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ORIGINAL ARTICLE

Evidence for solanidine as a dietary CYP2D6 biomarker: Significant correlation with risperidone metabolism

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Aims: The extensive variability in cytochrome P450 2D6 (CYP2D6) metabolism is mainly caused by genetic polymorphisms. However, there is large, unexplained variability in CYP2D6 metabolism within CYP2D6 genotype subgroups. Solanidine, a dietary compound found in potatoes, is a promising phenotype biomarker predicting individual CYP2D6 metabolism. The aim of this study was to investigate the correlation between solanidine metabolism and the CYP2D6-mediated metabolism of risperidone in patients with known *CYP2D6* genotypes.

Methods: The study included therapeutic drug monitoring (TDM) data from CYP2D6-genotyped patients treated with risperidone. Risperidone and 9-hydroxyrisperidone levels were determined during TDM, and reprocessing of the respective TDM full-scan high-resolution mass spectrometry files was applied for semi-quantitative measurements of solanidine and five metabolites (M402, M414, M416, M440 and M444). Spearman's tests determined the correlations between solanidine metabolic ratios (MRs) and the 9-hydroxyrisperidone-to-risperidone ratio.

Results: A total of 229 patients were included. Highly significant, positive correlationswere observed between all solanidine MRs and the 9-hydroxyrisperidone-to-risperidone ratio ($\rho > 0.6$, $P < .0001$). The strongest correlation was observed for the M444-to-solanidine MR in patients with functional CYP2D6 metabolism, i.e., genotype activity scores of 1 and 1.5 (ρ 0.72–0.77, $P < .0001$).

Conclusion: The present study shows strong, positive correlations between solanidine metabolism and CYP2D6-mediated risperidone metabolism. The strong correlation within patients carrying *CYP2D6* genotypes encoding functional CYP2D6 metabolism suggests that solanidine metabolism may predict individual CYP2D6 metabolism, and hence potentially improve personalized dosing of drugs metabolized by CYP2D6.

KEYWORDS
CYP2D6, phenotyping, precision medicine, solanidine biomarker

1 | INTRODUCTION

Cytochrome P450 2D6 (CYP2D6) is involved in the metabolism of 20–30% of commonly used drugs.[1] There is substantial interindividual variability in CYP2D6 expression and activity. This is caused by the highly polymorphic *CYP2D6* gene,[1] as well as nongenetic factors, e.g. pathophysiology of drug-eliminating organs (liver/-kidneys) or concomitant use of drugs inhibiting CYP2D6. Still, a large part of the observed variability in CYP2D6 activity is unexplained. Consequently, predicting the individual dose requirements of CYP2D6 substrates based solely on genotype is challenging.

To predict or measure the enzymatic activity of CYP2D6 for individualization of drug therapy, CYP2D6 genotyping and phenotyping may be applied, respectively. Clinical use of genotype-guided dosing of CYP2D6 substrates as a tool for personalized medicine is increasing[2,3]; however, genotyping is restricted to only capture known genetic determinants impacting the drug pharmacokinetics. Thus, phenotyping, which reflects the combined effects of genetic, environmental, and biological factors, is considered the most accurate method to measure real-time enzyme activity.[4] The traditional phenotyping approach requires administration of an external probe drug, where metabolism via the specific pathway is extensively mediated by the enzyme of interest. Debrisoquine, sparteine, metoprolol and dextromethorphan represent well-established probe drugs used to determine the CYP2D6 phenotype.[5] Still, this approach to measure enzyme activity is not straightforward either, as exogenous probe drug administration involves risk of probe-related adverse effects and is unsuitable for vulnerable populations. Furthermore, phenotyping based on probe drugs is associated with impracticalities such as pharmacokinetic sampling and the lag time needed for analysis to get the phenotype results.

