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Impact of *CYP2C:TG* haplotype on CYP2C19 substrates clearance *in vivo*, protein content and *in vitro* activity

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Abstract

A novel haplotype composed of two non-coding variants, *CYP2C18* NM_000772.3:c.*31T (rs2860840) and NM_000772.2:c.819+2182G (rs11188059), referred to as *"CYP2C:TG",* was recently associated with ultrarapid metabolism of various CYP2C19 substrates. As the underlying mechanism and clinical relevance of this effect remain uncertain, we analyzed existing *in vivo* and *in vitro* data to determine the magnitude of the *CYP2C:TG* haplotype effect. We assessed variability in pharmacokinetics of CYP2C19 substrates, including citalopram, sertraline, voriconazole, omeprazole, pantoprazole and rabeprazole in 222 healthy volunteers receiving one of these six drugs. We also determined its impact on CYP2C8, CYP2C9, CYP2C18 and CYP2C19 protein abundance in 135 human liver tissue samples, and on CYP2C18/CYP2C19 activity *in vitro*

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Author contributions

P.Z. and A.G. wrote the manuscript. P.Z. and A.G. designed the research. P.Z., P.S-C., E.C.B., B.P., J.D., W.Y.W., S.Z., A.R-L. E.G-I., J.S.L., F.A-S., and A.G. performed the research. P.Z. analyzed the data. B.P., J.D., and J.S.L. contributed new reagents/analytical tools.

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SUPPORTING INFORMATION

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using *N*-desmethyl atomoxetine formation. No effects were observed according to *CYP2C:TG* haplotype or to *CYP2C19*1+TG* alleles (i.e., *CYP2C19* alleles containing the *CYP2C:TG* haplotype). In contrast, CYP2C19 intermediate (e.g., *CYP2C19*1/*2*) and poor metabolizers (e.g., *CYP2C19*2/*2*) showed significantly higher exposure *in vivo*, lower CYP2C19 protein abundance in human liver microsomes, and lower activity *in vitro* compared to normal, rapid (i.e., *CYP2C19*1/*17*), and ultrarapid metabolizers (i.e., *CYP2C19*17/*17*). Moreover, a tendency towards lower exposure was observed in ultrarapid metabolizers compared to rapid metabolizers and normal metabolizers. Furthermore, when the *CYP2C19*17* allele was present, CYP2C18 protein abundance was increased suggesting that genetic variation in *CYP2C19* may be relevant to the overall metabolism of certain drugs by regulating not only its expression levels, but also those of CYP2C18. Considering all available data, we conclude that there is insufficient evidence supporting clinical *CYP2C:TG* testing to inform drug therapy.

Keywords

CYP2C:TG; *CYP2C18*; *CYP2C19*; drug exposure; *in vitro* activity; protein abundance

Introduction

Cytochrome P450 2C19 (CYP2C19) plays a principal role in the metabolism of many frequently prescribed medications. Genetic variation of *CYP2C19* contributes to the variability in drug metabolism and response and impacts drug safety and effectiveness. The Pharmacogene Variation Consortium (PharmVar)[1] defines allelic variation of pharmacogenes including *CYP2C19.* PharmVar-defined haplotypes are known as star alleles, which is a nomenclature system widely utilized by the community including the Pharmacogenomics Knowledgebase (PharmGKB)[2,3] and the Clinical Pharmacogenetics Implementation Consortium (CPIC)[4]. CPIC has published several clinical guidelines for CYP2C19 substrates, including clopidogrel, proton pump inhibitors, voriconazole, serotonin selective reuptake inhibitors and tricyclic antidepressants[5–9]. Based on the genotype-informed pharmacogenetic phenotype, therapeutic recommendations are issued. Although *CYP2C19* gene has been extensively characterized in the major population groups, novel variants within the gene may yet be discovered explaining some of the variability between patients.

Recently, Bråten *et al.* reported a novel *CYP2C18* haplotype ("*CYP2C:TG*") which was associated with the ultrarapid metabolism of escitalopram to a similar extent as the well-characterized *CYP2C19*17* allele [10]. This new haplotype is informed by *CYP2C18* rs2860840 T (NM_000772.3:c.*31C>T) and *CYP2C18* rs11188059 G (NM_000772.2:c.819+2182G>A) (Figure 1); this *CYP2C18* haplotype was only found on *CYP2C19*1* alleles. Kee *et al.*, 2022, confirmed that the *CYP2C:TG* haplotype occurs almost exclusively on *CYP2C19*1* alleles, but also on the nonfunctional *CYP2C19*4* allele[11]. Here, we refer to *CYP2C19*1* alleles that contain the *CYP2C:TG* haplotype as **1+TG*, to discriminate these from *CYP2C19*1* alleles without this haplotype (i.e., have *CYP2C:AT* or *GC*) (Figure 1). Moreover, Kee et al. found a higher prevalence of the *CYP2C:TG* haplotype but not *CYP2C19*17* in patients of gastroesophageal reflux disease

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(GERD) with therapeutic failure to omeprazole as compared with a healthy population control cohort. However, these differences were only observed for a subset of 39 patients with confirmed GERD and not in the general population of cases.

The underlying mechanism of how the *CYP2C:TG* haplotype increases the activity of *CYP2C19*1* alleles (**1+TG*) remains unknown. The extent of increased activity conveyed by the *CYP2C:TG* haplotype and its clinical relevance remains uncertain and requires independent confirmation before considering its clinical utility. Although rs2860840 and rs11188059 are in *CYP2C18*, Bråten *et al.* dismissed the possibility of CYP2C18 playing a role in escitalopram metabolism. However, since CYP2C18 participates in the metabolism of several drugs generally considered CYP2C19 substrates[12] a contribution of this enzyme to the metabolism of escitalopram and other CYP2C19 substrates should not be disregarded[12]. A recent editorial discussed possible mechanisms behind this association[12]. Briefly, two different mechanisms were proposed: a) *CYP2C18* is expressed in the liver at low levels and supplements CYP2C19-related metabolic capacity, with this effect being dependent on *CYP2C:TG* haplotype b) the *CYP2C:TG* haplotype influences CYP2C19 expression levels thereby increasing activity through one or more yet unidentified regulatory mechanism(s).

