Polymorphisms in *CYP1A2*, *CYP2C9* and *ABCB1* affect agomelatine pharmacokinetics

Miriam Saiz-Rodríguez[1], Dolores Ochoa[1,2], Carmen Belmonte[1,2], Manuel Román[1,2], Danilo Vieira[1], Pablo Zubiaur[1], Dora Koller[1] , Gina Mejía[1,2], Francisco Abad-Santos, [1,2,4]

[1]Clinical Pharmacology Department, Hospital Universitario de La Princesa, Instituto Teófilo Hernando, Universidad Autónoma de Madrid (UAM), Instituto de Investigación Sanitaria La Princesa (IP), Madrid, Spain

[2]UICEC Hospital Universitario de La Princesa, Plataforma SCReN (Spanish Clinical Research Network), Instituto de Investigación Sanitaria La Princesa (IP), Madrid, Spain.

[4]Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain

Corresponding author

Dr. Francisco Abad-Santos
Clinical Pharmacology Department,
Hospital Universitario de la Princesa
Diego de León 62. 28006 Madrid, Spain
Tel: 34 915202425; Fax: 34 915202540
E-mail: francisco.abad@salud.madrid.org

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Running title: Pharmacogenetics of agomelatine

ABSTRACT

BACKGROUND: Agomelatine is an agonist of the melatoninergic receptors used for the clinical management of depression. Our aim was to evaluate the effect of genetic polymorphisms in its metabolizing enzymes and the P-glycoprotein transporter on the pharmacokinetics and pharmacodynamics of agomelatine in healthy volunteers.

METHODS: Twenty-eight healthy volunteers (16 men and 12 women, 22 Caucasians, 5 Latin and 1 Black) receiving a single 25 mg oral dose of agomelatine, were genotyped for 9 polymorphisms in cytochrome P450 (CYP) enzymes (*CYP1A2, CYP2C9* and *CYP2C19)* and ATP binding cassette subfamily B member 1 (*ABCB1)*, by real-time PCR. Agomelatine plasma levels were measured by high-performance liquid chromatography combined with tandem mass spectrometry (HPLC-MS/MS).

RESULTS: *CYP1A2* activity score is directly correlated with agomelatine pharmacokinetic parameters. Individuals with a slower metabolism had a lower Cl/F and, consequently, accumulate higher concentrations of agomelatine. On the other hand, individuals with a high CYP1A2 inducibility showed an extensive Cl/F and lower concentrations of agomelatine. The apparently marked differences showed between races were due to the different *CYP1A2* genotype distribution among them. In addition, *CYP2C9* intermediate/poor metabolizers individuals showed a significantly higher area under the concentration-time curve and maximum concentration; however, no association was found between *CYP2C19* phenotype and agomelatine pharmacokinetics. *ABCB1* G2677T/A polymorphism affected the time to reach maximum concentration, as subjects carrying A/A or A/T genotype showed higher values. Agomelatine did not produce a significant change in blood pressure, heart rate or corrected QT interval.

CONCLUSION*: CYP1A2* phenotype inferred from the genotyping of *CYP1A2**1C, *1F and *1B alleles might be a potential predictor of agomelatine exposure. The influence of *ABCB1* G2677T/A could mean that this polymorphism enhances P-glycoprotein activity, as subjects with genotypes A/A+A/T had lower agomelatine concentration and thus it takes more time to reach the maximum concentration.

INTRODUCTION

Agomelatine is an antidepressant drug used for the clinical management of major depressive disorder (MDD), which is one of the most common mental disorders in the world (1). Agomelatine has a distinctive mechanism of action, acting synergistically as an agonist of the melatoninergic receptors MT1 and MT2 and as an antagonist of the post-synaptic serotonergic 5-HT2c receptor (2). MT1 and MT2 are involved in sleep regulation while 5-HT2c receptors inhibit the release of dopamine and norepinephrine in the prefrontal cortex. Agomelatine binds to MT1 and MT2 acting as a sleep inducer and blocks 5-HT2c, acting as an antidepressant (by augmenting the release of monoamines, whose levels are compromised in depressive patients) (3–5). These agomelatine combined actions help to resynchronize the circadian rhythm, normalize sleep patterns and resolve mood disorders (6).

After oral administration (25-50 mg), agomelatine is rapidly absorbed (80%) in the gastrointestinal tract, reaching peak plasma concentrations at approximately 2 hours. However, it has extensive first pass metabolism that reduces its bioavailability to less than 5% from the initial oral dose (7). It has a moderate volume of distribution (Vd), around 35 L, and a short plasma half-life (T½), around 1-2 h. It binds to plasma proteins albumin and glycoprotein alfa-1 (8). Agomelatine is metabolized in the liver by various cytochrome P450 (CYP) isoenzymes, about 90% of the drug is hydroxylazed by CYP1A2 while the remaining 10% is metabolized by demethylation via CYP2C9 and CYP2C19. It has at least four metabolites, but none of them has a pharmacological effect. These are conjugated with glucuronic acid and, thereafter, sulfonated. About 80% of the drug is eliminated through urinary excretion whereas a small amount of metabolites are excreted by faecal excretion (4).

Since CYP1A2 is the main enzyme responsible for the metabolism of agomelatine, variants in its coding gene could affect its disposition. Indeed, *CYP1A2**1C (rs2069514) has been associated with a decreased enzyme activity (9) while *1F (rs762551) and *1B (rs2470890) alleles were associated with a lower plasma concentration of agomelatine (10). Then, it is expected that subjects carrying *CYP1A2**1C allele show

higher agomelatine plasma levels. On the contrary, individuals with either *1F or *1B alleles would show lower levels of agomelatine, due to an enhanced metabolism.