Although recent development of low-dose phenotyping approaches and limited sampling strategies partly overcome the above-mentioned challenges associated with exogenous phenotyping probe drugs,[6] efforts have been made to discover endogenous biomarkers reflecting in vivo CYP2D6 activity.[7] A few endogenous compounds such as pinoline, 5-methoxytryptamine and endogenous codeine have been suggested to be potential biomarkers for CYP2D6, but the candidates have either proven to be unsuitable for phenotyping or the current data in humans are insufficient.[8–10] Metabolomics represents a new approach for biomarker discovery and recently, the alkaloid solanidine and its metabolites were discovered by Magliocco *et al.* as promising CYP2D6 markers.[11] Solanidine is the steroidal aglycone of the two major glycoalkaloids, α-solanine and α-chaconine, that are found in potato (*Solanum tuberosum*).[12] It has been reported that solanidine is present in nanomolar concentrations in human serum, and moreover, in amounts dependent of potato consumption.[13] With potatoes being a main staple food worldwide, and considering that solanidine has a long terminal elimination half-life,[13] it has a large potential as a non-drug CYP2D6 phenotypic marker.

Generally, CYP2D6 plays a major role in the metabolism of psychoactive drugs, including several antipsychotics, such as risperidone, which is predominantly metabolized by CYP2D6-mediated 9-hydroxylation, with minor contributions of CYP3A4.[14] Therapeutic drug monitoring (TDM) is often applied in patients receiving risperidone therapy as a tool to optimize dosing. Due to similar pharmacodynamic properties of risperidone and 9-hydroxyrisperidone, the latter is also included during TDM, as it is the sum of these concentrations, i.e., the active moiety, that is relevant for TDM-guided dosing.[15] Thus, the ratio between the 9-hydroxyrisperidone and risperidone constitutes a CYP2D6 metric expected to largely reflect in vivo enzyme activity,[16,17] that can be derived from TDM databases for clinical research on individual variability in CYP2D6 activity.

By using data from a TDM database, the aim of this study was to investigate the correlation between solanidine and risperidone metabolism in a large patient population with known *CYP2D6* genotypes.

What is already known about this subject

- CYP2D6 activity influences the individual dose requirement of several therapeutic drugs.
- There is substantial unexplained variability in CYP2D6 activity between individuals.
- Solanidine is a compound present in potato that has been suggested as a dietary CYP2D6 activity marker.

What this study adds

- Solanidine metabolism were significantly correlated with CYP2D6-mediated risperidone metabolism.
- The correlations were strong within *CYP2D6* genotype subpopulations encoding functional metabolism, which supports a potential of solanidine as a biomarker capturing all sources of CYP2D6 variability.
- Solanidine phenotyping could improve prediction of the individual clearance of CYP2D6-metabolized drugs.

2 | METHODS

2.1 | Subjects

This study included CYP2D6-genotyped patients receiving oral risperidone therapy in which serum concentrations of risperidone and its main metabolite 9-hydroxyrisperidone had been measured during routine TDM at Center for Psychopharmacology, Diakonhjemmet Hospital (Oslo, Norway) between March 2019 and August 2022. Information about *CYP2D6* genotype was retrieved from the laboratory database, while existing high-resolution mass spectrometry (HRMS) data files from the respective risperidone analyses of the included patients were used for detection and semi-quantitative measurements of solanidine and five solanidine metabolites; M402,

M414, M416, M440 and M444 (named by molecular mass). Patients were included if: (i) age was between 18 and 65 years; (ii) blood sampling time was within 10–48 h post risperidone dose intake; (iii) there was no concomitant use or prior use (<9 months) of paliperidone (9-hydroxyrisperidone) injectable formulations; and (iv) there were detectable serum levels of risperidone *and* 9-hydroxyrisperidone. If exact information about time interval since last dose intake and blood sampling was missing, but the requisition form confirmed daily administration of oral risperidone, it was assumed that the sampling criteria on the requisition form was met (i.e., 12–24 h after dose). For patients with >1 serum concentration measurement of risperidone during the time span of the study, the last eligible TDM measurement was used.

The use of existing TDM data from routine analysis was approved by the Regional Committee for Medical and Health Research Ethics and the Hospital Investigational Review Board.

