Clinical Pharmacokinetics (2025) 64:779–789
https://doi.org/10.1007/s40262-025-01502-7

ORIGINAL RESEARCH ARTICLE

Pharmacometabolomics Detects Various Unreported Metoprolol Metabolites in Urine of (Potential) Living Kidney Donors and Kidney Transplant Recipients

Wietske A. Heddema[1] · Marieke A. J. Hof[2] · Piotr Sosnowski[3] · Stephan J. L. Bakker[4] · Gérard Hopfgartner[5] · Frank Klont[1,6] on behalf of the TransplantLines Investigators

Accepted: 19 March 2025 / Published online: 26 April 2025

Abstract

Background and Objective Metoprolol is primarily metabolized via the polymorphic cytochrome P450-2D6 (CYP2D6) enzyme, which underlies interindividual variation in conversion rates and may benefit from pharmacogenetics-driven therapy personalization. However, the field relies heavily on knowledge of a drug's metabolism, often originating from early-phase clinical trials with single-dose administration in small samples of healthy volunteers. Pharmacogenetics could thus benefit from real-world drug metabolism studies.

Methods We conducted a real-world drug metabolism study for metoprolol in 18 (potential) living kidney donors and 374 kidney transplant recipients from the Transplant Lines Food and Nutrition Biobank and Cohort Study (NCT02811835) using existing liquid chromatography–high resolution mass spectrometry pharmacometabolomic data.

Results In both groups, we confirmed the presence of seven expected metabolites, including the high-abundance substances metoprolol acid and hydroxymetoprolol. We were unable to detect deisopropylmetoprolol and a metabolite known as "H 119/68". However, we did find putative further oxidized forms, namely the expected variant of deisopropylmetoprolol in which the primary amine is removed and the leftover methyl group is oxidized into a carboxylic acid ("H 104/83") and an unknown/unreported metoprolol metabolite that we refer to as "metoprolol benzoic acid". Moreover, we found nine other previously unknown/unreported metabolites, putatively reflecting *N*-glucuronidated metoprolol, four glucuronidated versions of hydroxymetoprolol, and a formylated, a glucuronidated, and two hydroxylated versions of metoprolol acid. Interestingly, the same metabolites were detected in potential living kidney donors and kidney transplant recipients, and metabolite profiles did not differ between both groups in principal component analysis.

Conclusion We found more metoprolol metabolites than previously reported, calling for replication studies and evaluation of pharmacogenetic testing approaches to realize safer, more effective metoprolol therapy.

1 Introduction

With the turn of the century, a new era of personalized medicine emerged, as was heralded by Langreth and Waldholz in *the Wall Street Journal* in 1999 [1]. Since then, healthcare has been moving from "one size fits all" to more individualized treatments, aiming to tailor therapies to an individual's unique characteristics by taking into account biological, lifestyle, environmental, and genetic factors [2]. Regarding the latter, genetics-guided decision-making is becoming more commonplace in clinical practice with the ongoing developments in 'omics' technologies, which aim to improve understanding of disease pathogenesis and associated therapy outcomes [3]. Taking the widely used antihypertensive drug metoprolol as an example, its efficacy and safety profiles can now be optimized based on a person's genetic information, which relates to the field of pharmacogenetics that is increasingly being integrated into routine medical practice [4].

Pharmacogenetics is the study of how a person's genetic makeup influences drug absorption, distribution, metabolism, and excretion (pharmacokinetics) as well as the therapeutic and adverse effects (pharmacodynamics) of a drug [4, 5]. Thereby, pharmacogenetics aims to improve the safety and efficacy of drug treatments [4]. With the availability of

Extended author information available on the last page of the article

Key Points

This study looked at how the widely used cardiovascular drug metoprolol is processed by the human body, focusing on the excretion of breakdown products in the urine of people who donated or received a kidney.

In both groups, researchers found many breakdown products that had not been reported before.

These discoveries could help make metoprolol treatment safer and more effective, particularly by enabling tailoring of therapy to individual patient characteristics.

genetic information, many genetic variants affecting pharmacokinetics and pharmacodynamics have been identified in recent years [6]. Moreover, genetic differences between individuals account for approximately 20–30% of variability in drug responses, and certain variants can lead to toxicity or treatment failure in some patients [7, 8]. Such variation has most commonly been found in genes encoding drug targets, transporters, and drug-metabolizing enzymes, such as the cytochrome P450 (CYP) enzymes, which play a major role in drug metabolism [9].

The CYP superfamily comprises numerous different enzymes, including the CYP2D6 subfamily, which is responsible for the metabolism of about 25% of all drugs [10]. The gene encoding CYP2D6 is highly polymorphic, with more than 100 variant alleles identified to date, and can result in increased or decreased drug conversion rates [11]. These variants occur in different frequencies across populations and between individuals, giving rise to a wide range of CYP2D6 activities and corresponding metabolizer phenotypes [9]. Notably, up to 15% of individuals of European descent are CYP2D6 poor or ultrarapid metabolizers and so are likely to convert drugs at rates that differ from those assumed during drug development when drug dosages were determined [12]. These metabolic effects can have implications for many CYP2D6-metabolized drugs, in both short-term and chronic therapies. For example, in chronic metoprolol use, poor metabolizers may have an increased risk of bradycardia due to overdosing, whereas ultrarapid metabolizers may require higher doses to realize a sufficient effect [11]. In fact, a large multicenter study evaluating (pre-emptive) genotype-guided treatments recently found that one of the highest extents of actionability was observed for metoprolol [13]. Moreover, the widely respected Clinical Pharmacogenetics Implementation Consortium issued a guideline in 2024 [14] indicating that "sufficient evidence exists to support clinical recommendations related to CYP2D6 and metoprolol", thus underscoring both the rationale and the timeliness of studying the metabolism of metoprolol and genetically predicted CYP2D6 phenotypes.

In dosing metoprolol, it may thus be beneficial to consider a person's CYP2D6 genotype, as about 80% of an oral metoprolol dose is metabolized by CYP2D6 into inactive metabolites that are subsequently eliminated from the body [10, 11]. The main metabolic pathway of metoprolol is demethylation to *O*-demethylmetoprolol, which is then further oxidized to metoprolol acid. Other metabolic pathways are also oxidative and lead to formation of α-hydroxymetoprolol and *N*-deisopropylmetoprolol, which may be further oxidized to a carboxylic acid [10, 12, 15–20]. Possible glucuronidation of metoprolol has also been reported, but direct evidence of the existence of metoprolol glucuronide is limited [21–23].

These metabolic data notably form the basis of pharmacogenetic testing, in conjunction with the genetic data of the patient. However, there may be limitations to the representativeness of the metabolic data obtained during (commercial) drug development for real-world metoprolol users [24]. For example, the corresponding clinical trials capture rather limited diversity, notably often studying single-dose administrations in very small samples of male volunteers [25–27]. There may also be metabolic differences between healthy and diseased states, whereas drug metabolism is typically elucidated in healthy volunteers [28]. Thus, re-evaluating metabolic data could provide further insight into the metabolic processes and may contribute to more effective drug dosing in real-world metoprolol users.

