFP pharmacokinetic parameters between the slowest metabolizer (C_{max} = 17.80 ng/mL and $AUC_{0\text{-}t}$ = 78.85 ng/mL/h) and the fastest metabolizer (C_{max} = 2.94 ng/mL and $AUC_{0\text{-}t}$ = 10.31 ng/mL/h). The pharmacokinetic profiles of the different metabolizer phenotypes are presented in Fig. 1B.

Pharmacogenetic tests. All gene polymorphisms were in Hardy Weinberg Equilibrium (HWE) ($P > 0.05$) and passed the quality control tests of genotyping. Genotype frequencies are presented in Table 3.

Association between genotypes and Amfepramone pharmacokinetics. Two of the analysed polymorphisms affected AFP pharmacokinetics. C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, and Ke were affected by *ABCB1*-rs1045642 and *CYP3A4*-rs2242480 under the co-dominant, dominant, and over-dominant models (Tables 3 and 4). For *ABCB1*-rs1045642, the values of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ in carriers of genotype C/C were significantly lower than those in carriers of the variant allele (T/T or C/T + T/T, $P \leq 0.042$; Table 3), but the values of Ke ($P = 0.046$) in carriers of genotype C/C were significantly higher than those in carriers of the homozygous variant allele (T/T). The regression analysis confirmed our findings. Regarding *CYP3A4*-rs2242480, $AUC_{0\text{-}t}$ was significantly lower in carriers of the heterozygous genotype (C/T) than in subjects with the C/C genotype ($P = 0.033$). Moreover,

Genotypes	n	Pharmacokinetics parameters						
		C_{max} (ng/mL)	AUC_{0-t} (ng/mL/h)	$AUC_{0-\infty}$ (ng/mL/h)	K_e	$T_{1/2}$ (h)	Cl (L/h/kg)	V_d (L/kg)
***ABCB1*-rs1045642**								
C/C	10	5.42 ± 1.88[a]	23.29 ± 9.57	29.02 ± 9.74	0.2193 ± 0.0533[b]	3.35 ± 0.90	2.94 ± 1.22	13.85 ± 5.13
C/T	13	8.15 ± 3.63	34.36 ± 15.42	42.10 ± 17.64	0.1901 ± 0.0628	4.20 ± 2.05	2.01 ± 0.63	11.85 ± 5.67
T/T	13	7.18 ± 2.11	30.45 ± 9.13	36.91 ± 9.01	0.1738 ± 0.0375	4.16 ± 0.95	2.17 ± 0.62	13.26 ± 5.78
C/T + T/T	26	7.66 ± 2.95[c]	32.40 ± 12.57[d]	39.50 ± 13.97[e]	0.1819 ± 0.0513	4.18 ± 1.57	2.09 ± 0.62	12.56 ± 5.65
***ABCG2*-rs2231142**								
C/C	25	6.77 ± 2.33	28.05 ± 9.10	35.04 ± 11.26	0.1928 ± 0.0577	4.01 ± 1.67	2.39 ± 0.94	13.26 ± 5.43
C/A	11	7.66 ± 3.87	34.01 ± 17.66	40.11 ± 18.14	0.1911 ± 0.0462	3.79 ± 0.84	2.17 ± 0.83	12.14 ± 5.75
***SLCO1B1*-rs4149056**								
T/T	32	7.09 ± 2.95	30.26 ± 12.84	37.01 ± 14.30	0.1959 ± 0.0553	3.90 ± 1.51	2.32 ± 0.95	12.68 ± 5.58
C/T	4	6.65 ± 2.26	26.75 ± 8.62	33.26 ± 6.84	0.1638 ± 0.0323	4.35 ± 0.84	2.32 ± 0.41	14.79 ± 4.75
***DRD3*-rs6280**								
C/C	8	7.10 ± 1.71	29.77 ± 8.09	35.48 ± 9.10	0.1994 ± 0.0521	3.69 ± 0.98	2.22 ± 0.51	11.73 ± 3.66
C/T	19	7.11 ± 3.38	30.47 ± 15.03	36.49 ± 14.91	0.1959 ± 0.0499	3.76 ± 1.00	2.37 ± 1.01	13.01 ± 6.42
T/T	9	6.85 ± 2.73	28.71 ± 10.20	37.78 ± 15.51	0.1784 ± 0.0665	4.58 ± 2.36	2.31 ± 1.01	13.76 ± 4.94
***CYP3A4*-rs2242480**								
C/C	13	8.49 ± 3.86	34.77 ± 15.64[f]	40.38 ± 16.27	0.1993 ± 0.0454	3.63 ± 0.76	2.10 ± 0.74	11.12 ± 4.88
C/T	14	5.52 ± 1.62[g]	24.60 ± 9.68	32.42 ± 13.77	0.1924 ± 0.0688	4.21 ± 2.07	2.70 ± 1.15	15.05 ± 5.90
T/T	9	7.32 ± 1.25	30.99 ± 8.00	37.60 ± 7.50	0.1821 ± 0.0412	4.00 ± 1.06	2.06 ± 0.39	12.20 ± 4.99
C/C + T/T	22	8.01 ± 3.08[h]	33.23 ± 12.95[i]	39.24 ± 36.59	0.1923 ± 0.0436	3.78 ± 0.89	2.08 ± 0.61	11.56 ± 4.84
***CYP3A5*-rs776746**								
A/A	1	6.30 ± NA	27.00 ± NA	34.56 ± NA	0.1500 ± NA	4.62 ± NA	2.17 ± NA	14.47 ± NA
A/G	13	6.44 ± 2.05	28.21 ± 11.55	36.18 ± 15.16	0.1913 ± 0.0605	4.16 ± 2.06	2.51 ± 1.27	13.73 ± 6.34
G/G	22	7.43 ± 3.29	30.99 ± 13.28	36.92 ± 13.40	0.1948 ± 0.0514	3.79 ± 1.01	2.22 ± 0.63	12.37 ± 5.11
***CYP2B6*-rs3745274**								
G/G	14	7.19 ± 3.66	32.17 ± 16.50	38.09 ± 16.16	0.2029 ± 0.0560	3.69 ± 1.14	2.26 ± 0.90	12.28 ± 6.57
G/T	18	6.84 ± 2.46	27.21 ± 8.88	34.69 ± 12.80	0.1845 ± 0.0545	4.18 ± 1.75	2.46 ± 0.98	13.88 ± 4.79
T/T	4	7.43 ± 1.57	33.80 ± 8.84	39.90 ± 8.30	0.1905 ± 0.0504	3.82 ± 0.98	2.32 ± 0.90	10.83 ± 4.35
***GSTM3*-rs1799735**								
*A/*A	29	7.18 ± 3.12	29.62 ± 13.59	36.66 ± 15.03	0.1922 ± 0.0572	4.00 ± 1.58	2.37 ± 0.98	13.26 ± 5.80
*A/*B	7	6.46 ± 1.28	30.93 ± 5.71	36.28 ± 5.78	0.1929 ± 0.0409	3.72 ± 0.75	2.12 ± 0.40	11.48 ± 3.83