The goal of this work was to investigate the contribution of the *CYP2C:TG* haplotype on CYP2C19 activity *in vivo* and *in vitro*. We examined the impact of *CYP2C18* rs2860840 and rs11188059 genotypes and haplotypes and *CYP2C19* genotype and genotype-predicted phenotypes on the variability of systemic exposure of six CYP2C19 substrates (citalopram, sertraline, voriconazole, omeprazole, pantoprazole and rabeprazole). Furthermore, we assessed the impact of these variants on CYP2C8, CYP2C9, CYP2C18 and CYP2C19 protein abundance in human liver tissue and on *N*-desmethyl atomoxetine (NDA-ATX) formation *in vitro*, which is a measure of CYP2C18 and CYP2C19 activity. *CYP2C18* haplotype information was integrated with *CYP2C19* genotype to evaluate if this combination is a superior predictor of CYP2C19 activity.

Materials and methods

Study participants, samples, and data sets

Pharmacokinetic data of 222 healthy volunteers participating in eight bioequivalence clinical trials investigating several drugs were available for this investigation: pantoprazole, n=60, EUDRA-CT: 2006–001162-17; omeprazole, n=31, 2010–024029-19; rabeprazole, n=35, 2007–002489-37 and 2007–002490-31; citalopram, n=21, EUDRA-CT: not registered, UECHUP code: CIT/02–5; sertraline, n=17, EUDRA-CT: not registered, UECHUP code: SER/02–1; and voriconazole, n=58. EUDRA-CT: 2014–001964-36 and 2014–005342-22. Several pharmacogenetic studies have been published using these data[13–18]. The clinical trials were conducted at the Clinical Trials Unit of Hospital Universitario de La Princesa (UECHUP), Madrid, Spain, according to Spanish and European legislation on research in humans and complied with Good Clinical Practice guidelines and the Declaration of Helsinki. Study protocols were approved by the Hospital's Research Ethics Committee and the Spanish Drugs Agency (AEMPS). All volunteers gave written informed consent to participate in the bioequivalence clinical trial and in pharmacogenetic study. Drugs with the potential for any type of pharmacological interaction with the drug under investigation

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were strictly prohibited by the study protocol to avoid introducing confounding factors while demonstrating bioequivalence. Additionally, the consumption of alcohol, tobacco, as well as any other recreational or illegal drugs, were disallowed. A single oral dose (pantoprazole 40 mg, rabeprazole 20 mg, omeprazole 40 mg, citalopram 40 mg, sertraline 100 mg and voriconazole 200 mg) was administered; blood was collected at different time points (from predose to up to 72 h after dosing) for the quantification of plasma levels and DNA isolation for genotyping. Only the reference formulations data were used for this study. Drug exposure was assessed as the area under the time-concentration curve (AUC); the AUC from pre-dose and the last sample collection time (AUC_t) was obtained by the trapezoidal method. The AUC from pre-dose to infinity ($AUC_{0-\infty}$) was determined by the sum of AUC_t and extrapolated AUC between t and infinite ($AUC_{t-\infty}$, calculated by the C_t/k ratio, where C_t is the concentration at t and k is the elimination slope of the curve).

Liver tissue samples (n=135 pediatric, donor median age, 7 years; range, 0.01–18 years) were procured from the National Institute of Child Health and Human Development (NICHD) Brain and Tissue Bank for Developmental Disorders at the University of Maryland (UMB, Baltimore, MD), the Liver Tissue Cell Distribution System (LCTD) at the University of Minnesota (Minneapolis, MN) and the University of Pittsburgh (Pittsburgh, PA). The use of these samples was approved and determined as nonhuman subject research by the Children's Mercy Kansas City institutional review board. Additional information on these tissue samples has been previously published[19–21].

Genotyping

Volunteers participating in bioequivalence clinical trials and liver tissue samples were genotyped for *CYP2C19*2, *3, *4, *17, CYP2C18* rs2860840 and *CYP2C18* rs11188059. Detailed methods are provided in Supplemental Materials. *CYP2C19* star alleles were defined in accordance with those published by PharmVar[1]. *CYP2C19* genotypes were translated into phenotype according to the CPIC/PharmGKB *CYP2C19* diplotype-phenotype table[22].

Proteomics and in vitro experiments

Protein abundance was measured by quantitative proteomics. Detailed methods are provided in the Supplemental Materials. Table S1 summarizes the chromatographic conditions used to separate surrogate peptides of CYP2C19, CYP2C18, CYP2C9 and CYP2C8; Table S2 provides the Multiple Reaction Monitoring (MRM) parameters for MS/MS analysis of the surrogate peptides.

The formation of NDM-ATX after the incubation with atomoxetine 1, 3 and 10 μM was used in the current investigation as a biomarker of CYP2C18/19 activity (n=116). Dinh *et al.*[23] demonstrated that heterologously expressed CYP2C18 and CYP2C19 have comparable rates of NDM-ATX formation, and therefore microsomal formation will be a function of the total CYP2C18+CYP2C19 content of individual samples. Sample preparation, incubations with ATX and UPLC-MS/MS analysis of metabolite formation are described in detail by Dinh *et al.*[23].

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Statistical analysis

To assess the impact of discrete variables on pantoprazole, rabeprazole, omeprazole, citalopram, sertraline, or voriconazole exposure, a normalized AUC (nAUC) variable was calculated. Individual AUC values were divided by the dose/weight (DW) ratio, yielding the AUC/DW variable, which was subsequently divided by the mean AUC/DW value within each clinical trial yielding the nAUC variable. The Shapiro–Wilk test was used to evaluate the normal distribution of dependent variables. Since AUC/DW and nAUC were not normally distributed, the nonparametric Kruskal–Wallis test was used for statistical inference. For the pairwise comparison of phenotypes or genotypes within the same variable, the Mann–Whitney U test was used. The statistical significance threshold was set at $p<0.05$. The Bonferroni correction for multiple comparisons was applied to control for type-1 error. A similar approach was utilized to evaluate protein abundances and CYP2C18/CYP2C19 activity. Where the median was not informative due to the small sample size, mean data were provided, and parametric tests were used (after logarithmic transformation of the dependent variable and demonstrating its normal distribution). The IBM SPSS software version 28 was used for statistical analysis. The Statistics Kingdom software[24] was used to create the figures.