We aimed to improve the understanding of metoprolol metabolism by studying its metabolites in a real-world setting. Specifically, we identified metoprolol metabolites using liquid chromatography coupled to high-resolution mass spectrometry-based pharmacometabolomics data from 24-h urine samples of individuals who were screened as potential living kidney donors (PLKDs) and kidney transplant recipients (KTRs). In particular, KTRs frequently uses metoprolol because of the increased risk of cardiovascular mortality after transplantation and their substantial risk of developing hypertension, often linked to their immunosuppressive drug treatments [29]. Moreover, drug kinetics should probably not be assumed to be normal in organ transplant recipients [30], highlighting the relevance of evaluating drug metabolism in this population.

2 Methods

2.1 Clinical and Pharmacometabolomics Data

This study used existing 24-h urine pharmacometabolomic data from 688 KTRs and 283 PLKDs included in the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT identifier NCT02811835) [31]. This study was approved by the institutional review board of the University Medical Center Groningen (decision METc 2008/186) and adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants. Clinical data and samples were collected between 2008 and 2010 during outpatient clinic visits to the University Medical Center Groningen. The pharmacometabolomic analyses concern untargeted data-independent acquisition analyses using reversed-phase liquid chromatography coupled to time-of-flight mass spectrometry operated in the "SWATH" acquisition mode, which was initially described by Hopfgartner et al. [32] and Gillet et al. [33] in 2012. All aspects of the pharmacometabolomic analyses are also described in full detail elsewhere [34]; the pharmacometabolomic data have been deposited in an open-access data repository and can be found at: https://doi.org/10.26037/yareta:ybdgdynykfe6rkjjxb7d6oynoa [35]. The clinical data collected were retrieved from medical records (e.g., age, sex, transplantation details) and through measurements during and shortly after each visit (e.g., body mass index, estimated glomerular filtration rate [Chronic Kidney Disease Epidemiology Collaboration creatinine equation 2009], serum albumin, serum alanine aminotransferase). Drug exposure statuses were also based upon self-reported drug use, with the exception of metoprolol, which was based upon the presence of metoprolol signals in the urinary pharmacometabolomic data (see Fig. S1 in the electronic supplementary material [ESM]). The latter drug exposure status was also used as a grouping variable in the evaluation of baseline characteristics, for which we used the Mann–Whitney *U* and Fisher's exact tests (two-sided, $\alpha = 0.05$) for continuous and categorical variables, respectively.

2.2 Feature Selection

We extracted and aligned two-dimensional features (i.e., mass-to-charge ratio [m/z], retention time) from raw mass spectrometry data using SCIEX MarkerView software (version 1.3.1) using the settings presented in Table S1 in the ESM. We subsequently used this software's t-test module to extract statistically significant features ($\alpha = 0.05$, with Bonferroni correction) for PLKDs and KTRs separately using the pharmacometabolomic-based metoprolol exposure status as grouping variable. Then, we excluded features in case of a negative log-fold change and when the median signal intensity value of a specific feature (in metoprolol-positive samples) was lower than 1.0% of the highest observed median value, thus excluding "downregulated" and low abundance features, respectively. We further cleaned the feature list by removing isotope signals, in-source fragments (e.g., due to in-source deconjugation), and adduct signals. However, when an $[M+H]^+$ signal was lower than the adduct signal, the adduct signal was kept.

2.3 Metabolite Identification

Given that the software we used for automatic feature detection is prone to combining closely eluting signals into one feature, we also manually assessed metoprolol-positive samples with SCIEX PeakView (version 2.2.0.11391) using the *m/z* values and retention times of the selected features. Specifically, we obtained extracted ion chromatograms and screened them for peaks that could reflect metoprolol metabolites by evaluating the underlying fragment spectra. In this process and the subsequent attempt to identify metoprolol metabolites, we considered several factors, including the chemical properties of metoprolol (e.g., molecular formula, molecular weight, known fragmentation patterns, retention times and fragmentation patterns of chemical reference standards [when available] measured as neat solutions), existing information on the metabolism of metoprolol (e.g., dealkylation, oxygenation, glucuronidation), general knowledge regarding drug metabolism in humans, and principles of the analytical workflow employed (e.g., reversed-phase liquid chromatography, positive electrospray ionization, collision-induced dissociation). This identification work was primarily done "manually" by two researchers independently, and their results were later compared with each other and with metabolites identified using the SCIEX Molecule Profiler software (version 1.3.1), which is a commercial tool commonly used for metabolite identification in drug development. Next, we manually extracted signals for all (putative) metoprolol metabolites using SCIEX MultiQuant software (version 2.1) with a ± 5 mDa mass extraction window and a 2.0-point Gaussian smoothing width [36]. In this process, MS1-level precursor peaks were integrated for quantitative purposes and MS2-level residual precursor and fragment peaks were integrated for qualitative purposes (see Table S2 in the ESM). We used the resulting data to generate a relative quantitative readout by dividing the signal intensity of each substance individually by the sum of signal intensities of all substances found per metoprolol user. We also then used these relative data for principal component analysis (PCA; with Pareto scaling) in the MarkerView software to visualize

variability and identify patterns in the metoprolol metabolite profiles. Lastly, we later requested representative samples for reanalysis to obtain complementary fragment spectra using the product ion scan acquisition mode. These samples were provided from an incomplete aliquot set, which is frequently used for confirmatory purposes.

3 Results

3.1 Study Groups

In the urine of 283 PLKDs for whom pharmacometabolomic data were available, we found molecular evidence of metoprolol exposure in 18 (6%) study participants. Among these (presumed) metoprolol users, ten were female and eight were male (Table S3 in the ESM). All users were aged ≥ 50 years, and ten eventually donated a kidney. The eight others were unable to donate because of various comorbidities. Lastly, drug use was poorly recorded in these individuals, seven of whom had no metoprolol or even any other drug reported at all.

Among the 688 KTRs included in this study, we found molecular evidence of metoprolol exposure in 374 (54%); this group had a median age of 56 (interquartile range 46–64) years and consisted of 40% females (Table 1). The groups of (presumed) metoprolol users and non-users were similar with regard to sex, serum albumin, serum alanine transaminase, and the time between transplantation and inclusion in the TransplantLines study. However, metoprolol users had a slightly but significantly higher body mass index and lower estimated glomerular filtration rate than non-users. Furthermore, not all users had reported using a β-blocker (96%), compared with 25% in the non-user group. Lastly, metoprolol users more frequently reported using a calcineurin inhibitor, a calcium antagonist, or a statin than did non-users.