2.2 | *CYP2D6* genotyping

The *CYP2D6* pharmacogenetic panel included the lack-of-function alleles *CYP2D6*3* (rs35742686), *CYP2D6*4* (rs3892097), *CYP2D6*6* (rs5030655); the reduced-function variants *CYP2D6*9* (rs5030656), *CYP2D6*10* (rs1065852) and *CYP2D6*41* (rs28371725), and copy number analysis to identify *CYP2D6*5* (whole gene deletion) and multiplication of alleles. Absence of analysed variant alleles was interpreted as a fully functional allele (*CYP2D6*1*). Analysis of *CYP2D6* variant alleles was performed using TaqMan-based real-time polymerase chain reaction assays, while TaqMan Copy number assay targeting exon 9, with RNase P as an endogenous control, was used for the copy number analysis of *CYP2D6*. The methods are implemented for routine pharmacogenetic analyses at Diakonhjemmet Hospital.

The patients were categorized by metabolizer status as predicted by the CYP2D6 genotype activity scores defined in joint consensus guidelines by the Dutch Pharmacogenomics Working Group and the Clinical Pharmacogenetics Implementation Consortium.[18] The variant alleles analysed in the present study were assigned to the following activity values: *CYP2D6*3–6* = 0, *CYP2D6*10* = 0.25, *CYP2D6*9* and *CYP2D6*41* = 0.5, and *CYP2D6*1* = 1. The CYP2D6 genotype activity score was calculated by summarizing the activity values assigned to each allele. The *CYP2D6*1/*1* xN genotype was assigned to a CYP2D6 activity score of >2.25. Patients with *CYP2D6* gene duplications in combination with reduced and/or nonfunctional variant alleles were not classified since the pharmacogenetic assays does not determine which allele is duplicated (inconclusive genotype result).

2.3 | Serum concentration analysis of risperidone and 9-hydroxyrisperidone

A targeted, multianalyte, ultra-high performance liquid chromatography–HRMS method was applied for serum concentration measurements of risperidone and 9-hydroxyrisperidone. Briefly, the serum samples were prepared by protein precipitation and the compounds were separated by an Xbridge BEH C18-column (2.6 µm, 2.1 × 75 mm; Waters, Milford, MA, USA) using gradient elution at 35°C with a mix of ammonium acetate buffer (pH 4.8) and acetonitrile (20–52%). The retention times were 1.2 and 1.4 min for 9-hydroxyrisperidone and risperidone, respectively. The QExactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was operated in positive ionization mode acquiring full-scan data at a resolution of 70 000 within the 100–1500 Da scan range. The compounds were quantified in full-scan acquisition mode, using the accurate mass of the protonated molecules (*m/z* 411.2191 and 427.2140 for risperidone and 9-hydroxyrisperidone, respectively) within a ±5 ppm mass tolerance, while accurate data dependent MS2 analysis was simultaneously triggered to permit confirmation of identification. The lower limit of quantification was 1 nM for risperidone and 3 nM for 9-hydroxyrisperidone. The method is validated and used in routine analyses at Diakonhjemmet Hospital and the interday imprecision and inaccuracy parameters at the lower limit of quantification were <15% for 9-hydroxyrisperidone and <20% for risperidone. Reference standards for risperidone and 9-hydroxyrisperidone were purchased from US Pharmacopeia (North Bethesda, MD, USA), while deuterated internal standards (d4-risperidone and d4-9-hydroxyrisperidone) were purchased from Alsachim (Illkirch-Graffenstaden, France).