Results.

Pharmacokinetics of CYP2C19 substrates

Data from 222 healthy volunteers were available, n=109 females and n=113 males, with median ages (quartiles 1–3) of 23 (22–25) and 24 (21–25) years, respectively ($p=0.684$). Ethnicity was self-reported and predominantly European (n=216) followed by Latin-American (n=5); one individual reported one African parent, which was merged with the Latin-American group for statistical analysis (“other” ethnicity, n=6). The following median AUC/DWs were observed for the six investigated drugs: 1632.03 kg*ng/h*ml*mg for voriconazole, n=58; 2740.28 kg*ng/h*ml*mg for citalopram, n=21; 475.90 kg*ng/h*ml*mg for sertraline, n=17; 8267.99 kg*ng/h*ml*mg for pantoprazole, n=60; 2849.71 kg*ng/h*ml*mg for rabeprazole, n=35, and 2359.60 kg*ng/h*ml*mg for omeprazole, n=31; the differences in AUC/DW were statistically significant as they correspond to different drugs ($p<0.001$). In contrast, nAUC values did not differ, confirming adequate normalization (Table S3).

Ethnicity and sex had no effect on nAUC (Table S3). *CYP2C19* genotype (without *CYP2C18* variants) and genotype-informed phenotype were significantly related to nAUC variability Table S3). A 39% statistically significant lower median nAUC was observed in rapid metabolizers (RMs) compared to intermediate metabolizers (IMs) (corrected $p<0.001$), an 78% lower value compared to poor metabolizers (PMs) (corrected $p=0.044$), and a 10% lower value in normal metabolizers (NMs) compared to IMs (corrected $p<0.001$). Similar differences were observed when comparing *CYP2C19* genotypes. In addition, ultrarapid metabolizers (UMs) (*CYP2C19*17/*17*) showed a 9% lower mean nAUC compared to RMs (*CYP2C19*1/*17*) and a 19% lower value compared to NMs (*CYP2C19*1/*1*). However, these differences did not reach statistical significance due to the low number of UMs (n=5). In contrast, the differences between UMs and PMs were large, i.e., UMs showed an 80%

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lower nAUC value compared to PMs, but due to the small sample size, only the level of nominal significance after nonparametric analysis was reached (p=0.011).

CYP2C18 rs2860840 (c.*31C>T) and rs11188059 (c.819+2182G>A) were unrelated to nAUC (Table S3). Although *CYP2C18* genotype (i.e., the combination of rs2860840 and rs11188059) was nominally related to nAUC variability (p=0.033, Table S3), no differences were observed after Bonferroni correction for multiple comparisons. No association (including nominal significance, p=0.202) was observed according to *CYP2C:TG* haplotype (Table S3), nor when it was investigated together with *CYP2C19* genotype, i.e., the impact of the *CYP2C19*1+TG* allele (Figure 2). No differences in the AUC/DW value according to the *CYP2C:TG* haplotype were observed either when analyzing the data of each clinical trial individually (Table S4). Table S5 summarizes the pairwise comparisons of the interrogated genotypes and nAUC (corrected p=0.003). Expectedly, genotypes with one or two *CYP2C19* no function alleles were associated with higher nAUC, but no differences were found among *CYP2C19*1* alleles regardless of whether they had the *CYP2C18 'TG', 'AT'* or '*GC'* haplotype nAUC was comparable for subjects having two *CYP2C19*1* alleles, two **1+TG* alleles or were heterozygous for these. Likewise, there were no statistically significant differences when comparing *CYP2C19*1/*17* with **1+TG/*17* or **17/*17* with **1+TG/*1+TG.* Healthy volunteers with the *CYP2C19*17/*17* genotype (n=5) showed a 20% lower nAUC compared to those with the **1/*17* genotype (n=20) and a 23% lower value compared to **1/*1* participants (n=33), but these differences were not statistically significant due to the relatively small sample size.

Liver tissue protein abundance and *CYP2C19* activity

The study cohort comprised liver tissue samples of 47 females and 87 males, and one unknown sex (n=135) with median ages (quartile 1 and 3) of 7.34 (3.08–15.53) and 5.00 (0.56–13.42) years, respectively (p=0.255). Individuals were identified as European (n=64), African American (n=29), Hispanic (n=4) or Other (n=2) while no data were available for the remaining samples. European donors were significantly older than African American donors (7.71 years [2.25–15.00] vs 2.75 [0.37–8.04] years, corrected p=0.040). The majority of tissue samples (n=74) were obtained from the LCTD while n=61 were obtained from UMB. LCTD donors were significantly older than those obtained from UMB (8.00 years [4.00–14.00] vs 4.56 [0.38–13.93], p=0.013).

Median and first and third quartiles of CYP2C19, CYP2C18, CYP2C8 and CYP2C9 protein abundance are shown in Table S6 which also provides CYP2C19 activity using NDA-ATX formation as a measure of CYP2C19 activity, according to *CYP2C18* genotype, *CYP2C19* genotype (with and without *CYP2C18* haplotypes) and CYP2C19 genotype-predicted phenotype. A trend between age and CYP2C18 protein content (R^2=0.161), but CYP2C19, CYP2C9 and CYP2C8 protein content were unrelated to age. CYP2C19 protein abundance was significantly higher in UMB samples compared to those sourced from LCTD (p=0.019). Variability in CYP2C19 protein abundance was strongly related to *CYP2C19* genotype (without *CYP2C18* information) (Figure 3a, Table S6). Expectedly, these differences were similar according to CYP2C19 phenotype (Table S6). No differences were observed according to *CYP2C18* variants or genotype (Table S6). When evaluating

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the impact of *CYP2C19* genotype including *CYP2C18* haplotype information, statistically significant differences were detected (p=0.002) (Figure 3b). The pairwise comparison revealed significant differences between *CYP2C19** 1/*1* and **2/*2* and **2/*4* genotypes (p=0.014); between **17/*17* and **2/*2* and **2/*4* genotypes (p=0.018), and between **1/*1* and **1/*2* and **1/*35* genotypes (p=0.047). That is, the differences observed were between samples with different *CYP2C19* genotype-informed phenotypes, while *CYP2C19*1+TG* alleles had no impact on CYP2C19 protein content (Table S7). Notably, the *CYP2C19*2/*4* sample had a *CYP2C18 CG*/*TG* genotype. We were unable to determine which *CYP2C19* star allele contained the *CYP2C:TG* haplotype, but based on Kee *et al.*[11], it is likely on the *CYP2C19*4* allele.