Table 3. Polymorphisms with significant effect on AFP pharmacokinetics. Data shown as mean ± standard deviation. [a]$P = 0.042$ (C/C *vs.* T/T); [b]$P = 0.046$ (C/C *vs.* T/T); [c]$P = 0.031$ (C/C *vs.* C/T + T/T); [d]$P = 0.041$ (C/C *vs.* C/T + T/T); [e]$P = 0.037$ (C/C *vs.* C/T + T/T); [f]$P = 0.033$ (C/C *vs.* C/T); [g]$P = 0.021$ (C/T *vs.* T/T); [h]$P = 0.005$ (C/T *vs.* C/C + T/T); [i]$P = 0.038$ (C/T *vs.* C/C + C/T). C_{max}, maximum plasma concentration; *AUC*, area under the plasma concentration-time curve; AUC_{0-t}, AUC from time 0 to the time of last measurement; $AUC_{0-\infty}$, AUC from time 0 extrapolated to infinity; *Ke*, elimination rate constant in the terminal drug phase; $T_{1/2}$, half-life drug; V_d, volume of distribution; *Cl*, total drug clearance.

Parameter	Predictors	Genetic model	R square	Adjusted R square	*P*-Value
K_e	rs1045642	Co-dominant	0.113	0.087	0.045*
C_{max}	rs1045642	Dominant	0.173	0.149	0.012*
AUC_{0-t}	rs1045642	Dominant	0.143	0.117	0.023*
$AUC_{0-\infty}$	rs1045642	Dominant	0.163	0.139	0.037*
Cl	rs1045642	Dominant	0.185	0.161	0.009*
C_{max}	rs224280	Over-dominant	0.223	0.200	0.004*
AUC_{0-t}	rs224280	Over-dominant	0.146	0.121	0.021*
C_{max}	rs224280, rs1045642	Over-dominant, dominant	0.248	0.202	0.009*
AUC_{0-t}	rs224280, rs1045642	Over-dominant, dominant	0.181	0.132	0.037*
Cl	rs224280, rs1045642	Over-dominant, dominant	0.240	0.194	0.011*

Table 4. Analysis of predictor models for the pharmacokinetic parameters of amfepramone. **P*-Value supported by automatic linear modeling and log-linear regression analysis and method. C_{max}, maximum plasma concentration; *AUC*, area under the plasma concentration-time curve; AUC_{0-t}, AUC from time 0 to the time of last measurement; $AUC_{0-\infty}$, AUC from time 0 extrapolated to infinity; *Ke*, elimination rate constant in the terminal drug phase; *Cl*, total drug clearance.

Gene	Polymorphism	Model	OR (95% CI)	*P*-Value	Pc-Value
ABCB1	rs1045642	Dominant (C/C vs. C/T + T/T)	C/C: Fast metabolizers	0.013	0.041*
			0.29 (0.11–0.75)		
			C/T + T/T: Slow/intermediate/normal metabolizers		
			2.19 (0.88–5.45)		
CYP3A4	rs224280	Co-dominant	C/T: Fast metabolizers	0.033	
CYP3A4	rs224280	Over-dominant (C/T vs. C/C + T/T)	C/C + T/T: slow metabolizers	0.019	0.055
			1.93 (1.36–2.75)		
CYP3A4	rs224280	Over-dominant (C/T vs. C/C + T/T)	C/T: Fast metabolizers	0.018	0.049*
			0.38 (0.19–0.77)		
			C/C + T/T: Slow/intermediate/normal metabolizers		
			2.86 (0.84–9.71)		
Combination	rs1045642, rs224280	Dominant (C/C vs. C/T + T/T), Over-dominant (C/T vs. C/C + T/T)	C/C-C/T: Fast metabolizers	0.004	0.014*
			0.14 (0.03–0.64)		
Combination	rs1045642, rs224280	Dominant (C/C vs. C/T + T/T), Over-dominant (C/T vs. C/C + T/T)	C/T + T/T-C/C + T/T: Slow metabolizers	0.003	0.012*
			2.64 (1.66–4.20)		

Table 5. Association between genotypes and metabolizer phenotypes *P-Value supported by logistic regression analysis. *OR*, odds ratio; *CI*, confidence interval; *Pc*, Bonferroni-corrected P-values.

the C/T genotype was associated with lower C_{max} values than the T/T genotype ($P = 0.021$). In addition, the C_{max} and $AUC_{0\text{-}t}$ values of the C/T genotype were significantly lower than those in the combined homozygous subjects (C/C + T/T; $P \leq 0.038$; Table 3).