3.2 Feature Selection

Starting with 102,106 features in the PLKD substudy, 448 were significantly associated with metoprolol use. After removing isotopes, adducts, in-source fragments, and low abundance features, only 12 features remained (Table 2). In the KTR substudy, 196 of 70,131 features were significantly associated with metoprolol use. Further feature removal resulted in a final list of 13 prioritized features (Table 2).

Table 1 Characteristics of kidney transplant recipients included in this study

Characteristic	Metoprolol users	Non-users	*P*-value
Number	374	314	–
Age, years	56 (46–64)	53 (42–61)	**0.005**
Female	40	47	0.09
BMI, kg/m^2	26 (24–30)	25 (23–29)	**0.008**
Serum albumin, g/L	43 (41–45)	43 (41–45)	0.07
Serum ALT, U/L	19 (15–26)	19 (14–26)	0.6
eGFR, mL/min/1.73 m^2	49 (36–64)	53 (40–68)	**0.007**
Years between transplantation and first study visit	5.7 (1.7–12.0)	5.4 (2.3–12.3)	0.6
Living kidney donation	30	38	0.3
Self-reported medication use			
Calcineurin inhibitor	62	51	**0.004**
Proliferation inhibitor	82	84	0.6
Angiotensin 2 receptor blocker	15	15	1.0
ACE inhibitor	32	34	0.7
Beta blocker	96	25	**< 0.001**
Calcium antagonist	29	19	**0.006**
Diuretic	42	39	0.4
Statin	59	45	**< 0.001**
Antidiabetics	16	15	0.8
Prednisolone dose, mg	10.0 (7.5–10.0)	10.0 (7.5–10.0)	0.6

Continuous and categorical data are presented as median (interquartile range) and valid percentage and are assessed using the Mann–Whitney *U* and Fisher's exact tests (two-sided, $\alpha = 0.05$, statistically significant *P*-values presented in bold), respectively

ACE angiotensin-converting enzyme, *ALT* alanine aminotransferase, *BMI* body mass index, *eGFR* estimated glomerular filtration rate

Between the two substudies, 11 features overlapped, and a full overlap would have been observed if a slightly lower relative abundance threshold (≥ 0.5 instead of ≥ 1.0) had been set. Moreover, the three features that were initially included in only one of the substudies all had borderline relative abundances (between 1.0 and 1.5) and thus were similar to the values observed in the other substudy.

3.3 Metabolite Identification

Manual assessment of metoprolol-positive samples using the *m/z* values and retention times of the 14 prioritized features (see Table 2) revealed 18 distinct signals associated with metoprolol exposure (see Table 3 and Figs. S2–S13 and Tables S4–S11 in the ESM). Two signals corresponded to metoprolol and its phase I metabolite metoprolol acid, as was confirmed using chemical reference standards. Their median relative abundances were approximately 18% and 48%, respectively, and these values were comparable for both PLKD and KTR, as was generally the case for all metabolites found in this study.

Table 2 Overview of selected features

m/z	RT (min)	PLKD		KTR	
		Rel. median (%)[a]	*P* value	Rel. median (%)[a]	*P* value
254.14	5.9	3.3	1.1×10^{-40}	6.3	2.2×10^{-87}
254.18	6.4	1.1	8.9×10^{-27}	0.9[c]	1.7×10^{-36}
258.13[b]	9.2	1.5	1.8×10^{-40}	1.7	3.1×10^{-51}
268.15	6.5	100	1.2×10^{-131}	100	7.0×10^{-143}
268.19	7.9	39.5	4.5×10^{-61}	36.5	3.1×10^{-90}
270.17	4.6	1.4	2.8×10^{-42}	1.9	1.6×10^{-67}
284.15	4.3	2.0	9.8×10^{-55}	2.2	5.6×10^{-71}
284.15	5.8	0.9[c]	5.3×10^{-62}	1.1	1.8×10^{-47}
284.15	7.8	0.5[c]	2.0×10^{-32}	1.3	3.2×10^{-70}
284.19	6.1	29.8	2.2×10^{-66}	41.3	1.8×10^{-116}
296.15	9.6	1.6	3.9×10^{-75}	1.2	8.6×10^{-70}
444.19	6.6	2.9	4.1×10^{-37}	3.3	4.7×10^{-52}
444.22	8.0	11.2	3.0×10^{-45}	11.5	4.6×10^{-69}
460.22	9.5	1.4	3.0×10^{-37}	2.5	2.9×10^{-64}

m/z mass-to-charge ratio, *RT* retention time, *rel.* relative

[a]For each substudy, the highest observed median intensity value (in the metoprolol users group) was set at 100%, and all other median values were expressed relative to this highest value

[b]Unlike the others, this feature represents an $[M+NH_4]^+$ adduction rather than an $[M+H]^+$ monoisotopic peak, which was markedly less intense for this substance

[c]These features were below the abundance threshold for the respective substudy but are shown because these were included in the other substudy

Two other expected phase I metabolites, namely hydroxymetoprolol and demethylmetoprolol, as well as the expected phase II metabolite metoprolol glucuronide were also found. These metabolites were identified based on their monoisotopic mass and spectral similarity with previously published [37] fragment spectra in the case of hydroxymetoprolol and demethylmetoprolol. Identification of metoprolol glucuronide was based on spectral similarity with metoprolol's fragment spectrum, along with the presence of the *m/z* +176.03 peak, as is indicative of glucuronide moieties. For these three substances, the median relative abundances were 18, 0.6, and 2.4%, respectively.

Median relative abundances of all other substances were (well) below 3.0%, for example, in the case of the expected metabolites demethylhydroxymetoprolol and hydroxymetoprolol acid. Their identification was based on monoisotopic mass and spectral similarity with fragment spectra of all four aforementioned substances. Regarding hydroxymetoprolol acid, it should be noted that only one oxygenated metoprolol acid metabolite was expected, namely an alpha-hydroxylated variant of metoprolol acid, previously denoted as metabolite "H 119/77" [17]. However, we found three putative variants of oxygenated metoprolol acid, representing differentially oxygenated species of the high-abundance metoprolol acid metabolite.

We also found other unknown/unreported metabolites, all of which represented phase II metabolites and showed spectral similarity with their nonconjugated precursors along with the presence of a peak indicating the respective conjugate. For example, we found a second metoprolol glucuronide signal, which may correspond to an N-linked conjugate given that its retention time was higher than that of unconjugated metoprolol. In addition, we found four glucuronides of hydroxymetoprolol, one glucuronide of metoprolol acid, and a putative formylated variant of metoprolol acid. Regarding the latter, formylation arguably is a rather uncommon (or underreported) metabolic conversion for xenobiotics. Still, the high degree of spectra similarity with metoprolol acid, along with its matching monoisotopic mass and the presence of an *m/z* +27.99 peak (see Fig. 1), would support the existence of such a metabolite.