Moreover, although CYP2C9 and CYP2C19 metabolize a significant lower proportion of the drug, they might also have an influence on its disposition. *CYP2C9**2 (rs1799853) and *3 (rs1057910) alleles are commonly related to a decreased enzyme activity (11), as well as *CYP2C19**2 (rs4244285) and *3 (rs4986893) alleles (12). On the contrary, *CYP2C19**17 (rs12248560) is associated with a higher enzyme activity (12).

Additionally, P-glycoprotein (P-gp), encoded by *ABCB1*, is an ATP-dependent efflux pump that exports substances outside the cell (13,14), thus, influencing the absorption and accumulation of several drugs. A particular polymorphism located in *ABCB1*, commonly known as C3435T (rs1045642), has been linked to differences in agomelatine response, suggesting that carriers of the T allele showed a lower response to the drug (15). Moreover, two other polymorphisms, C1236T (rs1128503) and G2677T/A (rs2032582), have been widely studied in the literature, but they have not been related to agomelatine response.

Not only polymorphisms in *CYP1A2* and *ABCB1* have been previously associated with the noticeable interindividual variability of agomelatine, but also its pharmacokinetic profile could be different among races (10).

Thus, this remarkable variability in agomelatine exposure implies the necessity to understand its pharmacogenetic background. Our aim was to evaluate the effect of genetic polymorphisms in metabolizing enzymes and one transporter on the pharmacokinetics and pharmacodynamics of agomelatine in healthy volunteers, in order to elucidate if there is any relevant pharmacogenetic factor affecting the disposition of agomelatine.

MATERIALS AND METHODS

Study population

The study population comprised 36 healthy volunteers from a clinical trial performed in the Clinical Trial Unit of Hospital Universitario de la Princesa (Madrid, Spain). The

protocol complied with the Spanish Legislation in clinical research in humans and was approved by the Research Ethics Committee, authorized by the Spanish Agency of Drugs and performed according to the guidelines of good clinical practice. All subjects provided their written informed consent for the clinical trial and 28 of them for the pharmacogenetic study.

Inclusion criteria were as follows: age 18-55 years, volunteers free from any psychiatric or organic conditions, normal vital signs and electrocardiogram (ECG), normal medical records and physical examination and no clinically significant abnormalities in serology, haematology, biochemistry, and urine test. Exclusion criteria were: subjects who had received pharmacological treatment in the last 15 days or any kind of medication in the 48 hours prior to receiving the study medication, body mass index (BMI) outside the 18.5-30 kg/m^2 range, having donated blood in the previous month, history of sensitivity to any drug, suspected consumption of controlled substances, smokers, daily consumers of alcohol and/or acute alcohol poisoning in the previous week, pregnant or breastfeeding women or subjects with abnormal blood pressure (BP) and pulse.

Study design and procedures

The data from a bioequivalence clinical trial of agomelatine 25 mg was analysed after a single oral dose under fasting conditions. The clinical trial was randomized, open, crossover, replicated, four-period, four-sequence, with a wash-out period of seven days and with blinded determination of agomelatine plasma concentrations. Agomelatine was administered with 200 ml of water. For pharmacokinetic analysis 20 blood samples were obtained between pre-dose and 24 hours post dose. Samples were centrifuged at 4 °C for 10 min. at 3500 rpm (1900 x g). All plasma samples were stored at -20 °C ± 5 °C until shipped to the external analytical laboratory.

Agomelatine plasma concentrations were determined in an external laboratory using high performance liquid chromatography coupled to a tandem mass spectrometry detector (HPLC/MS/MS). The method was validated according to EMA guidelines. The lower limit of quantification for agomelatine was achieved at 50.25 pg/mL. Chromatographic separation was performed on a reversed phase column (Zorbax

Eclipse Plus C18, 3.0 x 50mm, 3.5 µm, from Agilent Technologies). Ammonium formate 2.5mM, formic acid 0.02% prepared in water/acetonitrile (60/40 v/v) was used as the mobile phase. Isocratic separation of agomelatine was done at room temperature and a flow-rate of 0.70 mL/min. Protein precipitation was used as the sample preparation method for agomelatine and its internal standard. The extraction of agomelatine was performed in 100 µL of plasma by adding 50 µL of internal standard for each sample (study sample, calibration standard or quality control). Proteins were precipitated with formic acid 0.1% in acetonitrile.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by non-compartmental method using WinNonlin Professional Edition, version 7.0 (Pharsight Corporation, USA). The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (T_{max}) were obtained directly from raw data. The area under the curve (AUC) was calculated from administration to the last measured concentration (AUC_{0-t}) by linear trapezoidal integration. The total AUC from administration to infinity (AUC_{∞}) was calculated as the sum of AUC_{0-t} and the residual area (Ct divided by k_e, Ct being the last measured concentration and ke the apparent terminal elimination rate, which was estimated by log-linear regression). Half-life ($T_{1/2}$) was calculated by dividing 0.693 by k_e. Total clearance of drug adjusted for bioavailability (Cl/F) was calculated by dividing the dose by AUC_{∞} and adjusting for weight. The volume of distribution adjusted for bioavailability (Vd/F) was calculated as Cl/F divided by ke. AUC and C_{max} were adjusted for dose and weight (AUC/dW and C_{max}/dW, divided by dose/weight ratio) and logarithmically transformed for statistical analysis. For each parameter only the reference formulation (Valdoxan®) was analysed for each individual and, since it was a replicated clinical trial, we considered the mean of the two reference formulation values.