CYP2C18 protein abundance was significantly higher in the samples sourced from LCTD (corrected p<0.001). Significant variability in CYP2C18 protein abundance was observed according to CYP2C19 phenotype (p=0.029) (Figure 4); after the pairwise comparison and Bonferroni correction, no differences were observed. A similar trend was observed according to *CYP2C19* genotype (p=0.053) (Table S6). When analyzing CYP2C19+CYP2C18 protein abundance according to CYP2C19 phenotype, statistically significant differences were observed (p<0.001, data not shown), which remained significant after pairwise comparison and multiple comparison correction (PM vs RM, NM, and UM: p=0.006, p=0.003 and p=0.002, respectively; IM vs RM, NM, and UM: p=0.046, p=0.017 and p=0.022, respectively). The same differences were observed for CYP2C19 genotype.

No differences in CYP2C8 and CYP2C9 protein abundance were observed according to any variable, except for CYP2C9 abundance in samples with *CYP2C19*1/*2* and **1/*35* genotypes (IMs), which was lower than that observed for samples with the *CYP2C19*1+TG*/**17* genotype (RMs) (corrected p=0.031) (Table S6).

Samples from LCTD exhibited higher formation rates of NDA-ATX compared to those from UMB (corrected p<0.001) (Table S6). Significant variability in NDA-ATX formation rates was observed according to CYP2C19 phenotype (p=0.017) and *CYP2C19* genotype (p=0.034) after incubation with ATX 10 μM. The pairwise comparisons of subgroups revealed statistically significant differences between samples genotyped as *CYP2C19*2/*2* and **2/*4* vs **1/*1* (corrected p=0.014); vs **17/*17* (corrected p=0.018) and between those genotyped as **1/*2* and **1/*35* vs **1/*1* (corrected p=0.047). No differences were observed according to any other variable. Analyses were repeated with ATX 1 μM and 3 μM incubations and the same results were obtained (data not shown). Because liver tissue source was previously found to be a factor explaining variability[25], analyses were repeated including only LCTD-sourced tissue samples to further evaluate whether the source of donors affects the results. Similar results were observed compared to the overall analysis, with p values increasing in general due to the reduction of the sample size (data not shown).

Discussion

Bråten *et al.*[10] identified the *CYP2C:TG* haplotype by analyzing the GRCh37/hg19, Chr10:96442884–96522439 region in 24 patients with unexplained ultrarapid metabolism of escitalopram. This region includes *CYP2C18* and the *CYP2C18-CYP2C1*9 intergenic

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region (Figure 1). A 2-fold higher frequency of *CYP2C18* rs2860840 and rs11188059 variants was found in these patients[10] compared to their respective frequencies in the 1000 Genomes Project. In their work, authors affirm that *CYP2C:TG* predicts CYP2C19 UM phenotype and dismiss CYP2C18 contributing to metabolism. Interestingly, carriers of the *CYP2C:TG* haplotype showed a higher prevalence of rs34117282 (an indel variant located in the *CYP2C18-CYP2C19* intergenic region), but this variant was not further pursued. Furthermore, the haplotype related to the ultrarapid metabolism of escitalopram was rs2860840 (T) and rs11188059 (G), i.e., *CYP2C:TG*, but not rs2860840 (T) and rs11188059 (A), i.e., *CYP2C:TA*. This is surprising since both alleles (T and A) were apparently more prevalent among the UM-like patients compared to the 1000 Genomes Project prevalence. Nonetheless, since the within *CYP2C:TG* haplotype variability was several-fold greater than the between haplotype variability reported by Bråten *et al.* (same as in this work), it is unlikely that the differences between the groups have a meaningful clinical impact.

Here, no associations were found between the *CYP2C:TG* haplotype or *CYP2C19*1+TG* alleles on the exposure of six CYP2C19 substrates, *in vitro* activity, or protein content on any of the four CYP2C proteins in human liver microsomes. Similarly, the *CYP2C18* variants had no effect on any dependent variable. In contrast, *CYP2C19* genotype, especially nonfunctional alleles, had a significant impact on drug exposure, *in vitro* protein abundance, and activity in liver microsomes, as expected according to published data[26–28].

*CYP2C19*17* had no significant effect on drug exposure in this study either, although a tendency was observed. These results are consistent with Bråten *et al.*[10], who described a modest impact of *CYP2C19*17* while no function alleles had a considerable impact. For instance, patients with *CYP2C19*17/*17* and **1/*17* genotypes essentially displayed identical dose-normalized concentrations of escitalopram (an image interpretation software was used to calculate concentration means and ranges[29]): **17/*17*, 30 nM [7–140 nM] and **1/*17*, 31 nM [7–173 nM]) which were slightly lower (17% and 14%, respectively) compared to those measured for **1/*1* (36 nM [5–161 nM]). In contrast, when *CYP2C19*1/*1* patients were compared with **1/null* and *null/null* genotypes (**1/null*, 54 nM [14–190 nM]; *null/null*, 91 nM [34–184 nM]) drug exposure differed 1.5-fold and 2.5-fold, respectively. Patients with **1+TG/*1+TG* and ** 1/*1+TG* genotypes showed a 22% and 17% lower exposure (**1+TG/*1+TG*, 28 nM [1–144 nM]; **1/*1+TG*, 30 nM [4–121 nM]) compared to those with a **1/*1* genotype (36 nM [5–161 nM]). However, all genotypes exhibited concentrations in the range of 34 and 110 nM, all within the same wide range. Therefore, considering the large within-genotype variability of at least 374%, the mean differences among **1+TG/*1+TG, * 1/*1+TG* and **1/*1* appear negligible. In this case, statistical significance seems to be driven by the relatively large number of samples in the Bråten *et al.* study, which does, however, not necessarily translate into clinical relevance.