Lastly, two expected metabolites were not found, namely deisopropylmetoprolol and a metabolite known as "H 119/68" [17], which is an alpha-hydroxylated version of metoprolol acid minus the carboxylic acid (PubChem Chemical IDentifier 10354230) and which we refer to as "metoprolol alcohol". Regarding the former, we did appear to find the expected metabolite known as "metabolite II" [16] and "H 104/83" [17], which represents a variant of deisopropylmetoprolol in which the primary amine is removed, and the leftover methyl group is converted into carboxylic acid (PubChem Chemical

Table 3 Overview of (putatively) identified metoprolol metabolites

Identity[a,b]	Abbreviation	Molecular formula	*m/z*	RT (min)	Median metabolite abundance[c] in 18 PLKDs (%)	Median metabolite abundance[c] in 374 KTRs (%)
Metoprolol benzoic acid	M-BA	$C_{13}H_{19}NO_4$	254.14	5.8	2.5	2.9
Demethylmetoprolol	M-DM	$C_{14}H_{23}NO_3$	254.18	6.1	0.46	0.63
N-oxidized deisopropylmetoprolol	M-DI-O	$C_{12}H_{16}O_5$ ($[M+NH_4]^+$)	258.13	9.0	0.85	0.98
Metoprolol acid	MA	$C_{14}H_{21}NO_4$	268.15	6.2	48	48
Metoprolol	M	$C_{15}H_{25}NO_3$	268.19	7.8	16	19
Demethylhydroxymetoprolol	M-DM-O	$C_{14}H_{23}NO_4$	270.17	4.4	0.80	1.0
Hydroxymetoprolol acid	MA-O	$C_{14}H_{21}NO_5$	284.15	4.1	1.1	1.1
				5.6	0.31	0.52
				7.6	0.24	0.65
Hydroxymetoprolol	M-O	$C_{15}H_{25}NO_4$	284.19	6.0	18	17
Formylated metoprolol acid	MA-form	$C_{15}H_{21}NO_5$	296.15	9.5	0.71	0.57
Metoprolol acid glucuronide	MA-GLU	$C_{20}H_{29}NO_{10}$	444.19	6.5	1.1	1.2
Metoprolol glucuronide	M-GLU	$C_{21}H_{33}NO_9$	444.22	7.7	2.0	2.8
				8.1	1.9	1.8
Hydroxymetoprolol glucuronide	M-O-GLU	$C_{21}H_{33}NO_{10}$	460.22	5.8	0.05	0.06
				6.1	0.16	0.26
				9.1	0.03	0.34
				9.4	0.42	0.44

KTRs kidney transplant recipients, *PLKDs* potential live kidney donors, *RT* retention time

[a]Solely metoprolol, metoprolol acid, and metoprolol benzoic acid reflect substances for which the identity was verified using chemical reference standards. All other identities reflect putatively identified metabolites and are based upon spectral similarity with known substances

[b]Exemplary extracted ion chromatograms and fragment spectra are shown in Figs. S2 to S13 in the electronic supplementary material

[c]Median metabolite abundance values presented in the table reflect the median values per substudy of the relative quantitative readouts that were calculated by dividing the signal intensity of each substance individually by the sum of signal intensities of all substances found per metoprolol user

IDentifier 3043944). Notably, this substance was prioritized based on its $[M+NH_4]^+$ adduct ion, which gave markedly higher signals than the $[M+H]^+$ ion, as has also been described elsewhere for metabolites losing a nitrogen-containing moiety and gaining a carboxylic acid [36]. Furthermore, identification was based on its monoisotopic mass and spectral similarity with metoprolol's fragment spectrum. Regarding the latter expected but undetected metabolite (i.e., H 119/68), we did find a substance that could correspond to a further oxidized metabolite. This substance's molecular weight and fragment spectra matched a previously reported substance denoted as "hydroxy derivative 4-[2-hydroxy-3-(propan-2-ylamino)propoxy]benzaldehyde", which was previously detected during a forced in vitro metoprolol oxidative degradation test [39]. However, further testing with chemical reference standards revealed that this metabolite corresponds to a substance commercially known as bisoprolol (carboxylic) acid impurity (PubChem Chemical IDentifier 12540204; International Union of Pure and Applied Chemistry name, 4-[2-hydroxy-3-(propan-2-ylamino)propoxy] benzoic acid), which we refer to as metoprolol benzoic acid. Nonetheless, the relatively high abundance of this metabolite and the link to degradation experiments led to searches with metoprolol and its major metabolites using the open-source online BioTransformer Metabolism Prediction Tool [40]. These exploratory searches suggested that a metoprolol benzoic acid metabolite may be formed under specific conditions. This metabolite was proposed to be formed by "alpha-oxidation of carboxylic acid" from metoprolol by an "unspecified gut bacterial enzyme", which presents an interesting but preliminary finding that warrants further investigation, besides the proposed endogenous pathway via metoprolol acid, hydroxymetoprolol acid, and metoprolol alcohol.

3.4 Metabolite Profile Patterns and Variability

Unsupervised PCA indicated that there was no apparent differentiation between KTR and PLKD or between PLKD who did and PLKD who did not donate a kidney

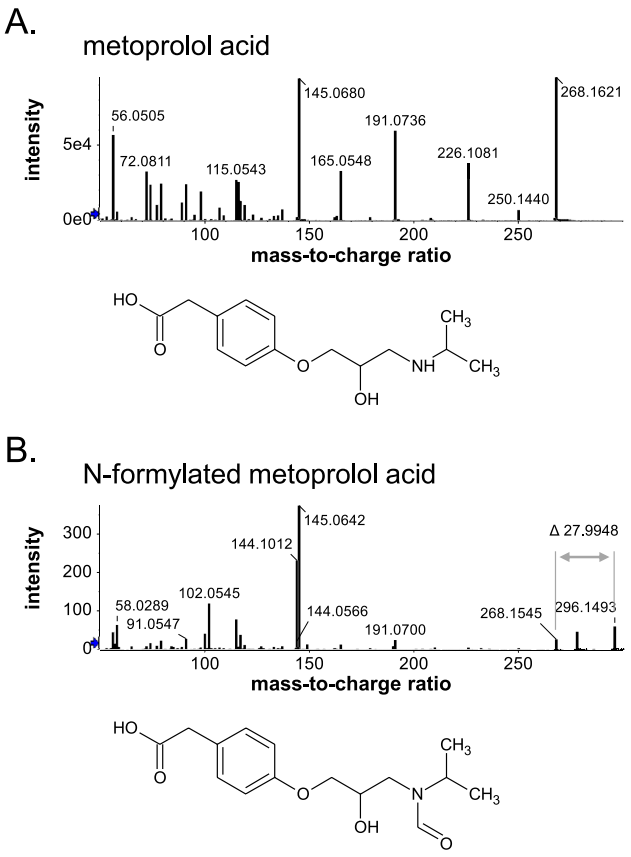

Fig 1 Chemical structures and product ion mass spectra of **a** metoprolol acid and **b** N-formylated metoprolol acid. Regarding the data shown for metoprolol acid, this reflects a 'level 1' identification in terms of the classification proposed by the Metabolomics Standards Initiative [38]. The data shown in panel **b** reflect a 'level 3' identification, and the presented structure is the proposed structural formula of the respective metoprolol metabolite candidate