Pharmacodynamic analysis and safety

BP, heart rate (HR) and 12-lead ECG were measured in supine position at pre-dose and 2 hours post-dose. The QT and HR values were calculated automatically by the ECG device. To correct the QT interval (QTc), the Bazett correction formula (16) was used.

According to the Guideline of the International Harmonization Council E14 (17), we considered a QTc interval prolongation an absolute QTc interval greater than 450 milliseconds or a change from baseline in QTc interval greater than 30 milliseconds.

Throughout the study, volunteers were asked about any experienced adverse event (AE). Additionally, those AEs that were spontaneously notified by the volunteers were documented. Causality was determined using the Karch and Lasagna criteria (18), according to five types of AE: definite, probable, possible, unlikely and unrelated. Only definite, probable or possible AEs were considered as adverse drug reactions (ADRs) and included in the statistical analysis. Time sequence, intensity and outcome of AEs were also recorded.

Genotyping

DNA was extracted from 1 mL of peripheral blood samples using a DNA automatic extractor (MagNa Pure® System, Roche Applied Science, Indianapolis, IN, USA) and quantified spectrophotometrically in NanoDrop® ND-1000 (Wilmington, Delaware). The 260/280 absorbance ratio was used to measure the purity of the samples.

All polymorphisms analysed were selected given the pharmacokinetic and pharmacodynamic parameters of agomelatine. *CYP2C9**2 and *3 and *CYP2C19**2, *3 and *17 polymorphisms were studied by real-time PCR using the LightCyler® 2.0 instrument (RocheDiagnostics, Mannheim, Germany). A set of primers and probes were designed by TIB MOLBIOL (Berlin, Germany) for this purpose. *ABCB1* (C3435T, C1236T and G2677T/A) and *CYP1A2**1C, *1F and *1B polymorphisms were genotyped using a StepOnePlus™ PCR instrument (Applied BiosystemsStepOne™ Real-Time PCR System, Forest City California) using TaqMan probes.

Statistical analysis

To simplify the analysis, *CYP2C9* and *CYP2C19* genotypes were classified according to the number of functional alleles in poor metabolizers (PM, carriers of two defective alleles), intermediate metabolizers (IM, carriers of one defective allele), normal

metabolizers (NM, carriers of two functional alleles) and rapid metabolizers (RM *CYP2C19* *17 carriers) (19).

Moreover, there is no functionality table regarding the activity of *CYP1A2* alleles that makes it easy to infer a phenotype. For that purpose, *CYP1A2* alleles were assigned an activity score based on their functionality, as table 1 shows. This activity score was after translated into a comprehensive phenotype to simplify the gene association analysis.

Statistical analysis was performed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at p values lower than 0.05. The Hardy-Weinberg equilibrium was estimated for all analysed variants. Balance deviations were detected by comparing the frequencies observed and expected using a Fisher exact test based on the De Finetti program (20). Differences in genotype frequencies according to sex and ethnic groups were determined using a corrected Pearson chi-square test. Differences in pharmacokinetic parameters between individuals with different sex, ethnic groups and genotypes were analysed by univariate parametric analysis (t-test or ANOVA). The correlation of AUC and C_{max} with changes in BP, QTc and HR was analysed by linear regression. Multiple linear regression models were used to study factors related to all the pharmacokinetic and pharmacodynamic dependent variables. For this purpose, variables with more than two categories, such as polymorphisms, were analysed using dummy variables.

RESULTS

Demographic and genotypic characteristics

Thirty-six healthy volunteers (19 men and 17 women) were included in the study. The mean age was 26.7 ± 7.1 years for men and 28.3 ± 8.5 years for women. Men were taller than women (1.74 ± 0.06 m vs. 1.63 ± 0.05 m, $p < 0.001$), weighed more (72.1 ± 10.6 kg vs. 59.8 ± 6.9 kg, $p < 0.001$) but exhibited a similar BMI (23.8 ± 2.9 kg/m^2 vs. 22.3 ± 2.6 kg/m^2 for women, p=0.106). Thirty subjects were Caucasians, 5 were Latin and 1 was Black.

From those, 28 healthy volunteers (16 men and 12 women, 22 Caucasian, 5 Latin and 1 Black) gave their written informed consent for the pharmacogenetic study. Table 2 shows genotypic frequencies according to sex and race. All the genetic variants were in Hardy-Weinberg equilibrium ($p > 0.05$), except for *ABCB1* C1236T.

No differences were observed between men and women. However, when stratifying individuals by ethnic group, some differences were observed in the main metabolizing enzyme, as *CYP1A2**1B allele frequency was lower in Caucasians (0.07) than in Latin (0.70) ($p=0.001$). Moreover, *CYP1A2**1F had an allele frequency of 0.32 in Caucasians but 0.0 in Latin ($p=0.060$). Finally, *CYP1A2**1B allele frequency was 0.6 in Caucasians but 0.2 in Latin ($p=0.056$). In relation to allele frequencies, after inferring phenotypes we observed that *CYP1A2* phenotype frequencies were statistically different among races ($p=0.002$), since all the individuals considered *CYP1A2* PM/IM were Latin and all individuals considered *CYP1A2* UM were Caucasian.