The importance of aforementioned variability in AFP pharmacokinetics was confirmed by a regression analysis. However, after this analysis, the effect of *ABCB1*-rs1045642 polymorphism on Cl was revealed using a dominant model (Table 4). The final prediction model included *ABCB1*-rs1045642 and *CYP3A4*-rs2242480 ($P \leq 0.037$; Table 4).

Association between genotypes and metabolizer phenotypes. The association between genotype and phenotype was evaluated under the various genetic models and combinations of genotypes. We found two polymorphisms associated with the fast metabolizer phenotype (Table 5). The C/C genotype of *ABCB1*-rs1045642 under the co-dominant and dominant model and the C/T genotype of *CYP3A4*-rs2242480 under the over-dominant model were significantly associated with the fast metabolizer phenotype (Table 5) after Bonferroni's correction ($P \leq 0.049$).

Discussion

In Mexico, the issue of obesity is alarming because of its high prevalence[1]; however, only a small fraction of the population goes to professional health services for diagnosis and treatment[32]. Pérez-Salgado *et al.* suggest that this could be because the government institutions in Mexico focus mainly on raising awareness among the population about diseases that derive from obesity[32] and, therefore, research focused on improving the effectiveness of treatments is needed.

In order to contribute to health needs, the pharmacokinetic variability of one of the drugs used in the treatment of obesity was tested in a Mexican population. In this study, AFP pharmacokinetic profiles of 36 healthy Mexican volunteers were assessed under controlled conditions. In our sample, the groups of men and women had similar age range and BMI and the same ethnic group. However, they were significantly different in terms of weight and height (Table 1). These differences that exist between men and women could affect the drug disposition[33] and, therefore, influence the pharmacokinetic variability[34]. Nevertheless, this variability could be eliminated if normalization by weight is performed[33]. Accordingly, in our findings, no sex differences were observed in pharmacokinetic parameters among males and females (Table 1). To our knowledge, no prior studies have reported the effect of sex on AFP pharmacokinetics.

To verify whether there exists variation in AFP pharmacokinetic profiles and investigate a possible association with polymorphisms in genes related to drug metabolism, we used a simple approach to classify the pharmacokinetic profiles using the parameters C_{max} and $AUC_{0\text{-}t}$. Four major metabolizer phenotypes were distinguished (slow, normal, intermediate, and fast) with significant differences in pharmacokinetic parameters among the metabolizer phenotype groups (Table 2, Fig. 1A,B). Although our metabolic classification should be validated by another phenotyping method such as pharmacometabolomics[35], the pharmacokinetic variability among metabolic phenotypes is evident. This variability is confirmed by a >6-fold difference in the pharmacokinetic parameters between the slowest and the fastest metabolizers. To the best of our knowledge, this is the first report on different AFP metabolizer types. In 1973, Testa and Beckett reported minor intersubject variations under standardized conditions of AFP metabolism; however, their trials were performed on only four subjects[36]. During the clinical trial and at the end, no volunteers showed any adverse effects.

Although several pharmacogenetics and pharmacogenomics studies have been published, this is the first study evaluating AFP. In this study, two polymorphisms had a significant impact on AFP pharmacokinetics ($P \leq 0.046$). One polymorphic variant involved a gene that encodes a transporter protein (*ABCB1*-rs1045642), and the other located polymorphism involved genes encoding drug-metabolizing enzymes (*CYP3A4*-rs2242480). It is known that *ABCB1* interacts with a multitude of structurally and biochemically unrelated substrates[37]. Therefore, *ABCB1* affects plasma concentrations of and, consequently, the pharmacological response to several drugs, with inconsistent results. The latter depends on the population as well as the substrate analyzed[20,37–44], for example, in a Chinese study population, the T/T genotype was associated with lower plasma concentrations of clopidogrel and its active metabolites[45]. However, in Korean subjects, the T/T genotype was associated with higher C_{max} and AUC values of fexofenadine hydrochloride[46]. These discrepancies may be due to the fact that polymorphisms with silent effects can affect the time of protein translation and, therefore, its folding, causing changes in substrate specificity[47]. Regarding anti-obesity drugs, Lloret *et al.* conducted a study on the role of *ABCB1*-rs1045642 gene variant on oral morphine pharmacokinetics; however, they did not find an association between the two[48]. Nevertheless, there is no information on the effect of *ABCB1* on AFP pharmacokinetics. For the Mexican population, the C/C genotype of the rs1045642 polymorphism has been associated with the atorvastatin fast metabolizer phenotype because of a significant effect on C_{max} and $AUC_{0\text{-}t}$ values among 60 healthy volunteers[20]. The results of the present study are consistent with the previous report; carriers of the C/C genotype showed significantly lower C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ values (Tables 3 and 4), significant variability in Ke and Cl values, and were associated with the AFP fast metabolizer phenotype after Bonferroni's correction (Table 5). These results suggest that the effect of rs1045642 could be related to abnormal intestinal absorption and clearance of AFP. Here, the C/C genotype carriers showed higher Ke and Cl values but lower $T_{1/2}$. However, only Ke was significantly different (Table 3). These findings are similar to those reported by Gonzalez-Vacarezza *et al.* in quetiapine pharmacokinetics[49]. To our knowledge, the present study is the first report on the effect of rs1045642 on AFP pharmacokinetics.