2.4 | Reprocessing of HRMS data files for solanidine metabolomics

The full-scan MS data generated from the TDM analyses of risperidone and 9-hydroxyrisperidone are not analyte specific, but rather general information characterized by the intrinsic characteristics of accurate mass and high resolution of all ionizable compounds in the purified serum sample that reaches the detector. This enables retrospective analysis of other compounds of interest in the HRMS data files, not only the drugs originally determined by the TDM analysis, without the need for additional injection of the samples. By using this methodological approach, serum levels of solanidine and five solanidine metabolites; M402, M414, M416, M440 and M444, were semi-quantified by retrospective reprocessing of the archived HRMS data files from the corresponding TDM analysis of risperidone and 9-hydroxyrisperidone, and subsequent extraction of the chromatographic peak areas, as described previously.[19] In short, solanidine and the metabolites were identified in the samples by accurate mass (the protonated molecule of solanidine and the metabolites were identified in the spectra within a ±5 ppm mass tolerance) and isotopic pattern. Liquid chromatograms from patients genotyped as *CYP2D6*1/*1* and *CYP2D6*4/*5* are provided in Figure S1. Molecular formulas, accurate *m/z* and average retention times for solanidine and the five metabolites are presented in Table S1. The identity of solanidine was confirmed using retention time and matched MS/MS spectrum by analysing a reference standard purchased from Phytolab (Vestenbergsgreuth, Germany). TraceFinder 5.1 (Thermo Fisher Scientific, Waltham, MA, USA) was used for data processing.

The chromatographic peaks were integrated automatically, but all chromatograms were checked manually by a trained technician (B.M.W.). The peak areas were used as response variable for the semi-quantitative measurements and in the calculation of the metabolite-to-solanidine ratios. Reprocessing of the HRMS data files was also used to detect use of the CYP2D6 inhibitors bupropion (limit of detection [LOD], 8 nM), fluoxetine (LOD, 20 nM), paroxetine (LOD, 6 nM) and levomepromazine (LOD, 1 nM).

2.5 | Analysed parameters and statistical analysis

The metabolic ratio (MR) of risperidone (9-hydroxyrisperidone-to-risperidone) was calculated and defined as a reference on CYP2D6 activity. Undetected risperidone concentrations were truncated to 0.4 nmol/L (= LOD). Metabolic ratios of solanidine (metabolite-to-solanidine) were calculated for each metabolite. Undetectable levels of solanidine and solanidine metabolites were truncated to half of the minimum value found in a previous project[19] for the corresponding analyte to enable proper calculations of MRs (i.e., solanidine, 1278 AUC; M402, 970 AUC; M414, 782 AUC; M416, 1117 AUC; M440, 963 AUC; M444, 629 AUC). All ratios were ln-transformed prior to the statistical analyses. Patients with undetectable serum levels of both solanidine *and* the metabolite of interest were excluded from the respective statistical analyses.

Spearman's correlation test was used to investigate correlations between various MRs of solanidine and the MR of risperidone. The correlations were initially performed for the whole population. The metabolite-to-solanidine ratio with the strongest association in the initial analysis was then carried forward to CYP2D6 activity score-defined subgroup analyses. Patients with inconclusive *CYP2D6* genotype results were not included in the subgroup analyses.

The correlation coefficients, ρ, are presented with 95% confidence intervals. GraphPad version 9 (GraphPad Software, San Diego, CA, USA) was used for statistical analyses and graphical presentations. $P < .05$ was considered statistically significant.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,[20] and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/2020.[21]

3 | RESULTS

Initially, 332 patients were considered for inclusion. Of these, 103 patients did not fulfil the inclusion criteria due to low or high age ($n = 75$), sampling time post risperidone dose intake <10 h ($n = 6$), concomitant use of injectable formulation of paliperidone ($n = 17$) and undetectable serum levels of both risperidone *and* 9-hydroxyrisperidone reflecting nonadherence ($n = 5$), leaving 229 patients eligible for analysis.

Patient characteristics of the included patients are given in Table 1. Five patients did not have detectable levels of solanidine or any of the solanidine metabolites in their serum sample and were not included in the correlation analyses. Metabolic ratios of M402, M414, M416, M440 and M444 could be calculated for 91.5, 99.6, 96.4, 82.6 and 92.9% of the remaining 224 patients, respectively. Details on detection frequencies and detected chromatographic peak areas of solanidine and the different metabolites are reported in Table S1.