CYP2C19 phenotype was significantly related to CYP2C19 protein abundance ($p<0.001$) and nominally to CYP2C18 protein abundance ($p=0.029$) in human liver microsomes. The combination of CYP2C18+CYP2C19 protein abundances was best predicted by CYP2C19 phenotype (compared to CYP2C19 or CYP2C18 individually), increasing the significance. CYP2C18 protein abundance ranges in UMs, RMs and IMs were not different, since

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CYP2C18 is present in some livers and absent in others. Many of the samples show extremely low or null abundance protein, therefore the lower value of the range is identical. However, four out of six CYP21C9 UMs showed high CYP2C18 levels, while in five of six PMs the protein was undetectable. This novel finding and its relevance regarding *in vivo* activity warrant further investigation. Additional research is warranted to determine the impact of *CYP2C18* genetic variation on the enzyme's abundance in other tissues, such as the gastrointestinal track, gallbladder, etc.

Variants unique to the *CYP2C19*17* allele could also affect CYP2C18 protein expression; a concerted increase in CYP2C18 and CYP2C19 protein levels may contribute to the *CYP2C19*17* allele presenting with increased function. Genetic variability of *CYP2C18* has not been systematically explored, but considering that *CYP2C8, CYP2C9* and *CYP2C19* are all highly polymorphic, *CYP2C18* is likely also polymorphic, and its activity may widely range. CYP2C18 has been traditionally excluded from being studied due to Läpple *et al.* who reported 20 years ago that this protein is not expressed[30]. More recent findings provide evidence to the contrary as discussed by Zubiaur & Gaedigk[12]. The proteomics data presented in this report further corroborates the presence of this enzyme in some livers while being absent in others. Taken together, it is plausible that CYP2C18 plays a role in the metabolism of some substrates which have traditionally been attributed to CYP2C19 in some patients. There is clearly a void of information regarding the contribution of CYP2C18 to drug metabolism. To close this knowledge gap, we call upon the scientific community to include this gene in their pharmacogenetics research.

Bråten *et al.* also published a study investigating the impact of the *CYP2C:TG* haplotype on sertraline exposure[31] concluding that "the *CYP2C:TG* haplotype status can prospectively be useful to clinicians in making more appropriate sertraline dosing decisions". However, again, the within *CYP2C:TG* haplotype variability in serum concentrations was several-fold greater than the between haplotype variability. Furthermore, *CYP2C19*1/*1* patients (n=142) showed a mean serum concentration of 72.2 nM/100mg per day while those with **1/*1+TG* (n=222) and *1/*17* (n=286) had concentrations of 71.6 nM/100mg and 59.5 nM/100mg, respectively, which appears to contrast their conclusion that this haplotype should be tested preemptively to guide dosing decisions.

Kee *et al.,* 2022[11] associated the *CYP2C:TG* haplotype with therapeutic failure to omeprazole in patients with GERD. However, this association was only found in a small subset of cases (n=39). Since *CYP2C19*17* is a well-known predictor of omeprazole therapeutic failure and the authors were unable to establish this association, their claims for *CYP2C:TG* are uncertain. Furthermore, a study describing the distribution of *CYP2C* haplotypes in Native American populations was published[32], however, no functional data were available.

Finally, although *CYP2C19*17/*17* individuals are traditionally considered functionally different from **1/*17* individuals (UMs vs. RMs), our data for CYP2C19 substrates suggest that differences in exposure are small and by far not as pronounced as those observed between NMs, IMs and PMs. Furthermore, recommendations in some CPIC guidelines do not differ for RMs and UMs (e.g., voriconazole[6] or sertraline[9]), reflecting the absence of

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literature supporting clinically meaningful differences among these two phenotype groups. In summary, no function alleles have a much greater impact on enzyme activity compared to *CYP2C19*17*. This opens the question of whether a RM phenotype classification is clinically useful or whether it should be merged into the NM group, as the Dutch Pharmacogenetics Working Group do[33].

This work has several limitations and strengths. First, the sample size is small and arbitrary due to the availability of data. As this is an observational study, some associations may not be truly significant while others may have been missed because the methodology employed does not guarantee sufficient statistical power. However, a positive control was employed throughout (i.e., *CYP2C19* genotype or genotype-informed phenotype), and it was repeatedly related to the interrogated dependent variables (e.g., nAUC), thus it can be assumed that there is indeed sufficient statistical power to determine clinically relevant associations. Therefore, unobserved associations may not be interpreted as “not statistically significant”, but as probably “not clinically relevant”. Second, we used multiple pharmacokinetic data sets from clinical trials performed with high methodological rigor, avoiding important confounding factors that are present in patients (e.g., concomitant medications, smoking, etc.), correcting for weight and controlling for several possible confounder factors such as ethnicity. In the Bråten *et al* studies, authors evaluated pharmacokinetic data using a less rigorous methodology, based on point concentrations, without weight correction. Furthermore, the AUC is most informative when the pathway of interest is the sole/major clearance pathway, which is indeed the case for escitalopram but is problematic for citalopram (i.e., the decline in citalopram concentrations is a composite of CYP2C19 effects on the S enantiomer and CYP2D6 on the R-enantiomer) or for voriconazole which is also metabolized by CY3A4 and FMO3. Third, the origin of the liver tissue samples was heterogeneous. However, differences did not vary significantly depending on the origin of the samples.

Conclusions

Consistent with the literature, CYP2C19 IM and PM metabolizer status impacted CYP2C19 drug exposure *in vivo*, protein abundance in human liver microsomes, and *in vitro* activity. In contrast, no effects were observed when C*YP2C19*1* alleles were stratified for the *CYP2C:TG* haplotype, i.e., *CYP2C19*1* alleles with ‘TG’ and those without. Considering all available data, we conclude that there is insufficient evidence supporting the clinical relevance of *CYP2C:TG* haplotype and its testing to inform drug therapy. Lastly, CYP2C18 should not be dismissed as it may contribute to the overall metabolism of some drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Highlights

What is the current knowledge on the topic?