CYP3A4 encodes one of the main drug metabolizing-enzymes[25]. Although the *CYP3A4**1B polymorphic variant is the most studied, there is no report of an association between drug metabolism and response in the Mexican population[50–52]. Therefore, other polymorphic variants in non-coding regions have been explored, for example, *CYP3A4*-rs2242480 in intron 10[50]. Interestingly, we found that *CYP3A4*-rs2242480 affected several parameters of AFP pharmacokinetics (Tables 3 and 4). Individuals with the heterozygous genotype (C/T) showed a significant effect on C_{max} and $AUC_{0\text{-}t}$ and a significant association with the fast metabolizer phenotype (Table 5), *i.e.* there is an increased capacity for plasma clearance. Nonetheless, our results suggest that the C/T genotype is associated with an increased biotransformation of AFP because rs2242480 significantly affects only C_{max} and $AUC_{0\text{-}t}$ values but not parameters such as Ke and Cl (Tables 3 and 4). These results are consistent with the previous report in a Mexican population. The rs2242480 polymorphism was the most important variant of the prediction model of the variation of AUC values of atorvastatin[21] and for the increased R-warfarin clearance in subjects with a heterozygous genotype in a population from the United Kingdom[53]. However, the present results differ from those reported by Danielak *et al.* in a Polish population where no effect of rs2242480 was observed on clopidogrel pharmacokinetics[50]. The T allele frequency (0.44) found in our study population was different from those reported in populations from the United Kingdom (0.09)[53], Poland (0.08)[50], and Asia (0.29)[54].

In summary, AFP pharmacokinetics differs among Mexicans, and we found two genetic polymorphisms with a significant effect on AFP pharmacokinetics, which were used in a log-linear regression analysis. Nevertheless, the interaction of these polymorphisms could be considered to produce a small or moderate effect because only 13–20% of the variability on C_{max}, $AUC_{0\text{-}t}$, and Cl can be predicted (Table 4). It is possible that other gene polymorphisms not evaluated in this study may contribute to AFP pharmacokinetic variability.

Furthermore, we did not measure the concentration of secondary metabolites. Such information might have provided an additional phenotyping method to support our results. Another study limitation was the small population size. A follow-up study with a larger population is required to validate our results.

Conclusion

This pilot study revealed variability in AFP pharmacokinetics in the Mexican population. Additionally, *ABCB1*-rs1045642 and *CYP3A4*-rs2242480 were found to significantly affect AFP plasma levels. These results suggest that genetic variants may be useful in studies predicting pharmacokinetic variability and those that evaluate the response to AFP treatment. Further studies in a larger population are required to validate our findings.

Material and Methods

Design. A controlled, randomized, crossover, single-blind, two-treatment, two-period, and two-sequence clinical study was conducted in healthy volunteers of Mexican origin to assess the bioequivalence of a single oral dose (75 mg) of AFP (extended-release capsule; Medix Products, S. A., Mexico City, CDMX, MEX) at Ipharma S.A. The study complies with the guidelines of the Declaration of Helsinki, the Good Clinical Practice Standards of Tokyo, and the Mexican regulations for studies of bioavailability and bioequivalence. The clinical protocol was approved by the Research and Ethics Committee of the Clinical and Experimental Pharmacology Center, Ipharma S. A. (Monterrey, NL, MEX), and the pharmacogenetic procedure was approved by the Ethics, Research, and Biosecurity Committees of the University of Monterrey (San Pedro Garza Garcia, NL, MEX; registry number 042014-CIE). The study was registered in the National Registry of Clinical Trials (RNEC; registry number /A394-16) at the Federal Commission for Protection Against Health Risks (COFEPRIS) and Australian New Zealand Clinical Trials Registry (ACTRN12619000391178; registration date: 12/03/2019). Written informed consent was obtained from all subjects or their parents/legal tutors.

Study population. From February 2017 to March 2017, 36 healthy volunteers were enrolled and randomized using R statistical software version 2.15.2. Inclusion criteria for this study were as follows: healthy males and

non-pregnant, non-breastfeeding females, aged 18–55 years, with a BMI in the range 18–27 kg/m^2, non-smokers, and willing to use adequate methods of contraception throughout the study. Exclusion criteria included electrocardiographic, clinical, biochemical, haematological, coagulation, or urinalysis test abnormalities; a positive test for drug or alcohol abuse, human immunodeficiency virus, hepatitis B and C viruses, and rapid plasma reagin; a medical history of serious adverse reaction or serious hypersensitivity to the test drug or to chemically related drugs; glaucoma, hypertension, anorexia and thyroid problems or existence of concurrent disease; a history of smoking, alcohol or drug abuse; the use of prescription or over-the-counter medication 3 weeks before enrolment; and participation in a clinical research study in the previous 3 months.

Randomization. The volunteers who fulfilled the requirements were selected by the clinical research coordinator of Ipharma, S.A. Before the start of the clinical study, the statistical division of Ipharma S.A. performed randomization for the allocation of drugs (R or P, file code, and subject code). The randomization was done in two blocks in a balanced design using the R statistical software version 2.15.2. and the Mersenne Twister algorithm. The participants were blinded to treatment allocation.

Sampling. A single dose of 75 mg of AFP extended-release capsule (Medix Products, S. A., Mexico City, CDMX, MEX) was administered orally after overnight fasting. The volunteers were under medical supervision throughout the protocol. Blood samples (4 mL) were collected in BD K_2EDTA-coated Vacutainers (BD Diagnostics, Franklin Lakes, NJ, USA) at pre-dose (time 0) and seventeen times after drug administration (0.25, 0.5, 0.75, 1, 1.25, 1.50, 1.75, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 h). Plasma was separated by centrifugation (10 min at 1600 × g at 4 °C) and stored at −65 ± 15 °C for subsequent analysis.