Pharmacokinetic analysis

Mean and standard deviation (SD) of pharmacokinetic parameters are shown in table 3. Agomelatine pharmacokinetic parameters were not affected by sex. However, when stratifying individuals by ethnicity, we observed a significantly higher Vd/F and Cl/F in Caucasians compared to Latin ($P<0.05$). Additionally, although not significant, Caucasians exhibited lower AUC and C_{max} (table 3). However, after correction for other covariates, such as *CYP1A2* phenotype, the multivariate analysis indicated that there was no association between race and any pharmacokinetic parameter (table 5).

The univariate analysis revealed an association between some pharmacokinetic parameters and polymorphisms in *CYP1A2*, *CYP2C9* and *ABCB1* (table 4). Although there were some tendencies not statistically significant regarding the influence of *CYP1A2* alleles alone, when analysing them in an inferred phenotype we observed that UM showed a significant lower AUC and higher Vd/F and CL/F (figure 1). The differences in AUC, Vd/F and Cl/F were confirmed in multivariate analysis after correction for other covariates (table 5). Moreover, we found that *CYP1A2* PM/IM had a significantly C_{max} (table 5).

Likewise, when analysing the pharmacokinetic values stratified by ethnicity, *CYP2C9* IM/PM showed a lower Cl/F compared to NM in Caucasians. Indeed, in the multivariate analysis, including race, CYP1A2 and sex as covariates, *CYP2C9* IM/PM showed a significant relationship towards a higher AUC and C_{max} (table 5). On the contrary, we found no significant association between polymorphisms in *CYP2C19* and agomelatine pharmacokinetics.

Regarding the transporter P-gp, we observed a significantly higher T_{max} in carriers of *ABCB1* G2677T/A A/A or A/T genotype (table 4), which continued to be statistically significant after multivariate correction (table 5). Moreover, when stratifying into races, *ABCB1* C3435T T/T and G2677T/A A/A or A/T carriers showed a significantly higher T_{max} in Caucasians.

Pharmacodynamic analysis

In relation to sex, women showed lower BP, which is a well-known aspect. Moreover, agomelatine did not produce a significant change in BP, HR and QTc. There was one subject with a prolonged QTc (an increase of 30.5 ms from baseline) at 2 hours postdose, which was not considered clinically relevant. According to European Medicines Agency criteria (21), an increase of 60 ms from the initial QTc interval is considered a risk of a potential fatal ventricular tachyarrhythmia, also known as torsade de pointes. No volunteer experienced this increase.

Regarding the safety profile, only one individual experienced one AE considered possibly related to agomelatine, which was abdominal pain of mild-moderate intensity. This ADR has been previously described in agomelatine drug label.

DISCUSSION

Agomelatine is an antidepressant that has demonstrated non-inferior effectiveness as compared to SSRIs or SNRIs (22,23) and has a better tolerability profile for sleep or sexual disorders (24,25). Given the limited efficacy of antidepressants, pharmacogenetic studies are crucial to determine markers that can predict treatment failure. In the case of agomelatine, whose pharmacokinetic features are extremely

variable (26), not many studies analysing its pharmacogenetics background have been performed as it is a relatively new antidepressant.

Agomelatine pharmacokinetics

In our study, consistent with the previously reported, agomelatine pharmacokinetic parameters varied widely among individuals, possibly due to its extensive first-pass metabolism and a large Vd with high lipophilicity (8). However, since agomelatine present a wide therapeutic window and an excellent safety profile, its high inter and intraindividual variability may not affect its use in the clinic (8).

The agomelatine pharmacokinetic parameters that we obtained were different from those found in other bioequivalence trial conducted in healthy Chinese subjects (8). It has been previously stated that agomelatine pharmacokinetic profile could be different among races (10), which was also shown in our study as Caucasians showed an approximately 50% less AUC compared to Latin, however, no association was found after correction for the studied genotypes. Thus, these apparently marked differences were due to the different *CYP1A2* genotype distribution among the two groups. However, all CYP1A2 allele frequencies were similar to those reported on 1000 genome database for each ethnic group (27).

Besides, as CYP1A2 isoenzyme plays a critical role in the hepatic metabolism of agomelatine, variations in its activity by any inducing or inhibitory factor could affect agomelatine pharmacokinetics and increase its variability. In our study, each polymorphism itself was not capable of predicting any significant change in agomelatine pharmacokinetic parameters maybe because of the small sample size. However, we demonstrated that a *CYP1A2* phenotype inferred from the presence of both inactivating and inducible polymorphisms predict agomelatine disposition. Compatible with the expected, we observed that individuals with a lower metabolism (PM/IM), carriers of *CYP1A2**1C, had a lower Cl/F and, consequently, accumulate higher concentrations of agomelatine. However, our results contradict the ones of Song *et al*., who performed a study in Chinese healthy volunteers, and did not find any difference in the pharmacokinetic parameters of *CYP1A2**1C carriers compared to the wild-type (10). On the other hand, in our study, individuals with a high inducibility

(UM), carriers of *CYP1A2**1F or *1B, showed an extensive Cl/F and lower concentrations of agomelatine. This is consistent with the study carried out by Song *et al*. where they found that carriers of *CYP1A2**1F and *1B presented a significantly lower level of agomelatine exposure (AUC, C_{max}) (10). Thus, *CYP1A2* phenotype might be a potential predictor of agomelatine exposure.

Further approaches need a larger sample size to better calculate a dose adjustment and demonstrate if it is a useful tool to reach a better response. However, according to these preliminary results, we propose to genotype *CYP1A2**1C, *1F and *1B and combine the genotyping results into a phenotype to predict agomelatine pharmacokinetic parameters.