(see Fig. 2). In addition, these analyses showed that by far the highest percentage of variance was explained by PC1 (64%), a still-substantial proportion was explained by PC2 (15%) and PC3 (8%), and vastly lower proportions were explained by the other PCs (≤ 3%). For PC1, major contributors were metoprolol on one side versus metoprolol acid and hydroxymetoprolol on the other side. For PC2, major contributors were hydroxymetoprolol versus metoprolol acid, and, for PC3, major contributors were metoprolol and hydroxymetoprolol versus glucuronidated forms of metoprolol and metoprolol acid. Respectively, these PCs thus showed separation based on the administered substance versus its major oxidation products, a singly oxidized major metabolite versus a doubly oxidized major metabolite, and nonconjugated substances versus conjugated metabolites.

Next, we calculated the signal intensities of the major metabolites metoprolol acid, (putative) hydroxymetoprolol, and (putative) metoprolol O-glucuronide relative to that of the administered drug metoprolol. In the case of the two phase I metabolites, we compared their ratios between study participants who did and those who did not report usage of a strong or intermediate CYP2D6 inhibitor, such as paroxetine or cinacalcet (see Figs. S14 and S15 in the ESM). Although only a few participants used these drugs, seemingly lower metabolite ratios were indeed observed in their users. These findings are thus consistent with the inhibitory effects of the drugs, which would be expected to reduce the relative proportion of these phase I metabolites compared with the administered drug. For metoprolol O-glucuronide, its ratio was associated with kidney function (see Fig. S16 in the ESM), showing a trend of increased glucuronidation in individuals with reduced kidney function. This observation logically links to the expectation that drug molecules may undergo additional metabolic processing in organs such as the liver when renal excretion is impaired.

4 Discussion

Using urinary pharmacometabolomic data from 374 KTRs and 18 PLKDs, we detected nearly all metabolites previously identified, either directly [16–18, 21, 41] or by inference [22, 23] in 86 patients with uncontrolled hypertension [21], in 36 patients with liver cirrhosis [23], in an undisclosed patient population [41], and/or in studies including up to 12 healthy volunteers [16–18, 22, 23] (see Fig. 3). For the two expected metabolites that were not found, namely deisopropylmetoprolol and a hydroxylated variant of metoprolol acid lacking the carboxylic acid moiety (also known as metabolite H 119/68), we did find signals of potential further metabolized substances. In the case of deisopropylmetoprolol, this refers to a variant of deisopropylmetoprolol in which the primary amine is removed and the leftover methyl group is converted into carboxylic acid (also known as metabolite II or H 104/83), as has previously been found in human urine [16, 17]. In the case of H 119/68, this concerns a variant featuring a benzoic acid instead of a benzyl alcohol substructure, which furthermore is known as an impurity of bisoprolol. We thus describe its presence in human samples of metoprolol users for the first time, as we also do for a putative N-glucuronidated variant of metoprolol, a glucuronidated variant and a formylated variant of metoprolol acid, and four glucuronidated variants of hydroxymetoprolol.

Comparison of pharmacometabolomic signal intensity values indicated that metoprolol acid was generally the most abundant substance, followed by metoprolol and