Determination of plasma Amfepramone concentration. Plasma AFP concentration was measured using a validated method developed by Ipharma S.A., according to Mexican regulations (NOM-177-SSA1-2013)[55] and guidelines of European Medicines Agency (EMA) regarding bioanalytical method validation[56].

In brief, the plasma proteins were eliminated by acetonitrile precipitation. Then, 300 µL acetonitrile was added to a 50 µL plasma sample, vortexed (60 rpm, 4 min), and centrifuged (9600 × *g*, 10 min, 10 °C). The supernatant (200 µL) was injected into a high-performance liquid chromatography (HPLC) system coupled to a tandem mass spectrometer 6410B (MS/MS) with a triple quadrupole detector (Agilent Technologies, Santa Clara, CA, USA). The chromatographic separation was performed at 45 °C with a Gemini C18 pre-column (Phenomenex, Torrance, CA, USA) and a Zorbax Eclipse XDB C-18 column (3.5 µm, 80 Å, 4.6 × 150 mm) (Agilent Technologies, Santa Clara, CA, USA). The injection sample volume was 5 µL. The mobile phase had a flow rate of 0.6 mL/min and consisted of 5.0 mM ammonium formate/0.1% formic acid and acetronitrile (15:85). The detection system used an ESI MS/MS precursor ion (+) 206.2 m/z and a (+) product ion 105.0 m/z. The interday linearity was assessed with calibration curves (curve range, 1 to 100 ng/mL: 1, 2.5, 5, 10, 25, 50 and 100 ng/mL). The intraday quality control was evaluated by control samples of 1.7, 7.5, 35, and 75 ng/mL each. The validated method had a coefficient of variation (CV) ≤5% for the precision and an error ≤5% for the accuracy.

Pharmacokinetic analysis. The pharmacokinetic parameters were calculated by non-compartmental methods. The maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were obtained from the concentration-time data of the plasma. The area under the plasma AFP concentration-time curve (AUC) from time 0 to the time of last measurement ($AUC_{0\text{-}t}$), AUC from time 0 extrapolated to infinity ($AUC_{0\text{-}\infty}$) were calculated by log-linear trapezoidal rule. Ke was estimated by log-linear regression from the terminal portion of the log-transformed concentration-time plots. $T_{1/2}$ was estimated by dividing 0.693 by Ke. The total drug clearance was calculated by dividing dose by $AUC_{0\text{-}\infty}$ and adjusting for weight. Volume of distribution was calculated as Cl divided by Ke. The AUC and C_{max} values were adjusted for dose and weight (AUC/dW and C_{max}/dW). The pharmacokinetic analysis was performed using R statistical software version 2.15.2.

Classification of Amfepramone metabolism. The metabolizer phenotypes were determined according to a multivariate analysis of the combined pharmacokinetic parameters C_{max} and $AUC_{0\text{-}t}$ using a hierarchical cluster analysis (HCA)[20,29]. Since the clustering algorithms are dependent on the data size, we used a modified method to estimate the ideal number of clusters[30,31]. First, we introduced the data set of the unadjusted C_{max} and $AUC_{0\text{-}t}$ values together with the adjusted data set. Second, C_{max} and $AUC_{0\text{-}t}$ were standardized to minimize the effect of scale differences, and a distance matrix was made from the combined C_{max} and $AUC_{0\text{-}t}$ values. Third, hierarchical cluster analysis (HCA) using the Ward linkage method was performed on individual C_{max} and $AUC_{0\text{-}t}$ values. Finally, the interindividual Manhattan distances were computed. Minitab 16 software (Minitab Inc., State College, PA, USA) was used for standardization and HCA. We identified the subjects of each cluster and calculated the means of all adjusted pharmacokinetic parameters by cluster. According to the mean values of the pharmacokinetic parameters of the clusters, they were classified into metabolizer phenotypes[20].

Pharmacogenetic tests. The Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI, USA) was used to isolate DNA according to manufacturer's instructions. Genomic DNA was quantified by UV absorbance using Nanodrop (Thermo Fisher Scientific Inc., Wilmington, MA, USA). The DNA purity was evaluated with the A260/280 and A260/230 ratios, and the samples were stored at −20 °C until use. The DNA samples were subjected to genotyping for the polymorphisms *ABCB1*-rs1045642, *ABCG2*-rs2231142, *SLCO1B1*-rs4149056, *DRD3*-rs6280, *CYP3A4*-rs2242480, *CYP3A5*-rs776746, *CYP2B6*-rs3745274, and *GSTM3*-rs1799735[57] using real-time polymerase chain reaction and Taqman probes (Applied Biosystems; Thermo Fisher Scientific Inc., Wilmington, MA, USA) according to the manufacturer's protocol. Three quality control thresholds were applied: a genotype call rate equal to 1, an HWE test with $P > 0.05$, and minor allele frequency >0.01.