The *"CYP2C:TG"* haplotype was recently identified and related to the ultrarapid metabolism of CYP2C19 substrates. However, the underlying mechanism behind this effect and its clinical relevance remain uncertain.

What question did this study address?

We evaluated the impact of the *CYP2C:TG* haplotype on the pharmacokinetic variability of six CYP2C19 substrates (citalopram, sertraline, voriconazole, omeprazole, pantoprazole and rabeprazole), CYP2C18, CYP2C19, CYP2C8 and CYP2C9 protein abundance in human liver samples and CYP2C18/CYP2C19 activity *in vitro.*

What does this study add to our knowledge?

CYP2C19 genotype was related to drug exposure *in vivo,* CYP2C19 protein abundance, and activity in human liver tissue. We were unable, however, to confirm an association between the *CYP2C:TG* haplotype (i.e., *CYP2C19*1+TG* alleles) and increased CYP2C19 activity. Increased levels of CYP2C18 protein were detected in liver samples with one or two *CYP2C19*17* alleles, suggesting a possible intricate interplay between CYP2C19 and CYP2C18 expression levels and activity.

How might this change clinical pharmacology or translational science?

There is insufficient evidence supporting the clinical relevance of *CYP2C:TG* haplotype and its testing to inform drug therapy. The contribution of variable CYP2C18 expression levels and genetic variation in its gene may not be fully recognized and warrants further investigation.

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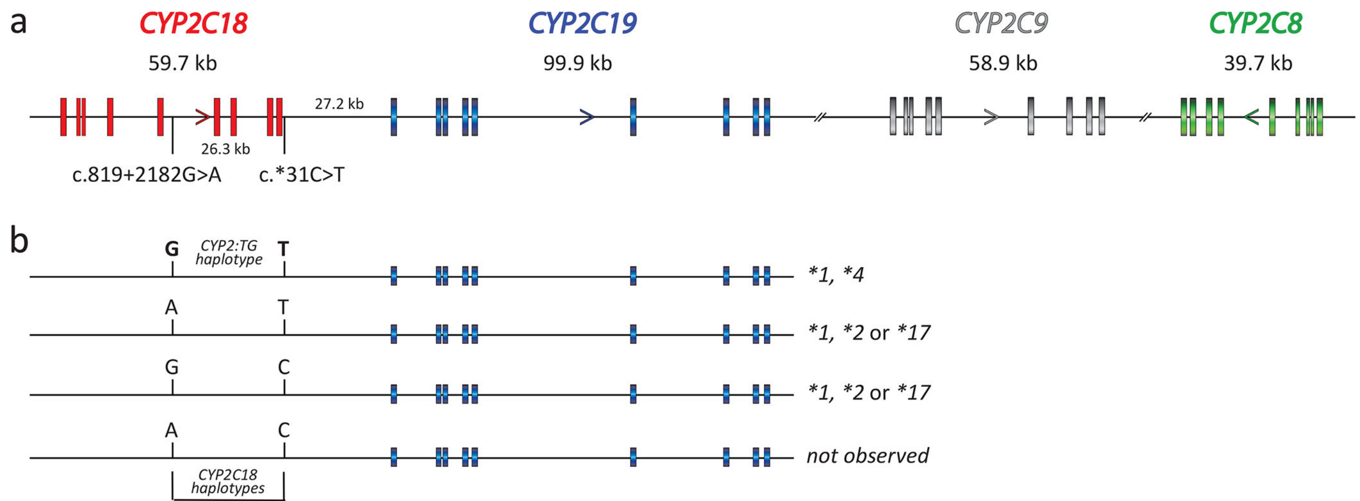

Figure 1. Overview of the *CYP2C* gene locus (a) and *CYP2C:TG* haplotype and its linkage with *CYP2C19* star alleles

(b). Boxes represent exons and arrows indicate whether the gene is encoded on the positive or negative stand. The size of each gene is provided in kilobase pairs (kb); “CYP2C:TG” haplotype refers to the combination of the following genotypes: T at CYP2C18 c.*31C>T (rs2860840) and G at c.819+2182G>A (rs11188059).