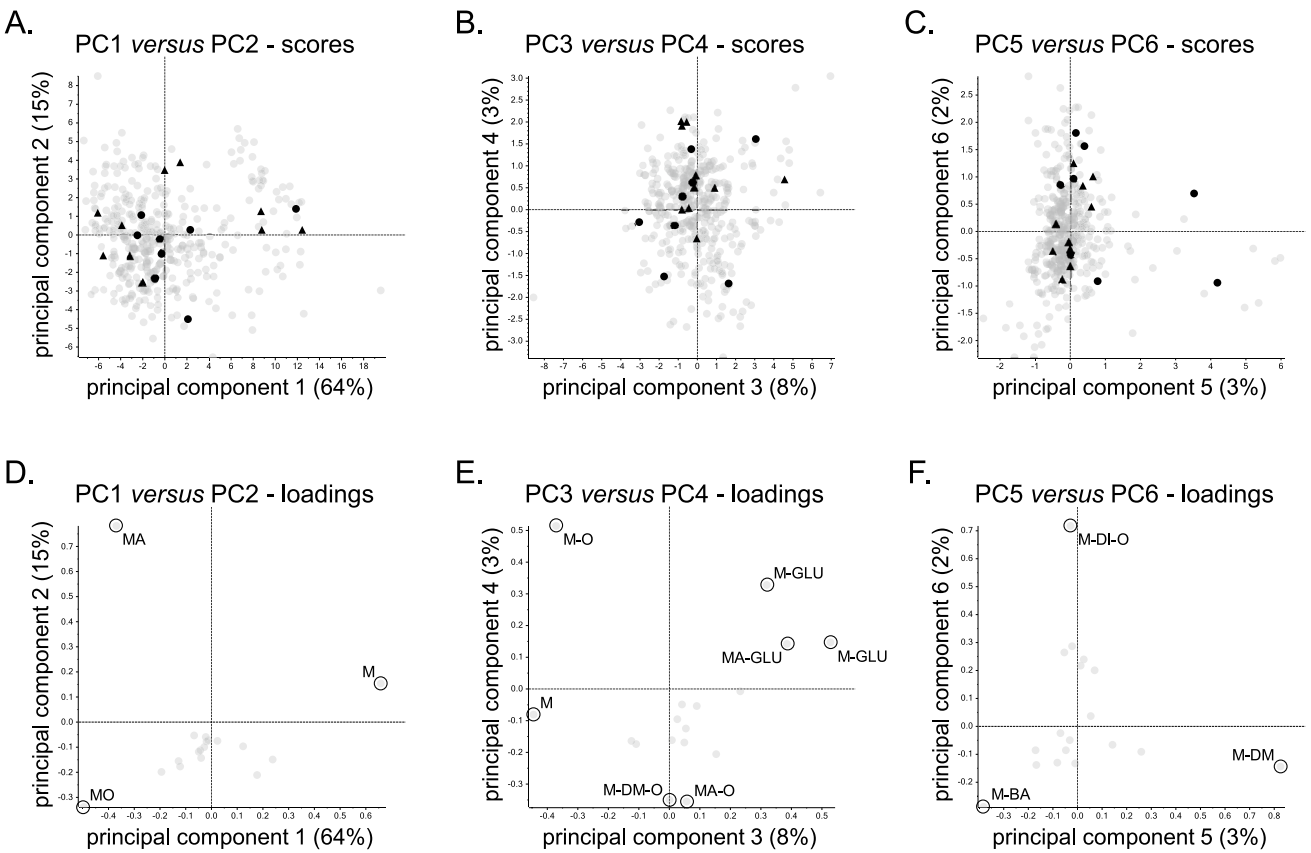

Fig. 2 Pareto-scaled (**a**–**c**) scores and (**d**–**f**) loadings plots for unsupervised principal component (PC) analysis of relative metabolite profile data of 374 kidney transplant recipients (grey dots), eight potential living kidney donors who were unable to donate a kidney (black dots), and 10 potential living kidney donors who donated a kidney (black triangles). This figure includes all PCs showing a percentage of variance explained above 1%. The substances highlighted in the bottom panes are metoprolol (M) and its glucuronidated variants (M-GLU), metoprolol acid (MA) and its hydroxylated (MA-O) and glucuronidated (MA-GLU) variants, hydroxymetoprolol (M-O) and its demethylated variant (M-DM-O), N-oxidized deisopropylmetoprolol (M-DI-O), and metoprolol benzoic acid (M-BA)

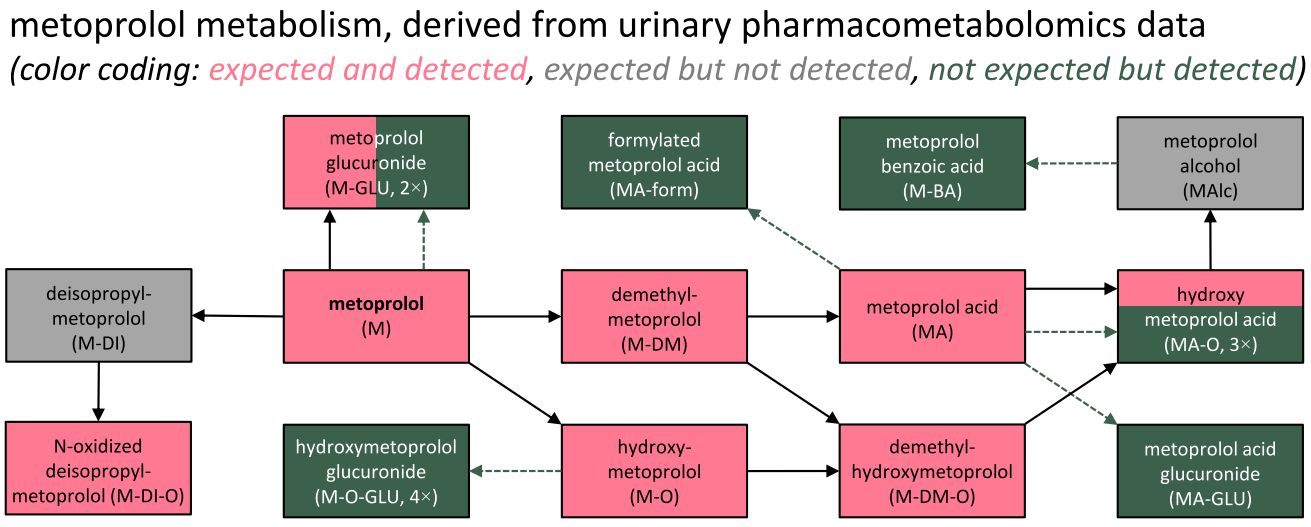

Fig. 3 Summary of study findings. A more detailed overview of putative human metabolism of metoprolol based on this study's findings, including structural formulas, is included as Fig. S17 in the electronic supplementary material

hydroxymetoprolol at two to three times lower signal intensity values, metoprolol benzoic acid and the two metoprolol glucuronides at 15–25 times lower, and the other metabolites at ≥ 40 times lower signal intensity values. Interestingly, the metabolite patterns were similar between KTRs and PLDKs, as was also observed based on the results of PCA. This analysis also indicated that by far the largest degree of variance in metoprolol metabolite patterns was due to the relative levels of metoprolol itself in relation to those of its main oxidation products, metoprolol acid and hydroxymetoprolol. These results link to a previous study in which ratios between these substances were indicative of a person's CYP2D6 genotype (i.e., intermediate metabolizer, normal metabolizer, ultrarapid metabolizer) [21], as represents a key determinant of conversion rates in the case of metoprolol's metabolism. Unfortunately, genetic data were not available for the present study. Hence, future studies aimed at unveiling both genetic and nongenetic factors explaining variability in metoprolol metabolism should be conducted in different cohorts. Future studies should also focus on the pharmacological activity of the newly found metabolites, which admittedly have rather low relative abundances. However, it is known that the primary action of several therapeutic drugs, such as clopidogrel, relies on (very) low-abundance metabolites, thus indicating that low abundance does not necessarily equate to low relevance in the case of xenobiotic metabolites.

A strength of this study is that it includes a large number of real-world metoprolol users, thereby capturing (but not correcting for) various sources of variability, notably relating to drug use (e.g., dosage, therapy duration, adherence) but, for example, also relating to anthropometric, demographic, dietary, genetic, lifestyle, and (patho)physiological factors. We also confirmed the presence of several expected metabolites, which could strengthen existing pharmacokinetic modeling or pharmacogenetic testing approaches, and we identified several previously unreported metabolites, which could be considered for future refinements of these modeling and testing approaches. Additionally, the newly described substances could be used to enhance the identification potential of metabolomics techniques by addressing the analytical "dark matter", which still constitutes a major proportion of detected signals in untargeted metabolomics experiments. Admittedly, most of the substances detected reflect level 3 identifications, or "putatively characterized compound classes", according to the Metabolomics Standards Initiative. However, all of them were only observed in metoprolol-positive samples, thus adding evidence to their status as potential metoprolol metabolites (or at least metoprolol use-associated substances).

Our study's real-world design also links to a weakness of our study, namely that it inevitably captures variability that we are unaware of and thus cannot study in detail. In addition, our study captures considerable interindividual variability, yet it still only includes individuals from a single, confined geographical area, which is not representative of all other areas across the globe and calls for replication studies. Furthermore, we used biobanked samples and untargeted analytical techniques (employed in a nonregulatory environment), which are inherently associated with different types of (pre)analytical bias. In this regard, we want to emphasize the associations we found between phase I metabolite ratios and concomitant CYP2D6 inhibitor use as well as between phase II metabolite ratios and kidney function, as discussed in Sect. 3.4. These findings suggest that our data do seem to capture pharmacologically plausible trends despite the many potential sources of uncontrolled (pre)analytical variability pertaining to our study setting. Lastly, it should be considered that the metabolite patterns detected may not fully represent those at the time of urine collection, as they are influenced by various (pre)analytical factors, including the exact timing and frequency of metoprolol intake within the 24-h collection period. This underscores the need to replicate our study in larger and better characterized cohorts, preferably also using different analytical workflows (allowing for absolute quantification).