Statistical analysis. For sample size calculation, the intrasubject CV obtained in a previous pilot study (Amfepramone/A359-16P) was considered. It was assumed that CV was 25.77% for both C_{max} and AUC. Considering a confidence level of 95%, a significance level of 5%, and a minimum power of 80%, a sample size of 30 would suffice. One-way analysis of variance (ANOVA); Kruskal Wallis test; automatic linear modelling using forward stepwise method, Akaike Information Criterion (AICC) and Overfit Prevention Criterion (ASE); and linear and logistic regression analysis model were applied to validate the phenotyping model. The HWE was determined by comparing the genotype frequencies with the expected values using the maximum likelihood method[58]. Differences between males and females regarding genotype frequencies were determined using a corrected χ^2 test. To assess the effects of polymorphisms on the AFP pharmacokinetic parameters, comparisons between two and three groups were made. The Student's *t*-test and one-way analysis of variance were used for parametric distributions, whereas Mann-Whitney U and Kruskal-Wallis H tests were used for nonparametric distributions. Post-hoc tests (Bonferroni correction and Tamhane's T2 test) were used for pairwise comparisons. To confirm the contribution of genetic factors to the variability of pharmacokinetic parameters, automatic linear modelling using forward stepwise method, AICC and ASE as well as linear regression analysis using enter, stepwise, remove, backward, and forward methods were performed. Possible associations of genotypes or combinations of genotypes with phenotypes were evaluated using χ^2 and Fisher's exact tests and validated by logistic regression analysis. The evaluation effects of polymorphisms and associations were assessed under three different models (co-dominant, dominant, over-dominant, and recessive). The odds ratio (OR) was estimated with a 95% confidence interval (95% CI). All *P* values were two-tailed. Corrected *P* values (Pc) were obtained using the Bonferroni correction for exclusion of spurious associations. $P < 0.05$ was interpreted as statistically significant. The statistical analyses were performed with SPSS for Windows, V.20 (IBM Corp., NY, USA).

Trial registration. Australian New Zealand Clinical Trials Registry: ACTRN12619000391178, date registered: 12/03/2019.

Data availability

All data generated and analysed during this study are included in this published article, but if necessary, some additional information is available from the corresponding author upon reasonable request.

Received: 28 March 2019; Accepted: 12 November 2019;
Published online: 28 November 2019

References

1. Organisation for Economic Co-operation and Development. *Obesity Update 2017*, https://www.oecd.org/els/health-systems/Obesity-Update-2017.pdf (2017).
2. Rivera-Dommarco, J. *et al.* (Instituto Nacional de Salud Publica (MX), Cuernavaca, México, 2016).
3. World Health Organization. *Obesity and overweight*, http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (2017).
4. Bray, G. A. & Greenway, F. L. Pharmacological treatment of the overweight patient. *Pharmacological reviews* **59**, 151–184, https://doi.org/10.1124/pr.59.2.2 (2007).
5. Wilbert, B., Mohundro, B. L., Shaw, V. & Andres, A. Appetite suppressants as adjuncts for weight loss. *American family physician* **83**, 1–2 (2011).
6. Manning, S., Pucci, A. & Finer, N. Pharmacotherapy for obesity: novel agents and paradigms. *Therapeutic advances in chronic disease* **5**, 135–148, https://doi.org/10.1177/2040622314522848 (2014).
7. Kang, J. G. & Park, C. Y. Anti-Obesity Drugs: A Review about Their Effects and Safety. *Diabetes & metabolism journal* **36**, 13–25, https://doi.org/10.4093/dmj.2012.36.1.13 (2012).
8. Thomas, E. A. *et al.* Greater hunger and less restraint predict weight loss success with phentermine treatment. *Obesity* **24**, 37–43, https://doi.org/10.1002/oby.21244 (2016).
9. Bray, G. A. Lifestyle and pharmacological approaches to weight loss: efficacy and safety. *The Journal of clinical endocrinology and metabolism* **93**, S81–88, https://doi.org/10.1210/jc.2008-1294 (2008).
10. Hendricks, E. J. Off-label drugs for weight management. *Diabetes, metabolic syndrome and obesity: targets and therapy* **10**, 223–234, https://doi.org/10.2147/DMSO.S95299 (2017).
11. Arias, H. R., Santamaria, A. & Ali, S. F. Pharmacological and neurotoxicological actions mediated by bupropion and diethylpropion. *International review of neurobiology* **88**, 223–255, https://doi.org/10.1016/S0074-7742(09)88009-4 (2009).
12. Suplicy, H. *et al.* A comparative study of five centrally acting drugs on the pharmacological treatment of obesity. *International journal of obesity* **38**, 1097–1103, https://doi.org/10.1038/ijo.2013.225 (2014).
13. Cercato, C. *et al.* A randomized double-blind placebo-controlled study of the long-term efficacy and safety of diethylpropion in the treatment of obese subjects. *International journal of obesity* **33**, 857–865, https://doi.org/10.1038/ijo.2009.124 (2009).
14. Kalyanasundar, B. *et al.* The efficacy of the appetite suppressant, diethylpropion, is dependent on both when it is given (day vs. night) and under conditions of high fat dietary restriction. *Appetite* **100**, 152–161, https://doi.org/10.1016/j.appet.2016.01.036 (2016).
15. Garcia-Mijares, M., Bernardes, A. M. & Silva, M. T. Diethylpropion produces psychostimulant and reward effects. *Pharmacology, biochemistry, and behavior* **91**, 621–628, https://doi.org/10.1016/j.pbb.2008.10.001 (2009).
16. Soto-Molina, H. *et al.* Six-month efficacy and safety of amfepramone in obese Mexican patients: a double-blinded, randomized, controlled trial. *International journal of clinical pharmacology and therapeutics* **53**, 541–549, https://doi.org/10.5414/CP202135 (2015).
17. Leon-Cachon, R. B., Ascacio-Martinez, J. A. & Barrera-Saldana, H. A. Individual response to drug therapy: bases and study approaches. *Revista de investigacion clinica; organo del Hospital de Enfermedades de la Nutricion* **64**, 364–376 (2012).
18. Herrera-Gonzalez, S. *et al.* Effect of AGTR1 and BDKRB2 gene polymorphisms on atorvastatin metabolism in a Mexican population. *Biomedical reports* **7**, 579–584, https://doi.org/10.3892/br.2017.1009 (2017).
19. Leon-Cachon, R. B. *et al.* Application of Genomic Technologies in Clinical Pharmacology Research. *Revista de investigacion clinica; organo del. Hospital de Enfermedades de la Nutricion* **67**, 212–218 (2015).
20. Leon-Cachon, R. B. R. *et al.* A pharmacogenetic pilot study reveals MTHFR, DRD3, and MDR1 polymorphisms as biomarker candidates for slow atorvastatin metabolizers. *BMC cancer* **16**, 74, https://doi.org/10.1186/s12885-016-2062-2 (2016).