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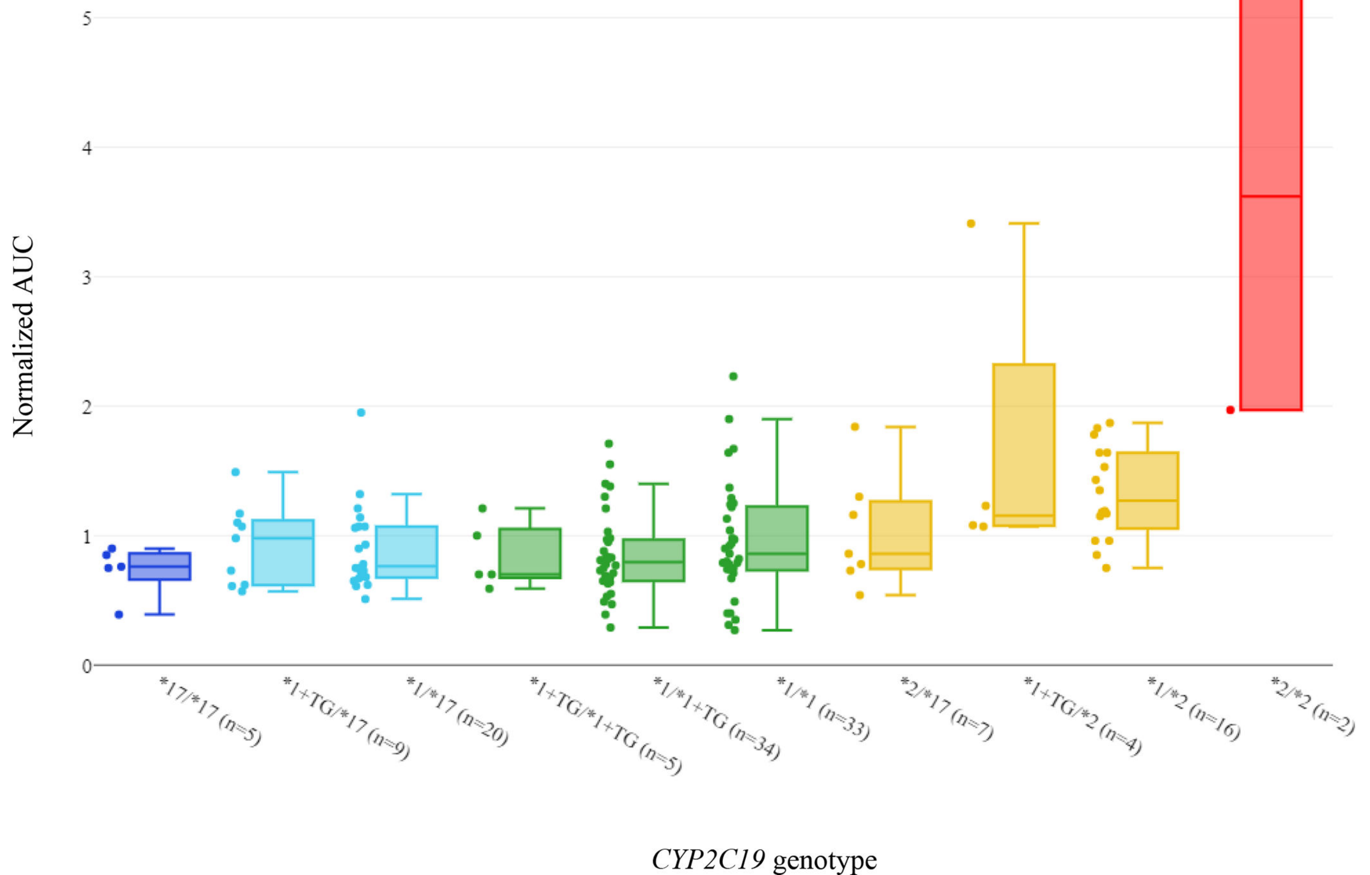

Figure 2. Normalized AUC according to *CYP2C19* genotype and including *CYP2C:TG* haplotype information.

*CYP2C19*1+TG* refers to *CYP2C19*1* alleles with the *CYP2C:TG* haplotype. *CYP2C:TG* haplotype refers to the combination of T at *CYP2C18* c.*31C>T (rs2860840) and G at c.819+2182G>A (rs11188059). *CYP2C19*1* alleles with *CYP2C18 AT* or *GC* haplotypes are referred as *CYP2C19*1.* Statistical inference values are shown in Table S5. Genotypes colored in dark blue correspond to CYP2C19 ultrarapid metabolizers (UMs); light blue denote rapid (RMs), green normal (NMs), yellow intermediate (IMs) and red poor metabolizers (PMs). Points represent real values of nAUC. Box borders and the central line represent quartiles 1, 2 and 3. The whiskers represent the maximum and minimum values outside the q1-q3-range