Regarding *CYP2C9* phenotype, it was found that IM/PM Caucasian subjects showed a lower agomelatine Cl/F compared to NM, as they have a lower enzyme activity. Moreover, in the multivariate analysis we found that IM/PM showed a significantly higher AUC and C_{max}, consistent with the expected. Since CYP2C9 and CYP2C19 play a minor role in agomelatine metabolism, this finding could be irrelevant. Further research is needed to confirm if there is any association between polymorphisms in *CYP2C9* and *CYP2C19* and agomelatine pharmacokinetics.

As regards to *ABCB1*, we found that C3435T and G2677T/A affect agomelatine T_{max} in Caucasian individuals. Indeed, after multivariate analysis corrected by race, sex and polymorphisms, G2677T/A continued to be a significant factor affecting T_{max}, as subjects carrying A/A or A/T genotype showed higher values. The influence of *ABCB1* C3435T polymorphism on antidepressants disposition has been widely studied and has arisen contradictory results (28). As our group has previously reviewed, *ABCB1* C3435T can affect the elimination of some drugs in different ways: an enhanced elimination has been found in some antipsychotics as risperidone and dehydro-aripiprazole while a diminished elimination was found in olanzapine and citalopram (28). *ABCB1* C3435T, which is a synonymous variant, is in partial linkage disequilibrium with G2677T/A, which is a missense polymorphism. Thus, is it more likely that G2677T/A is the responsible polymorphism affecting the transporter activity. The fact that individuals carrying A/A or A/T genotype showed a higher T_{max}, could mean that this

polymorphism enhance P-gp activity, as they show a lower drug concentration and thus it takes more time to reach the C_{max}. Further studies with sufficient statistical power are needed to determine the clinical relevance of *ABCB1* polymorphisms in agomelatine treatment.

Study limitations

The study was performed after single-dose administration to healthy subjects, which prevents us from assessing long-term effectiveness and safety. Agomelatine pharmacokinetics and pharmacodynamics might vary in depressive patients receiving chronic treatment. However, a single-dose design in healthy subjects can assess the effect of genetic polymorphisms over agomelatine without other confounding factors such as smoking or concomitant treatment. As this is an exploratory study, it is of importance that these results are interpreted with caution given the small sample size. Larger studies are needed to increase the statistical power of these results.

CONCLUSIONS

CYP1A2 activity score is directly correlated with agomelatine pharmacokinetic parameters. Thus, *CYP1A2* phenotype inferred from the genotyping of CYP1A2*1C, *1F and *1B alleles might be a potential predictor of agomelatine exposure. Based on this activity score, individuals with a slower metabolism had a lower Cl/F and, consequently, accumulate higher concentrations of agomelatine. On the other hand, individuals with a high CYP1A2 inducibility showed an extensive Cl/F and lower concentrations of agomelatine. In addition, individuals *CYP2C9* IM/PM showed a significantly higher AUC and $C_{max;}$ however, no association was found between CYP2C19 phenotype and agomelatine pharmacokinetics. Regarding the transporter P-gp, ABCB1 G2677T/A polymorphism was a significant factor affecting T_{max}, as subjects carrying A/A or A/T genotype showed higher values, which could mean that this polymorphism enhance P-gp activity, as they show a lower drug concentration and thus it takes more time to reach the C_{max}. Agomelatine did not produce a significant change in blood pressure, heart rate or corrected QT interval.

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Table 1. CYP1A2 alleles proposed activity score and inferred phenotype

CYP1A2 allele	Value assigned to the allele		Activity score	Inferred phenotype
*1	1		1-1.5	PM
*1C	0.5		1.75-2.5	NM/RM
*1F	1.5		2.75-3	UM
*1B	1.25			

The activity score for a genotype is calculated as the sum of the values assigned to each allele (e.g. *CYP1A2* *1C/*1C genotype has an activity score of 1, considered then a PM). Abbreviation: PM, poor metabolizer; NM, normal metabolizers; RM, rapid metabolizers; UM, ultra-rapid metabolizers.

Table 2. Genotype frequencies of enzymes and the transporter in the study subjects, stratified by sex and races.