The correlations between the MRs of the five solanidine metabolites and MR of risperidone are presented in Figure 1. Highly significant ($P < .0001$), positive correlations were observed between risperidone MR and all the metabolite-to-solanidine MRs (M402: ρ 0.68 [confidence interval 0.59, 0.75]; M414: ρ 0.60 [0.51, 0.68];

TABLE 1 Patient characteristics.

Female/male	106/123
Age (years), median (range)	38 (18–65)
Risperidone dose (mg), median (range)[a]	3 (0.5–10)
Risperidone serum conc. (nM), median (range)[b]	7 (1–144)
9-hydroxyrisperidone serum conc. (nM), median (range)	36 (3–178)
Time since last intake (h), median (range)[c]	14 (10–37)
CYP2D6 inhibitor users (*n*)	15
(Bupropion, fluoxetine, levomepromazine, *n*)	4, 4, 7
CYP2D6 activity score	*CYP2D6* genotype (n)
0	**4/*4* (5), **4/*5* (5), **4/*6* (1)
0.25	**4/*10* (1)
0.5	**4/*9* (2), **3/*41* (1), **4/*41* (3)
0.75	**9/*10* (1)
1	**1/*3* (6), **1/*4* (54), **1/*5* (10), **1/*6* (3), **10/*41* (1), **41/*41* (2)
1.25	**1/*10* (1)
1.5	**1/*9* (8), **1/*41* (24)
2	**1/*1* (95)
>2.25	**1/*1xN*(4)
Inconclusive genotype result	**1/*4xN(1)*,**1/*41xN*(1)

Note: CYP2D6 activity scores according to joint consensus guidelines by the Dutch Pharmacogenomics Working Group and the Clinical Pharmacogenetics Implementation Consortium.[18]
Abbreviation: Conc., concentration.
[a]Unknown dose, $n = 2$.
[b]Concentrations below the limit of detection, $n = 27$.
[c]Unknown time, $n = 43$.