21. Cruz-Correa, O. F., Leon-Cachon, R. B., Barrera-Saldana, H. A. & Soberon, X. Prediction of atorvastatin plasmatic concentrations in healthy volunteers using integrated pharmacogenetics sequencing. *Pharmacogenomics* **18**, 121–131, https://doi.org/10.2217/pgs-2016-0072 (2017).
22. Wishart, D. S. *et al*. DrugBank: a knowledgebase for drugs, drug actions and drug targets. *Nucleic acids research* **36**, D901–906, https://doi.org/10.1093/nar/gkm958 (2008).
23. Wishart, D. S. *et al*. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic acids research* **46**, D1074–D1082, https://doi.org/10.1093/nar/gkx1037 (2018).
24. Beckett, A. H. & Stanojcic, M. Re-evaluation of the metabolism and excretion of diethylpropion in non-sustained and sustained release formulations. *The Journal of pharmacy and pharmacology* **39**, 409–415 (1987).
25. Klein, K. & Zanger, U. M. Pharmacogenomics of Cytochrome P450 3A4: Recent Progress Toward the "Missing Heritability" Problem. *Frontiers in genetics* **4**, 12, https://doi.org/10.3389/fgene.2013.00012 (2013).
26. Kobayashi, K. *et al*. Human buprenorphine N-dealkylation is catalyzed by cytochrome P450 3A4. *Drug metabolism and disposition: the biological fate of chemicals* **26**, 818–821 (1998).
27. Wang, J. S., Backman, J. T., Taavitsainen, P., Neuvonen, P. J. & Kivisto, K. T. Involvement of CYP1A2 and CYP3A4 in lidocaine N-deethylation and 3-hydroxylation in humans. *Drug metabolism and disposition: the biological fate of chemicals* **28**, 959–965 (2000).
28. Jancova, P., Anzenbacher, P. & Anzenbacherova, E. Phase II drug metabolizing enzymes. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia* **154**, 103–116 (2010).
29. Lindon, J. C. & Nicholson, J. K. The emergent role of metabolic phenotyping in dynamic patient stratification. *Expert opinion on drug metabolism & toxicology* **10**, 915–919, https://doi.org/10.1517/17425255.2014.922954 (2014).
30. Green, M. A. *et al*. Who are the obese? A cluster analysis exploring subgroups of the obese. *Journal of public health* **38**, 258–264, https://doi.org/10.1093/pubmed/fdv040 (2016).
31. Hu, C. W., Kornblau, S. M., Slater, J. H. & Qutub, A. A. Progeny Clustering: A Method to Identify Biological Phenotypes. *Scientific reports* **5**, 12894, https://doi.org/10.1038/srep12894 (2015).
32. Perez-Salgado, D., Valdes Flores, J., Janssen, I. & Ortiz-Hernandez, L. Diagnosis and treatment of obesity among Mexican adults. *Obesity facts* **5**, 937–946, https://doi.org/10.1159/000346325 (2012).
33. Soldin, O. P., Chung, S. H. & Mattison, D. R. Sex differences in drug disposition. *Journal of biomedicine & biotechnology* **2011**, 187103, https://doi.org/10.1155/2011/187103 (2011).
34. Gandhi, M., Aweeka, F., Greenblatt, R. M. & Blaschke, T. F. Sex differences in pharmacokinetics and pharmacodynamics. *Annual review of pharmacology and toxicology* **44**, 499–523, https://doi.org/10.1146/annurev.pharmtox.44.101802.121453 (2004).
35. Huang, Q. *et al*. A Pharmacometabonomic Approach To Predicting Metabolic Phenotypes and Pharmacokinetic Parameters of Atorvastatin in Healthy Volunteers. *Journal of proteome research* **14**, 3970–3981, https://doi.org/10.1021/acs.jproteome.5b00440 (2015).
36. Testa, B. & Beckett, A. H. Metabolism and excretion of diethylpropion in man under acidic urine conditions. *The Journal of pharmacy and pharmacology* **25**, 119–124 (1973).
37. Hodges, L. M. *et al*. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). *Pharmacogenetics and genomics* **21**, 152–161, https://doi.org/10.1097/FPC.0b013e3283385a1c (2011).
38. Dessilly, G., Panin, N., Elens, L., Haufroid, V. & Demoulin, J. B. Impact of ABCB1 1236C> T-2677G> T-3435C> T polymorphisms on the anti-proliferative activity of imatinib, nilotinib, dasatinib and ponatinib. *Scientific reports* **6**, 29559, https://doi.org/10.1038/srep29559 (2016).
39. Gregers, J. *et al*. Polymorphisms in the ABCB1 gene and effect on outcome and toxicity in childhood acute lymphoblastic leukemia. *The pharmacogenomics journal* **15**, 372–379, https://doi.org/10.1038/tpj.2014.81 (2015).
40. Miura, M. *et al*. Influence of ABCB1 C3435T polymorphism on the pharmacokinetics of lansoprazole and gastroesophageal symptoms in Japanese renal transplant recipients classified as CYP2C19 extensive metabolizers and treated with tacrolimus. *International journal of clinical pharmacology and therapeutics* **44**, 605–613 (2006).
41. Suthandiram, S. *et al*. Effect of polymorphisms within methotrexate pathway genes on methotrexate toxicity and plasma levels in adults with hematological malignancies. *Pharmacogenomics* **15**, 1479–1494, https://doi.org/10.2217/pgs.14.97 (2014).