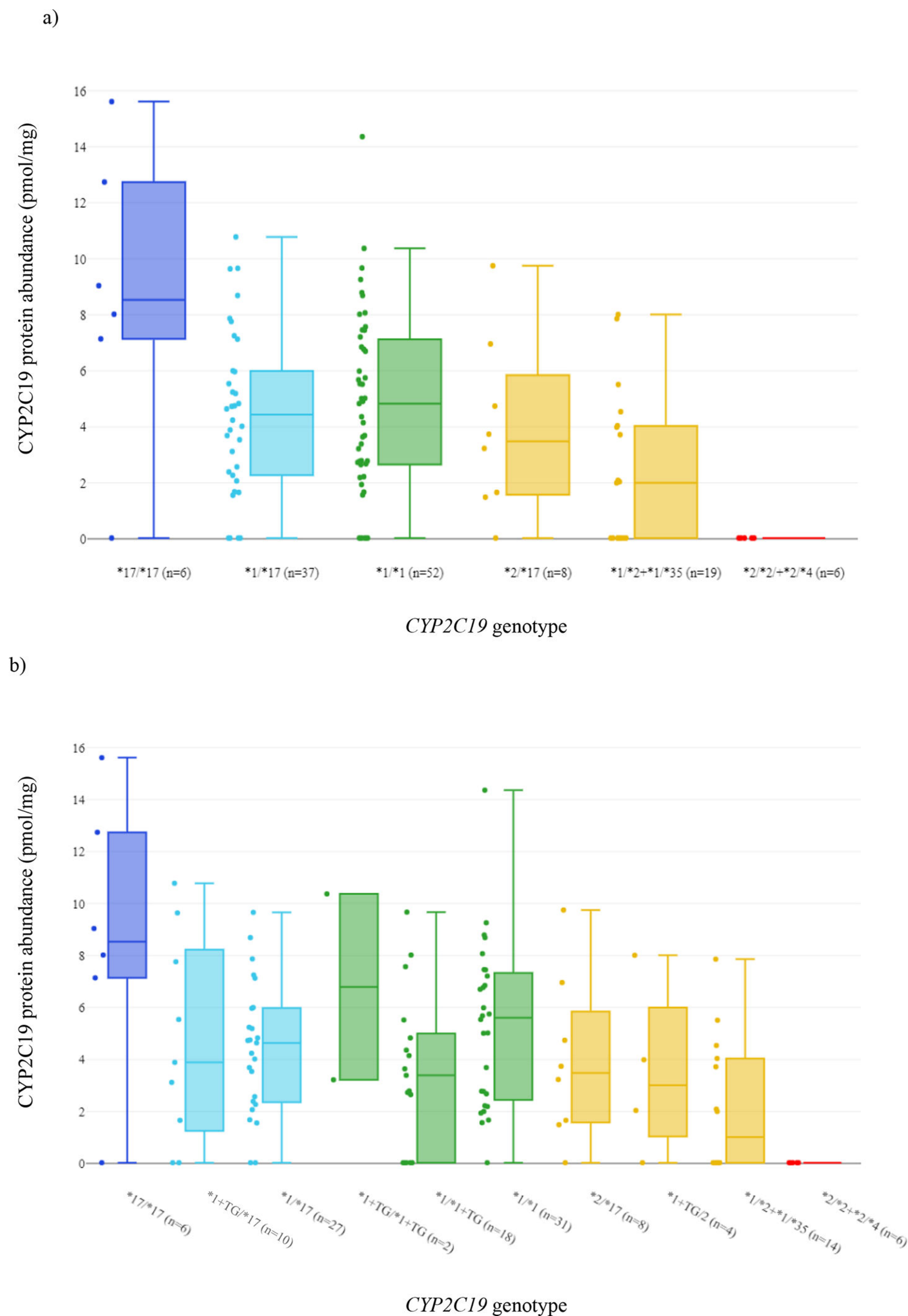

Figure 3. CYP2C19 protein abundance (pmol/mg microsomal protein) according to *CYP2C19* genotype (a) and *CYP2C19* genotype including *CYP2C:TG* haplotype information (b).
CYP2C19 genotype does not comprise *CYP2C18* variants in panel a); *CYP2C19*1* includes *CYP2C19*1+TG* alleles and **1* alleles with *CYP2C18 AT* or *GC* haplotypes. In panel b), *CYP2C19*1+TG* refers to *CYP2C19*1* alleles with the *CYP2C:TG* haplotype; *CYP2C:TG* haplotype refers to the combination of T at *CYP2C18* c.*31C>T (rs2860840) and G at c.819+2182G>A (rs11188059); *CYP2C19*1* alleles with *CYP2C18 AT* or *GC* haplotypes are referred as *CYP2C19*1.* Statistical inference values are shown in Table S7. Genotypes colored in dark blue correspond to CYP2C19 ultrarapid metabolizers (UMs); light blue

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denotes rapid (RMs), green normal (NMs), yellow intermediate (IMs) and red poor metabolizers (PMs). Outliers were excluded from the plot according to the Tukey fences technique. Points represent real values of nAUC. Box borders and the central line represent quartiles 1, 2 and 3. The whiskers represent the maximum and minimum values outside the q1-q3-range

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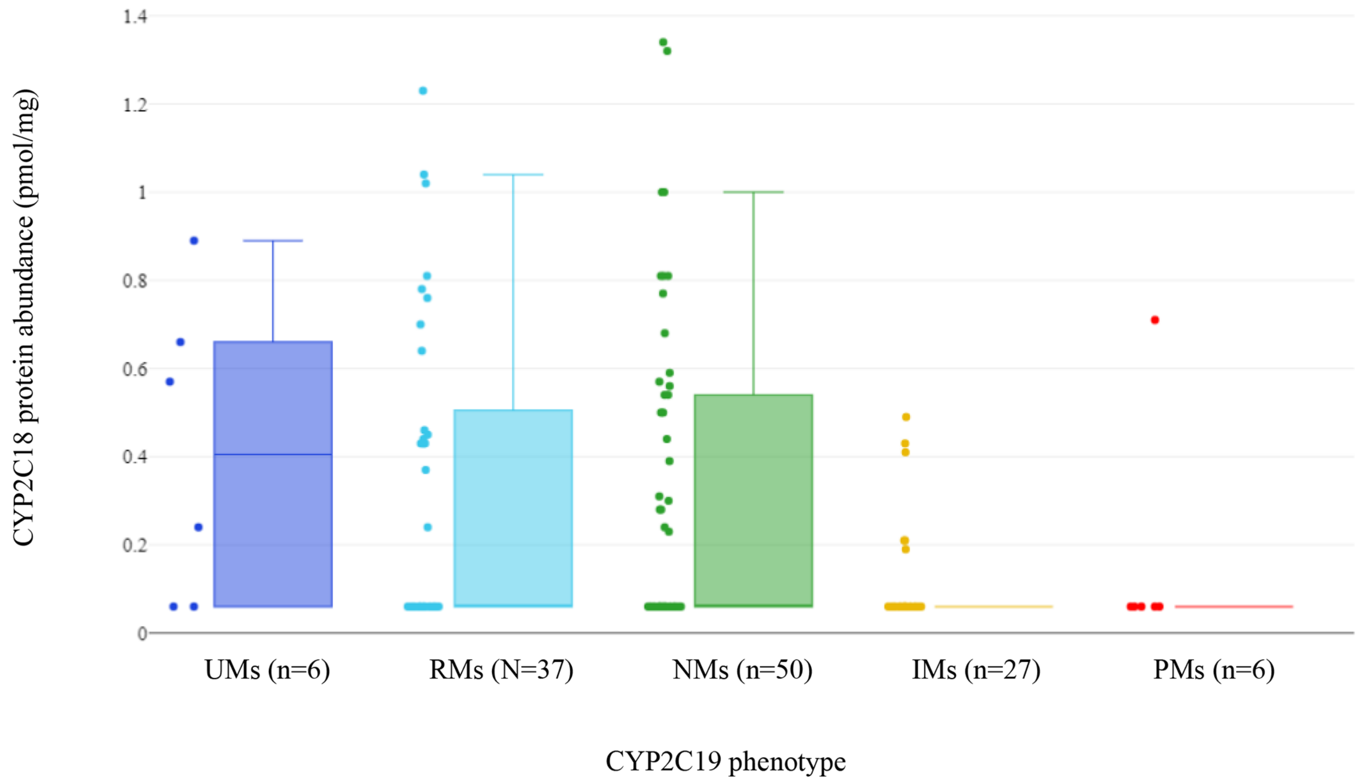

Figure 4. CYP2C18 protein abundance (pmol/mg microsomal protein) according to CYP2C19 phenotype.

CYP2C19 genotype does not comprise *CYP2C18* variants. Statistical inference values are shown in Table S6. Outliers are excluded from the plot according to the Tukey fences technique. Points represent real values of nAUC. Box borders and the central line represent quartiles 1, 2 and 3. The whiskers represent the maximum and minimum values outside the q1-q3-range