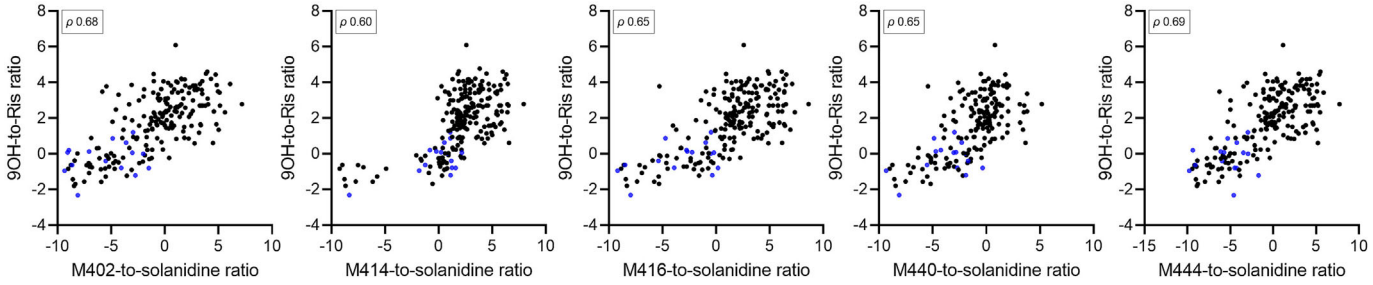
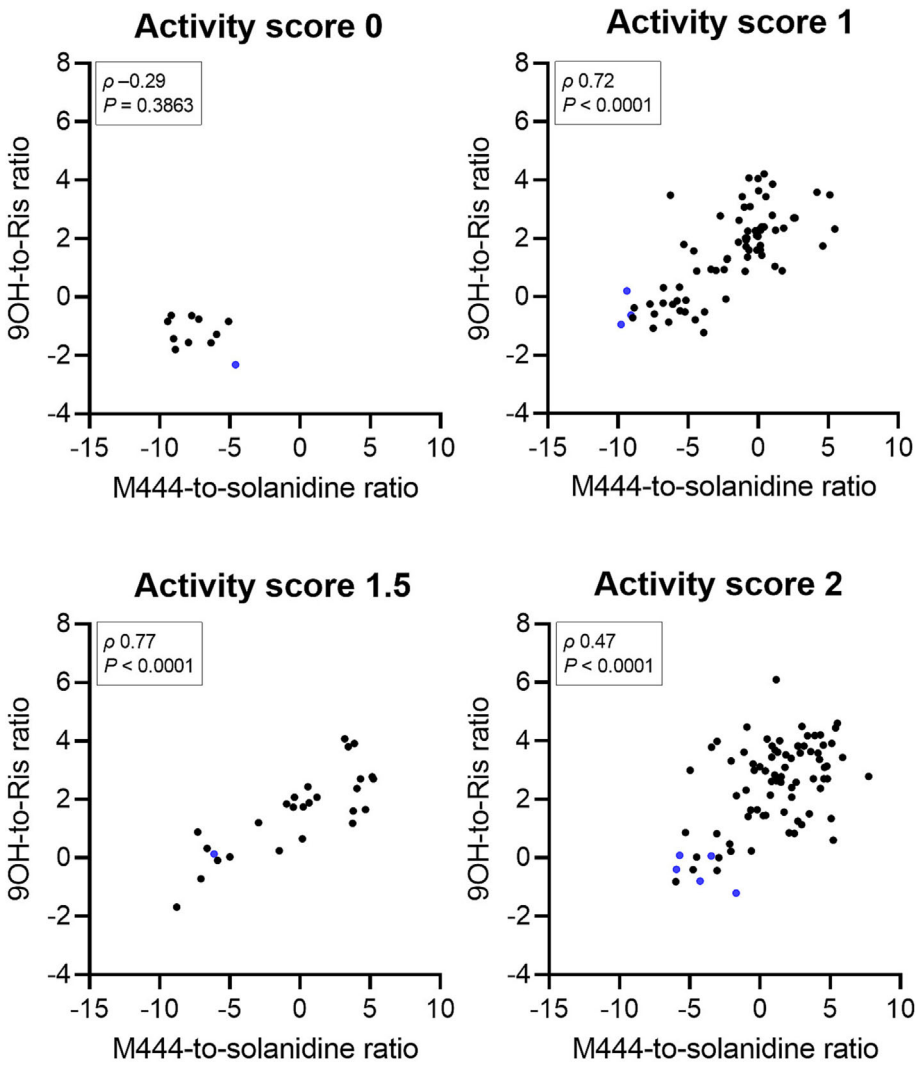


FIGURE 1 Correlations between the metabolite-to-solanidine ratios of the five solanidine metabolites (M402, M414, M416, M440 and M444) and the metabolic ratio of risperidone (9-hydroxyrisperidone-to-risperidone, 9OH-to-Ris). Each dot represents a patient sample and blue dots represent CYP2D6 inhibitor users. Spearman ρ from Spearman's correlation tests is added on each plot. All correlations were significant ($P < .0001$).

FIGURE 2 Correlations between the metabolic ratios of M444-to-solanidine and 9-hydroxyrisperidone-to-risperidone (9-OH-to-Ris ratio) within CYP2D6 genotype-predicted activity score-defined subpopulations. *P* and ρ values from Spearman's correlation tests are added on each plot. Each dot represents a patient sample and blue dots represent CYP2D6 inhibitor users.



M416: ρ 0.65 [0.57, 0.72]; M440: ρ 0.65 [0.55, 0.72]; and M444: ρ 0.69 [0.61, 0.76]; Figure 1).