42. Saiz-Rodriguez, M. *et al*. Effect of Polymorphisms on the Pharmacokinetics, Pharmacodynamics and Safety of Sertraline in Healthy Volunteers. *Basic & clinical pharmacology &. toxicology* **122**, 501–511, https://doi.org/10.1111/bcpt.12938 (2018).
43. Su, J. *et al*. ABCB1 C3435T polymorphism and response to clopidogrel treatment in coronary artery disease (CAD) patients: a meta-analysis. *PloS one* **7**, e46366, https://doi.org/10.1371/journal.pone.0046366 (2012).
44. Calderon-Cruz, B. *et al*. C3435T polymorphism of the ABCB1 gene is associated with poor clopidogrel responsiveness in a Mexican population undergoing percutaneous coronary intervention. *Thrombosis research* **136**, 894–898, https://doi.org/10.1016/j.thromres.2015.08.025 (2015).
45. Wang, X. Q. *et al*. Genetic polymorphisms of CYP2C19 2 and ABCB1 C3435T affect the pharmacokinetic and pharmacodynamic responses to clopidogrel in 401 patients with acute coronary syndrome. *Gene* **558**, 200–207, https://doi.org/10.1016/j.gene.2014.12.051 (2015).
46. Yi, S. Y. *et al*. A variant 2677A allele of the MDR1 gene affects fexofenadine disposition. *Clinical pharmacology and therapeutics* **76**, 418–427, https://doi.org/10.1016/j.clpt.2004.08.002 (2004).
47. Kimchi-Sarfaty, C. *et al*. A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* **315**, 525–528, https://doi.org/10.1126/science.1135308 (2007).
48. Lloret-Linares, C. *et al*. Oral Morphine Pharmacokinetic in Obesity: The Role of P-Glycoprotein, MRP2, MRP3, UGT2B7, and CYP3A4 Jejunal Contents and Obesity-Associated Biomarkers. *Molecular pharmaceutics* **13**, 766–773, https://doi.org/10.1021/acs.molpharmaceut.5b00656 (2016).
49. Gonzalez-Vacarezza, N. *et al*. MDR-1 genotypes and quetiapine pharmacokinetics in healthy volunteers. *Drug metabolism and drug interactions* **28**, 163–166, https://doi.org/10.1515/dmdi-2013-0008 (2013).
50. Danielak, D. *et al*. Impact of CYP3A4*1G Allele on Clinical Pharmacokinetics and Pharmacodynamics of Clopidogrel. *European journal of drug metabolism and pharmacokinetics* **42**, 99–107, https://doi.org/10.1007/s13318-016-0324-7 (2017).
51. Shi, W. L., Tang, H. L. & Zhai, S. D. Effects of the CYP3A4*1B Genetic Polymorphism on the Pharmacokinetics of Tacrolimus in Adult Renal Transplant Recipients: A Meta-Analysis. *PloS one* **10**, e0127995, https://doi.org/10.1371/journal.pone.0127995 (2015).
52. Zochowska, D., Wyzgal, J. & Paczek, L. Impact of CYP3A4*1B and CYP3A5*3 polymorphisms on the pharmacokinetics of cyclosporine and sirolimus in renal transplant recipients. *Annals of transplantation* **17**, 36–44 (2012).
53. Lane, S. *et al*. The population pharmacokinetics of R- and S-warfarin: effect of genetic and clinical factors. *British journal of clinical pharmacology* **73**, 66–76, https://doi.org/10.1111/j.1365-2125.2011.04051.x (2012).
54. He, B. X. *et al*. A functional polymorphism in the CYP3A4 gene is associated with increased risk of coronary heart disease in the Chinese Han population. *Basic & clinical pharmacology &. toxicology* **108**, 208–213, https://doi.org/10.1111/j.1742-7843.2010.00657.x (2011).
55. Secretaría de Salud & Comisión Federal para la Protección Contra Riesgos Sanitarios. (Secretaría de Gobernación, México, DF, 2013).
56. European Medicines Agency. Science Medicines Health. (European Medicines Agency, London, UK, 2011).
57. Martinez-Trevino, D. A. *et al*. Rapid Detection of the GSTM3 A/B Polymorphism Using Real-time PCR with TaqMan((R)) Probes. *Archives of medical research* **47**, 142–145, https://doi.org/10.1016/j.arcmed.2016.04.002 (2016).
58. Reed, T. E. & Schull, W. J. A general maximum likelihood estimation program. *American journal of human genetics* **20**, 579–580 (1968).

Acknowledgements
The authors would like to thank QBP Marcelino Aguirre Garza, Dr. Lizeth Alejandra Martínez Jacobo from University of Monterrey and technical staff of Ipharma, S.A. for facilities, advice and technical support; and Dr. Irene Meester for reviewing and improving the manuscript. Clinical trial Pharmaceutical Research S.A.,/A394-16. Pharmacogenetic study University of Monterrey, UIN19516.

Author contributions
M.G.-S., A.L.-G. and R.B.R.L.-C. designed research and conception. E.P.-G. and R.V.-Z. collected samples and acquired clinical data of volunteers. M.G.-S., E.P.-G. and R.V.Z. conducted analytical determination M.B.L. performed genetic studies of the study subjects. M.E.G.-P. carried out the pharmacokinetic analysis data. R.B.R.L.-C. and M.E.G.-P. analyzed data. R.B.R.L.-C., M.B.L. and A.L. data interpretation. M.G.-S., A.L.-G. R.B.R.L.-C. and A.L. wrote and critically reviewed the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to R.B.R.L.-C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.