Genotype	Total	Sex		Races		
		Men	Women	Caucasian	Latin	Black
CYP1A2*1C	N=28	n=16	n=12	n=22	n=5	n=1[α]
*1/*1	19 (67.9)	12 (75.0)	7 (58.3)	19 (86.4)	0 (0.0)	0 (0.0)
*1/*1C	7 (25.0)	2 (12.5)	5 (41.7)	3 (13.6)	3 (60.3)	1 (100.0)
*1C/*1C	2 (7.1)	2 (12.5)	0 (0.0)	0 (0.0) *	2 (40.0)	0 (0.0)
CYP1A2*1F						
*1/*1	16 (57.1)	8 (50.0)	8 (66.7)	11 (50.0)	5 (100.0)	0 (0.0)
*1/*1F	9 (32.1)	7 (43.8)	2 (16.7)	8 (36.4)	0 (0.0)	1 (100.0)
*1F/*1F	3 (10.7)	1 (6.3)	2 (16.7)	3 (13.6)	0 (0.0)	0 (0.0)
CYP1A2*1B						
*1/*1	7 (25.0)	4 (25.0)	3 (25.0)	3 (13.6)	3 (60.0)	1 (100.0)
*1/*1B	14 (50.0)	8 (50.0)	6 (50.0)	12 (54.5)	2 (40.0)	0 (0.0)
*1B/*1B	7 (25.0)	4 (25.0)	3 (25.0)	7 (31.8) *	0 (0.0)	0 (0.0)
CYP1A2 phenotype						
PM/IM	3 (10.7)	2 (12.5)	1 (8.3)	0 (0.0)	3 (60.0)	0 (0.0)
NM/RM	15 (53.6)	8 (50.0)	7 (58.3)	12 (54.5)	2 (40.0)	1 (100.0)
UM	10 (35.7)	6 (37.5)	4 (33.3)	10 (45.5)*	0 (0.0)	0 (0.0)
CYP2C9 phenotype						
NM	16 (57.1)	9 (56.3)	7 (58.3)	11 (50.0)	4 (80.0)	1 (100.0)
IM	11 (39.2)	6 (37.5)	5 (41.7)	10 (45.5)	1 (20.0)	0 (0.0)
PM	1 (3.7)	1 (6.3)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
CYP2C19 phenotype						
NM	14 (50.0)	9 (56.3)	5 (41.7)	11 (50.0)	3 (60.0)	0 (0.0)
IM	7 (25.0)	3 (18.7)	4 (33.3)	6 (27.3)	1 (20.0)	0 (0.0)
PM	1 (3.7)	0 (0.0)	1 (8.3)	1 (4.5)	0 (0.0)	0 (0.0)
RM	6 (21.4)	4 (25.0)	2 (16.7)	4 (18.1)	1 (20.0)	1 (100.0)
ABCB1 C3435T						
C/C	8 (28.6)	4 (25.0)	4 (33.3)	6 (27.3)	1 (20.0)	1 (100.0)
C/T	18 (64.3)	11 (68.8)	17 (58.3)	15 (68.2)	3 (60.0)	0 (0.0)
T/T	2 (7.1)	1 (6.3)	1 (8.3)	1 (4.5)	1 (20.0)	0 (0.0)
ABCB1 C1236T						
C/C	9 (32.1)	6 (37.5)	3 (25.0)	7 (31.8)	1 (20.0)	1 (100.0)
C/T	18 (64.3)	9 (56.3)	9 (75.0)	14 (63.3)	3 (60.0)	0 (0.0)
T/T	1 (3.7)	1 (6.3)	0 (0.0)	1 (4.5)	1 (20.0)	0 (0.0)
ABCB1 G2677T/A						
C/C	10 (35.7)	6 (37.5)	4 (33.3)	8 (36.4)	1 (20.0)	1 (100.0)
C/A	16 (57.1)	8 (50.0)	8 (66.7)	13 (59.0)	3 (60.0)	0 (0.0)
A/A	1 (3.7)	1 (6.3)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)
A/T	1 (3.7)	1 (6.3)	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)

Values are expressed as number of individuals (%). Abbreviation: PM, poor metabolizer; NM, normal metabolizers; IM, intermediate metabolizers; RM, rapid metabolizers.

* $P < 0.05$ compared to Latin [α]This subject was excluded from the chi-square analysis

Table 3. Pharmacokinetics parameters of agomelatine after a single oral dose of 25 mg.

Pharmacokinetic parameter	Total (N=36)	Men (n=19)	Women (n=17)	P-value	Caucasian (n=30)	Latin (n=5)	Black (n=1)[α]	P-value
AUC (ng·h/mL)	261.2 (230.4)	236.9 (201.0)	288.4 (263.1)	0.590	223.5 (218.0)	459.2 (235.3)	403.9	0.033
AUC/dW (ng·h·mg/mL·kg)	684.3 (594.7)	665.4 (527.9)	705.4(677.7)	0.981	587.8 (553.4)	1129.5 (666.7)	1353.9	0.060
C_{max} (ng/mL)	211.3 (220.1)	176.5 (142.7)	250.2 (282.9)	0.633	182.6 (212.3)	385.1 (230.8)	203.8	0.060
C_{max}/dW (ng·mg/mL·kg)	548.5 (546.2)	492.0 (363.2)	611.6 (704.3)	0.950	475.5 (518.1)	959.5 (639.0)	683.3	0.100
T_{max} (h)	1.2 (0.5)	1.2 (0.5)	1.2 (0.5)	0.967	1.3 (0.6)	1.0 (0.2)	1.0	0.262
$T_{1/2}$ (h)	0.9 (0.1)	0.9 (0.2)	0.9 (0.1)	0.788	0.9 (0.2)	0.9 (0.2)	1.0	0.696
Vd/F (L/kg)	52.8 (62.4)	50.4 (59.0)	55.4 (67.8)	0.699	59.6 (66.2)	19.2 (15.7)	15.9	0.045
Cl/F (L/h·kg)	38.6 (41.3)	36.0 (38.0)	41.5 (45.7)	0.616	43.6 (43.4)	14.2 (11.7)	9.9	0.028

Values are shown as mean (SD). Abbreviation: AUC, area under the curve; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration; $T_{1/2}$, Half-life; Cl/F, Total drug clearance adjusted for bioavailability; Vd/F, volume of distribution adjusted for bioavailability; dW, adjusted for dose and weight.

[α]This subject was excluded from the ANOVA analysis

Table 4. Association between agomelatine pharmacokinetic parameters and polymorphism in the studied enzymes and transporter.