5 Conclusions

This pharmacometabolomics study analyzed the metabolism of metoprolol in a real-world setting and found more metoprolol transformation products than expected. The detected metabolites and metabolite patterns were also similar between KTRs and PLKDs. Moreover, the largest degree of variance in metoprolol metabolite patterns was due to the relative levels of metoprolol itself in relation to those of its main oxidation products, metoprolol acid and hydroxymetoprolol. Markedly lower but still relevant degrees of variance were due to (singly oxidized) hydroxymetoprolol versus (doubly oxidized) metoprolol acid, and nonconjugated versus conjugated substances. Future studies including genetic (e.g., pharmacogenetic genotypes) and nongenetic (e.g., comedication, lifestyle exposures, comorbidities) data are warranted to elucidate key real-world determinants of metoprolol metabolism, to study metabolic variability in light of therapy efficacy and safety, and to assess whether additional metabolic conversions may necessitate pharmacogenetic monitoring.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40262-025-01502-7.

Acknowledgements The authors extend their gratitude to Desanka Stoppels, whose preliminary work inspired this study. They also gratefully acknowledge the TransplantLines investigators: Coby Annema, Stephan J. L. Bakker, Stefan P. Berger, Hans Blokzijl, Frank A. J. A. Bodewes, Marieke T. de Boer, Kevin Damman, Martin H. de Borst, Arjan Diepstra, Gerard Dijkstra, Caecilia S. E. Doorenbos, Rianne M. Douwes, Michele F. Eisenga, Michele E. Erasmus, C. Tji Gan, Antonio W. Gomes Neto, Eelko Hak, Bouke G. Hepkema, Frank Klont, Tim J. Knobbe, Daan Kremer, Henri G. D. Leuvenink, Willem S. Lexmond, Vincent E. de Meijer, Hubert G. M. Niesters, Gertrude J. Nieuwenhuijs-Moeke, L. Joost van Pelt, Robert A. Pol, Adelita V. Ranchor, Jan Stephan F. Sanders, Marion J. Siebelink, Riemer J. H. J. A. Slart, J. Casper Swarte, Daan J. Touw, Marius C. van den Heuvel, Coretta van Leer-Buter, Marco van Londen, Erik A. M. Verschuuren, Michel J. Vos, and Rinse K. Weersma.

Declarations

Funding Frank Klont gratefully acknowledges the funding provided by the Netherlands Organisation for Scientific Research NWO (domain Applied and Engineering Sciences) under Veni grant agreement no. 19060. The funder had no role in the study design, data collection, analysis, reporting, or the decision to submit for publication. Furthermore, the generation of the TransplantLines Food and Nutrition Biobank and Cohort Study (TxL-FN), trial registration number NCT02811835, was funded by the Top Institute Food and Nutrition, grant number A-1003. Continuation of the TransplantLines Food and Nutrition Biobank and Cohort Study was supported by grants from Astellas BV and Chiesi Pharmaceuticals BV and co-financed by the Dutch Ministry of Economic Affairs and Climate Policy by means of the public–private partnership allowance made available by Top Sector Life Sciences & Health to stimulate public–private partnerships. The funders had no role in the study design, data collection, analysis, reporting, or decision to submit for publication.

Conflict of interest The authors have no financial or proprietary interests in any material discussed in this article.

Ethics approval This study used existing data of KTRs and PLKD included in the TransplantLines Food and Nutrition Biobank and Cohort Study (NCT identifier NCT02811835), which was approved by the institutional review board of the University Medical Center Groningen (decision METc 2008/186) and adhered to the Declaration of Helsinki.

Consent to participate All participants provided written informed consent before study participation.

Consent for publication Not applicable.

Availability of data The pharmacometabolomic data used have been deposited in an open-access data repository, as can be found at: https://doi.org/10.26037/yareta:ybdgdynykfe6rkjjxb7d6oynoa [35]. Patient-level data for data on file in this manuscript are not available for sharing as there is a reasonable likelihood that individual patients could be re-identified. Further information can be requested from the authors.

Author contributions W.A.H., M.A.J.H., P.S., S.J.L.B., G.H., and F.K. wrote the manuscript. F.K. designed the research. W.A.H., M.A.J.H., and F.K. performed the research. W.A.H. and F.K. analyzed the data. S.J.L.B. and G.H. contributed new reagents/analytical tools.

References

1. Langreth R, Waldholz M. New era of personalized medicine: targeting drugs for each unique genetic profile. Oncologist. 1999;4:426–7.
2. Jørgensen JT. Twenty years with personalized medicine: past, present, and future of individualized pharmacotherapy. Oncologist. 2019;24:e432–40.
3. Chen R, Snyder M. Promise of personalized omics to precision medicine. Wiley Interdiscipl Rev Syst Biol Med. 2013;5:73–82.
4. Duarte JD, Cavallari LH. Pharmacogenetics to guide cardiovascular drug therapy. Nat Rev Cardiol. 2021;18:649–65.
5. Hasanzad M, Sarhangi N, Ehsani Chimeh S, et al. Precision medicine journey through omics approach. J Diabetes Metab Disord. 2021;21:881–8.
6. Schärfe CPI, Tremmel R, Schwab M, et al. Genetic variation in human drug-related genes. Genome Med. 2017;9:117.
7. Jian J, He D, Gao S, Tao X, Dong X. Pharmacokinetics in pharmacometabolomics: towards personalized medication. Pharmaceuticals. 2023;16:1568.
8. Tremmel R, Zhou Y, Schwab M, Lauschke VM. Structural variation of the coding and non-coding human pharmacogenome. NPJ Genom Med. 2023;8:24.
9. Lauschke VM, Milani L, Ingelman-Sundberg M. Pharmacogenomic biomarkers for improved drug therapy—recent progress and future developments. AAPS J. 2018;20:4.
10. Rüdesheim S, Wojtyniak JG, Selzer D, et al. Physiologically based pharmacokinetic modeling of metoprolol enantiomers and α-hydroxymetoprolol to describe CYP2D6 drug-gene interactions. Pharmaceutics. 2020;12:1–18.
11. Meloche M, Khazaka M, Kassem I, et al. *CYP2D6* polymorphism and its impact on the clinical response to metoprolol: a systematic review and meta-analysis. Br J Clin Pharmacol. 2020;86:1015–33.
12. Zhou S-F. Polymorphism of human cytochrome P450 2D6 and its clinical significance. Clin Pharmacokinet. 2009;48:689–723.
13. Swen JJ, van der Wouden CH, Manson LE, et al. A 12-gene pharmacogenetic panel to prevent adverse drug reactions: an open-label, multicentre, controlled, cluster-randomised crossover implementation study. Lancet. 2023;401:347–56.
14. Duarte JD, Thomas CD, Lee CR, et al. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for *CYP2D6*, *ADRB1*, *ADRB2*, *ADRA2C*, *GRK4*, and *GRK5* genotypes and beta-blocker therapy. Clin Pharmacol Ther. 2024;116:939–47.
15. Berger B, Bachmann F, Duthaler U, et al. Cytochrome P450 enzymes involved in metoprolol metabolism and use of metoprolol as a CYP2D6 phenotyping probe drug. Front Pharmacol. 2018;9:774.
16. Borg KO, Carlsson E, Hoffmann K-J, Jönsson T-E, Thorin H, Wallin B. Metabolism of metoprolol-(3H) in man, the dog and the rat. Acta Pharmacol Toxicol (Copenh). 1975;36:125–35.