The CYP2D6 activity score distribution of the patients with available MRs of M444 generated the following CYP2D6 activity score subgroups: score 0 ($n = 11$), 0.25 ($n = 1$), 0.5 ($n = 6$), 0.75 ($n = 1$), 1 ($n = 73$), 1.25 ($n = 1$), 1.5 ($n = 27$), 2 ($n = 82$) and >2.25 ($n = 4$). The correlations between risperidone MR and M444-to-solandine MR were further assessed within the four CYP2D6 activity score-defined subgroups with >10 patients (Figure 2). The positive correlations between M444-to solanidine MR and risperidone MR remained significant within the *CYP2D6* genotype subgroups with activity score of 1 (ρ 0.72 [0.58, 0.82], $P < .0001$), 1.5 (ρ 0.77 [0.55, 0.89], $P < .0001$)

and 2 (ρ 0.47 [0.28, 0.63], $P < .0001$). In the remaining subgroup with an activity score of zero (CYP2D6 poor metabolizers), there was no apparent correlation ($P > .05$).

4 | DISCUSSION

In this study we show that metabolic ratios of five different solanidine metabolites are strongly and significantly correlated with CYP2D6-mediated metabolism of risperidone. These results further support the potential usefulness of measuring solanidine and its metabolites as a biomarker for individual CYP2D6 metabolism, reflecting both genetic and nongenetic sources of interindividual variability. Importantly, significant and positive correlations were found within subgroups with *CYP2D6* genotypes encoding functional CYP2D6 metabolism. Thus, these findings suggest that solanidine metabolism may also predict individual CYP2D6 metabolism within patient subgroups with similar genotype-predicted activity scores.

Solanidine metabolism as a potential CYP2D6 activity marker was recently reported in a global metabolomics study.[11] The authors found strong correlations between the metabolic ratios of three of the solanidine metabolites investigated in the present study, i.e., M414, M416 and M444, and the urinary metabolic ratio of dextromethorphan (*O*-demethylation to dextrorphan), which is a well-established exogenous CYP2D6 biomarker, in 43 adults before and after a CYP2D6 inhibition session with paroxetine.[11] Prior to this, a significant difference was also reported between M444 levels in urine, normalized to creatinine, and the urinary metabolic ratio of dextromethorphan in 10 adult participants before and after fluoxetine intake.[22]

One of the objectives of the present study was to assess whether the solanidine metabolic ratios and CYP2D6-mediated metabolism of risperidone were correlated within subgroups assigned to the same CYP2D6 metabolizer subgroup based on the CYP2D6 genotype-derived activity scores, in which the patients are assumed to exhibit similar enzyme activity. The large variability in the individual metabolic ratios of risperidone within the different CYP2D6 activity score-defined subgroups demonstrates that prediction of CYP2D6 activity based on activity scores alone is not sufficient to account for all aspects of variability in CYP2D6 metabolism. Encouragingly, the strong, positive correlations between risperidone and M444-to-solanidine MRs observed for the whole population, independently of *CYP2D6* genotype, were maintained in the subgroup analyses, exemplifying how solanidine metabolic ratios also could add information on CYP2D6 phenotype in patients who are assumed to exhibit similar enzyme activity based on CYP2D6 genotyping. Such sources of variability could be rare genetic variants and additional genetic variants influencing the expression or activity of CYP2D6 not yet identified, as well as potential comedication with interacting drugs and other nongenetic factors. Notably, the correlation was poor in the subgroup with a CYP2D6 activity score of zero (i.e., poor metabolizers), which supports the CYP2D6 specificity of the biomarker.

To our knowledge solanidine is the first dietary CYP2D6 probe to be described, and one of the most promising nondrug biomarkers of CYP2D6 metabolizer phenotype. The detection rate of solanidine (75%) was the same as reported elsewhere.[11] However, because the detection rates of the metabolites are typically higher, we were able to calculate at least one of the solanidine MRs in 224 of 229 (98%) of the included patients. Although individual potato consuming habits were unknown in this study, this high frequency of detecting metabolites indicates that the potato content in western diets is sufficient for deriving a solanidine-based CYP2D6 activity prediction in almost all of individuals. This demonstrates the great potential of solanidine as an easy-to-use and side-effect-free CYP2D6 activity marker, which also may be applied for phenotype predictions in vulnerable patient populations. Future studies in residents of regions with typical diets containing less potato-derived food products would be informative to understand the global usefulness of this biomarker.

Novel phenotyping probes need validation, and the correlation between the candidate phenotyping metric and another validated reference metric is regarded as a key assessment when evaluating the ability of exogenous and endogenous probes to reflect CYP2D6 phenotype.[5,7] Although risperidone MR is not a formally accepted reference metric for CYP2D6 activity, its previously demonstrated strong correlation with debrisoquine[17] and CYP2D6 genotype[23,24] support that it should reflect CYP2D6 activity reasonably well. Therefore, the results from the current large-scale study add to the emerging evidence of solanidine metabolism as a potential CYP2D6 activity marker as previously suggested based on smaller cohorts.[11,22] Nevertheless, additional studies examining the correlation between solanidine MRs and other CYP2D6 activity metrics would be of interest to further support these findings. The availability of a validated, quantitative analytical assay would also be necessary before the use of solanidine could be implemented in clinical practice for assessing individual CYP2D6 metabolism. Development and validation of such assay have in part been impeded by the lack of structural information of the solanidine metabolites. M444 was recently identified as 3,4-seco-solanidine-3,4-dioic-acid[25] and it is expected that structure elucidation of the other metabolites will follow.

A limitation of this study is that individual CYP2D6-mediated partial clearance of risperidone cannot be accurately estimated based on single-point pharmacokinetic data.[26] The metabolic ratio reflects the activity of the enzymatic pathway of interest, but this derived metric is sensitive to sampling time differences as well as interindividual variability in metabolite clearance, which is mainly renal for 9-hydroxyrisperidone. Although elderly subjects were excluded to reduce the impact of age-dependent renal impairment, differences in renal function may still have caused non-CYP2D6-related variability in the risperidone MRs. To what extent similar limitations apply to the derived MRs of solanidine is difficult to assess, considering the scarce pharmacokinetic information currently available for its metabolites. Another important limitation is the use of a semi-quantitative method for the analysis of solanidine and its metabolites, which is also likely to introduce additional variability to the solanidine MRs. Although the use of metabolic ratios rather than the individual chromatographic peak areas is likely to increase the robustness of our semi-quantitative data by compensating for inconsistency during the sample

preparation, poor injection reproducibility and differences in detector response between runs, the lack of internal standards does not allow correction for factors affecting the ionization efficacy. Still, the correlations were indeed strong, and the availability of more accurate individual estimates of CYP2D6-mediated clearance as well as a quantitative analytical assay for solanidine and metabolite measurements may have further strengthened them. The major strength of this study is the large number of included subjects and that the results of this naturalistic study setting reflect real-life. An additional strength is that reprocessing of the HRMS data files could identify comedication of the CYP2D6 inhibiting drugs bupropion, fluoxetine, paroxetine and levomepromazine. As shown in the presented graphics, these individuals typically present low metabolic ratios of both solanidine and risperidone and align well with subjects who have lowered CYP2D6 metabolism for unknown reasons. As information about concomitant use of CYP2D6-inhibiting drugs may not always be available in a clinical setting, this indicates that solanidine metabolic ratios would also be applicable to identify abnormal metabolism due to CYP2D6-related drug interactions.

In conclusion, the present study shows that solanidine and its metabolites measured in serum samples may provide suitable biomarkers of CYP2D6 metabolizer phenotype, as demonstrated by their strong, positive correlations with CYP2D6-mediated metabolism of risperidone. The strong correlations remained within *CYP2D6* genotype subpopulations encoding functional metabolism, which supports a potential of solanidine MR as a biomarker capturing all sources of CYP2D6 variability and thus could improve prediction of the individual clearance of drugs that are CYP2D6 substrates.

AUTHOR CONTRIBUTIONS

Birgit M. Wollmann wrote the manuscript. Birgit M. Wollmann, Robert L. Smith, Marianne Kristiansen Kringen, Magnus Ingelman-Sundberg, Espen Molden, and Elisabet Størset designed the research. Birgit M. Wollmann and Marianne Kristiansen Kringen performed the research. Birgit M. Wollmann and Elisabet Størset analysed the data.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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