Gene	n	AUC (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	Vd/F (L/kg)	Cl/F (L/h·kg)
CYP1A2*1C							
*1/*1	19	214.3 (226.0)	185.0 (245.1)	1.2 (0.5)	0.9 (0.2)	57.6 (56.2)	42.6 (36.0)
*1/*1C	7	320.8 (255.2)	255.9 (244.2)	1.2 (0.7)	1.0 (0.1)	52.6 (76.4)	37.1 (53.4)
*1C/*1C	2	426.3 (184.5)	333.9 (70.1)	1.2 (0.0)	0.8 (0.2)	15.7 (10.5)	12.4 (5.2)
CYP1A2*1F							
*1/*1	16	289.1 (210.6)	235.9 (182.3)	1.2 (0.6)	0.9 (0.2)	39.2 (51.5)	29.3 (37.0)
*1/*1F	9	241.6 (298.6)	218.0 (341.9)	1.1 (0.5)	0.9 (0.2)	68.5 (60.4)	50.8 (41.6)
*1F/*1F	3	123.3 (84.2)	79.5 (49.9)	1.3 (0.5)	0.9 (0.4)	84.2 (95.2)	56.0 (45.6)
CYP1A2*1B							
*1/*1	7	338.7 (248.9)	241.4 (192.2)	1.1 (0.4)	0.9 (0.3)	44.3 (66.6)	29.7 (36.2)
*1/*1B	14	248.5 (274.4)	223.3 (302.8)	1.1 (0.6)	0.9 (0.2)	67.9 (68.3)	50.0 (47.2)
*1B/*1B	7	188.4 (75.3)	165.5 (103.8)	1.4 (0.6)	0.9 (0.2)	33.8 (16.5)	27.0 (18.9)
CYP1A2 phenotype							
PM/IM	3	532.3 (225.3)	415.9 (150.4)	1.0 (0.3)	1.0 (0.3)	13.8 (8.2)	10.0 (5.6)
NM/RM	15	238.9 (164.9)	186.7 (157.8)	1.3 (0.6)	0.9 (0.1)	41.5 (52.5)	31.2 (37.6)
UM	10	198.9 (282.2)*	192.6 (328.2)	1.1 (0.5)	0.9 (0.3)	83.4 (67.2)*	59.6 (40.3)*
CYP2C9 phenotype							
NM	16	264.6 (275.1)	219.9 (283.5)	1.1 (0.4)	0.9 (0.2)	67.7 (73.7)	47.8 (46.5)
IM/PM	12	244.6 (172.5)	204.6 (163.4)	1.4 (0.7)	0.9 (0.2)	34.5 (23.5)	27.4 (25.0)§
CYP2C19 phenotype							
NM	14	281.0 (277.0)	239.6 (292.9)	1.2 (0.6)	0.9 (0.2)	40.9 (26.2)	31.6 (24.7)
IM/PM	7	276.4 (227.7)	244.4 (2111.7)	1.1 (0.4)	0.8 (0.2)	53.7 (72.2)	41.0 (46.1)
RM	7	185.9 (135.6)	129.9 (92.9)	1.3 (0.5)	1.0 (0.2)	78.3 (89.8)	52.1 (56.9)
ABCB1 C3435T							
C/C	8	256.6 (234.1)	196.4 (206.2)	1.0 (0.1)	1.0 (0.2)	78.1 (88.8)	53.9 (59.7)
C/T	18	267.7 (249.3)	235.0 (261.1)	1.3 (0.5)	0.9 (0.2)	44.3 (44.0)	33.7 (29.3)
T/T	2	149.3 (0.7)	86.8 (44.5)	1.8 (1.2)§	0.9 (0.0)	37.5 (10.7)	28.0 (8.1)
ABCB1 C1236T							
C/C	9	256.2 (215.0)	211.1 (188.1)	1.0 (0.2)	1.0 (0.2)	53.8 (59.0)	35.4 (34.2)
C/T+T/T	19	256.0 (246.6)	214.5 (260.1)	1.3 (0.6)	0.9 (0.2)	53.3 (61.2)	40.8 (42.6)
ABCB1 G2677T/A							
C/C	10	233.4 (215.2)	192.9 (186.5)	1.0 (0.2)	1.0 (0.2)	72.6 (80.6)	50.2 (54.2)
C/A	16	274.4 (261.4)	227.7 (278.9)	1.3 (0.5)	0.9 (0.2)	44.8 (44.9)	34.6 (29.8)
A/A+A/T	2	222.3 (103.9)	201.3 (117.3)	1.9 (1.0)* §	0.9 (0.0)	26.6 (4.8)	19.2 (4.4)

Values are shown as mean (SD). Abbreviation: Values are shown as mean (SD). Abbreviation: AUC, area under the curve; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration; $T_{1/2}$, Half-life; Cl/F, Total drug clearance adjusted for bioavailability; Vd/F, volume of distribution adjusted for bioavailability;

PM, poor metabolizer; NM, normal metabolizers; IM, intermediate metabolizers; RM, rapid metabolizers; UM, ultra-rapid metabolizers.

* $p<0.05$

§ $p<0.05$ in Caucasians

Table 5. Results from the multivariate analysis.

	Pharmacokinetic parameters					
Independent variable	AUC (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	Vd/F (L/kg)	Cl/F (L/h·kg)
Sex	---	---	---	---	---	---
Age	β=0.064; p=0.001	β=0.079; p<0.001	---	---	---	---
Race	---	---	---	---	---	---
CYP1A2 PM/IM	β=10577; p=0.004	β=2.055; p<0.001	---	---	---	---
CYP1A2 UM	β=-0.589; p=0.052	---	---	---	β=0.953; p=0.006	β=1.002; p=0.004
CYP2C9 PM/IM	β=0.673; p=0.029	β=0.903; p=0.004	---	---		
CYP2C19 PM/IM	---	---	---	---	---	---
CYP2C19 RM	---	---	---	---	---	---
ABCB1 C3435T C/T	---	---	---	---	---	---
ABCB1 C3435T T/T	---	---	---	---	---	---
ABCB1 C1236T C/T+T/T	---	---	---	---	---	---
ABCB1 G2677T/A C/A	---	---	---	---	---	---
ABCB1 G2677T/A A/A+A/T	---	---	β=0.765; p=0.047	---	---	---
R^2	0.558	0.599	0.144	---	0.258	0.283

Abbreviation: β, non-standardized β coefficient; AUC, area under the curve; C_{max}, maximum plasma concentration; T_{max}, time to reach the maximum plasma concentration; $T_{1/2}$, Half-life; Cl/F, Total drug clearance adjusted for bioavailability; Vd/F, volume of distribution adjusted for bioavailability; PM, poor metabolizer; NM, normal metabolizers; IM, intermediate metabolizers; RM, rapid metabolizers; UM, ultra-rapid metabolizers.

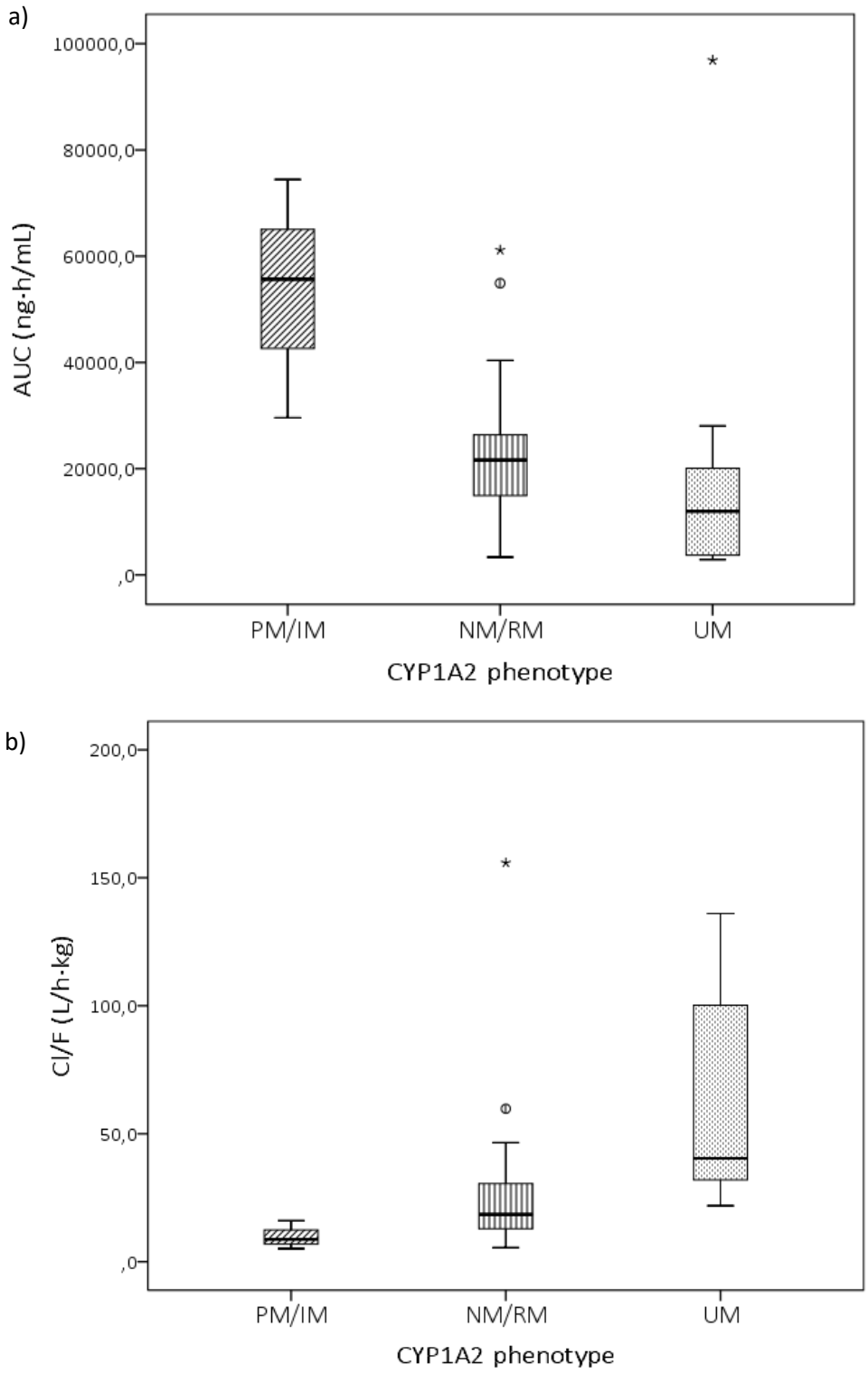

Figure 1. a) Agomelatine AUC in different *CYP1A2* phenotypes. b) Agomelatine Cl/F in different *CYP1A2* phenotypes. Abbreviation: PM, poor metabolizer; NM, normal metabolizers; IM, intermediate metabolizers; RM, rapid metabolizers; UM, ultra-rapid metabolizers. Sample size: PM/IM n=3; NM/RM n=15; UM n=10. The bottom and top of the box represent the first and third quartiles, and the band inside the box corresponds to the second quartile (the median). Whiskers extend to the maximum and minimum values of the series or up to 1.5 times the interquartile range. Outliers and extreme outliers (3 times the interquartile range from the box) are plotted with a circle or a star, respectively.