17. Hoffmann K-J, Gyllenhaal O, Vessman J. Analysis of α-hydroxy metabolites of metoprolol in human urine after phosgene/trimethylsilyl derivatization. Biol Mass Spectrom. 1987;14:543–8.
18. Gyllenhaal O, Hoffmann K. Simultaneous determination of metoprolol and metabolites in urine by capillary column gas chromatography as oxazolidineone and trimethylsilyl derivatives. J Chromatogr. 1984;309:317–28.
19. Bodor N, Buchwald P. Ophthalmic drug design based on the metabolic activity of the eye: soft drugs and chemical delivery systems. AAPS J. 2005;7:79.
20. Zamir A, Hussain I, ur Rehman A, et al. Clinical pharmacokinetics of metoprolol: a systematic review. Clin Pharmacokinet. 2022;61:1095–114.
21. Brocker CN, Velenosi T, Flaten HK, et al. Metabolomic profiling of metoprolol hypertension treatment reveals altered gut microbiota-derived urinary metabolites. Hum Genomics. 2020;14:10.
22. Bachmann F, Duthaler U, Krähenbühl S. Effect of deglucuronidation on the results of the Basel phenotyping cocktail. Br J Clin Pharmacol. 2021;87:4608–18.
23. Duthaler U, Bachmann F, Ozbey AC, et al. The activity of members of the UDP-glucuronosyltransferase subfamilies UGT1A and UGT2B is impaired in patients with liver cirrhosis. Clin Pharmacokinet. 2023;62:1141–55.
24. Klont F, Hof MAJ, Nijdam FB, et al. Pharmacometabolomics may be the next stamp in the pharmacogenetic passport. Pharmacol Res. 2024;204: 107191.
25. Hughes JH, Woo KH, Keizer RJ, Goswami S. Clinical decision support for precision dosing: opportunities for enhanced equity and inclusion in health care. Clin Pharmacol Ther. 2023;113:565–74.
26. Corpas M, Siddiqui MK, Soremekun O, et al. Addressing ancestry and sex bias in pharmacogenomics. Annu Rev Pharmacol Toxicol. 2024;64:53–64.
27. Ramamoorthy A, Bende G, Chow ECY, et al. Human radiolabeled mass balance studies supporting the FDA approval of new drugs. Clin Transl Sci. 2022;15:2567–75.
28. Li Y, Meng Q, Yang M, et al. Current trends in drug metabolism and pharmacokinetics. Acta Pharm Sin B. 2019;9:1113–44.
29. Weir MR, Burgess ED, Cooper JE, et al. Assessment and management of hypertension in transplant patients. J Am Soc Nephrol. 2015;26:1248–60.
30. Venkataramanan R, Habucky K, Burckart GJ, Ptachcinski RJ. Clinical pharmacokinetics in organ transplant patients. Clin Pharmacokinet. 1989;16:134–61.
31. Van Den Berg E, Pasch A, Westendorp WH, et al. Urinary sulfur metabolites associate with a favorable cardiovascular risk profile and survival benefit in renal transplant recipients. J Am Soc Nephrol. 2014;25:1303–12.
32. Hopfgartner G, Tonoli D, Varesio E. High-resolution mass spectrometry for integrated qualitative and quantitative analysis of pharmaceuticals in biological matrices. Anal Bioanal Chem. 2012;402:2587–96.
33. Gillet LC, Navarro P, Tate S, et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics. 2012;11:O111.016717.
34. Klont F, Stepanović S, Kremer D, et al. Untargeted 'SWATH' mass spectrometry-based metabolomics for studying chronic and intermittent exposure to xenobiotics in cohort studies. Food Chem Toxicol. 2022;165: 113188.
35. Klont F, Hopfgartner G. Mass spectrometry-based clinical metabolomics data of urine from kidney transplant recipients (KTR), potential living kidney donors (PLKD), and living kidney donors (LKD). Yareta. 2022. https://doi.org/10.26037/yareta:64ruex2sxff5nenyfyexurzs3.
36. Klont F, Sosnowski P, Kremer D, et al. Assessing the potential of untargeted SWATH mass spectrometry-based metabolomics to differentiate closely related exposures in observational studies. Metabolites. 2022;12:942.
37. Ma YR, Rao Z, Shi AX, et al. Simultaneous determination of metformin, metoprolol and its metabolites in rat plasma by LC-MS-MS: application to pharmacokinetic interaction study. J Chromatogr Sci. 2016;54:1–9.
38. Sumner LW, Amberg A, Barrett D, et al. Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). Metabolomics. 2007;3:211–21.
39. Abramović B, Kler S, Šojić D, Laušević M, Radović T, Vione D. Photocatalytic degradation of metoprolol tartrate in suspensions of two TiO_2-based photocatalysts with different surface area. Identification of intermediates and proposal of degradation pathways. J Hazard Mater. 2011;198:123–32.
40. Wishart DS, Tian S, Allen D, et al. BioTransformer 3.0—a web server for accurately predicting metabolic transformation products. Nucleic Acids Res. 2022;50:W115–23.
41. Szultka-Młyńska M, Bajkacz S, Baranowska I, Buszewski B. Structural characterization of electrochemically and in vivo generated potential metabolites of selected cardiovascular drugs by EC-UHPLC/ESI-MS using an experimental design approach. Talanta. 2018;176:262–76.

Authors and Affiliations

Wietske A. Heddema[1] · Marieke A. J. Hof[2] · Piotr Sosnowski[3] · Stephan J. L. Bakker[4] · Gérard Hopfgartner[5] · Frank Klont[1,6] on behalf of TransplantLines Investigators

✉ Frank Klont
frank.klont@rug.nl

[1] Unit of Pharmacotherapy, Epidemiology and Economics, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands

[2] Department of Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands

[3] Faculty of Chemistry, University of Warsaw, Warsaw, Poland

[4] Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[5] Life Sciences Mass Spectrometry, Department of Inorganic and Analytical Chemistry, University of Geneva, Geneva, Switzerland

[6] Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands