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SHORT COMMUNICATION

Common UGT1A9 polymorphisms do not have a clinically meaningful impact on the apparent oral clearance of dapagliflozin in type 2 diabetes mellitus

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Dapagliflozin is an inhibitor of human renal sodium-glucose cotransporter 2 (SGLT2), first approved for the treatment of type 2 diabetes mellitus (T2DM). Dapagliflozin is primarily metabolized by uridine diphosphate glucuronosyltransferase 1A9 (UGT1A9). The effect of UGT1A9 polymorphisms on dapagliflozin apparent oral clearance (CL/F) was studied with dapagliflozin population pharmacokinetic data and UGT1A9 genotype data (I.399C>T, rs2011404, rs6759892, rs7577677, rs4148323, UGT1A9*2 and UGT1A9*3) from a Phase 2 study conducted in subjects with T2DM ($n = 187$). An analysis of covariance (ANCOVA) model accounting for known covariates influencing dapagliflozin CL/F was applied to these data to quantify the impact of each UGT1A9 polymorphism relative to the wildtype UGT1A9 genotype. The analysis showed that the geometric mean ratios of dapagliflozin CL/F for all of the UGT1A9 polymorphisms studied were within the range of wildtype UGT1A9 CL/F values. Consequently, the polymorphisms of UGT1A9 studied had no clinically meaningful impact on the CL/F of dapagliflozin.

KEYWORDS
dapagliflozin, oral clearance, polymorphism, type 2 diabetes mellitus, UGT1A9

1 | INTRODUCTION

Dapagliflozin is a potent, highly selective and orally active inhibitor of human renal sodium-glucose cotransporter 2 (SGLT2), the transporter responsible for the majority of renal glucose reabsorption.[1] Dapagliflozin lowers blood plasma glucose concentrations by inhibiting renal reabsorption of glucose in the proximal tubule, thus promoting urinary glucose excretion. Dapagliflozin is readily absorbed with a high absolute oral bioavailability (78%), with dose-proportional systemic exposures for doses ranging from 0.1 to 500 mg. The half-life of dapagliflozin is about 12.5 hours following oral administration. Sixty-one percent[2] of the administered dose of dapagliflozin is metabolized through glucuronidation via uridine diphosphate glucuronosyltransferase 1A9 (UGT1A9).[3]

The UGT1A9 gene is encoded by the UGT1A gene cluster on human chromosome 2q37. This highly complex locus produces nine unique enzymes (UGT1A1, UGT1A3–10), with different N-termini

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The authors confirm that James List, MD, PhD was the Study Director of Study MB102008, the study in which the pharmacogenomic and pharmacokinetic samples were collected. Dr List was an employee of Bristol-Myers Squibb (BMS) when he was involved in the study. The manuscript for this exploratory analysis objective of the study was written after Dr List left BMS and company policy is not to have ex-employees as authors if they did not fully participate in the writing of the manuscript.

and identical C-termini, via exon sharing and alternative splicing. Each protein comprises a unique alternate exon 1 that encodes the substrate binding site and is regulated by its own promoter.[4]

Although UGT1A9 is a polymorphic gene, there are no reported common amino acid changing or protein truncating UGT1A9 variants. UGT1A9*2 (p.Cys3Tyr; rs145084767) and UGT1A9*3 (p.Met33Thr; rs72551330) are both relatively rare (global minor allele frequencies: 0.001 and 0.009, respectively) (Supplementary Table S1), but have been shown to decrease the metabolism of some substrates. For example, it has been reported that UGT1A9*3 reduces the rate of glucuronidation of SN-38, an antineoplastic drug, to 3.8% of the activity of the wildtype (UGT1A9*1) allele.[5,6] In addition, the common intron I.399C>T polymorphism in UGT1A9 (global minor allele frequency: 0.382) (Supplementary Table S1) has been found to increase glucuronidation of SN-38 both in vivo[6] and in vitro[7] but did not account for the interindividual differences in the pharmacokinetics of the UGT1A9 substrate mycophenolic acid.[8] Hence, the overall functional relevance of the I.399C>T polymorphism cannot be generalized across substrates. Furthermore, the functional significance of five other common (global minor allele frequency >0.1) intronic polymorphisms (rs2011404, rs1105880, rs6759892, rs7577677 and rs4148323) have not been comprehensively studied. Since the impact of UGT1A9 polymorphisms on activity may not be generalizable, and with UGT1A9 being the major clearance mechanism of dapagliflozin, this analysis assesses the potential of several common single nucleotide polymorphisms (SNPs) of UGT1A9 to affect the apparent oral clearance of dapagliflozin.

What is already known about this subject

- Dapagliflozin is an SGLT2 inhibitor that lowers blood plasma glucose by inhibiting renal glucose reabsorption.
- Dapagliflozin is primarily metabolized through glucuronidation via UGT1A9.
- Specific UGT1A9 single nucleotide polymorphisms (SNPs) have been shown to have altered metabolic activity for some substrates.

What this study adds

- The analysis showed that the geometric mean ratio of dapagliflozin apparent oral clearance for all of the UGT1A9 polymorphisms studied were within the range of wildtype UGT1A9 apparent oral clearance values.
- The polymorphisms of UGT1A9 studied had no clinically meaningful impact on the apparent oral clearance of dapagliflozin.

2 | METHODS

The dataset is based on a subset of a randomized, double-blind, placebo-controlled, dose-ranging, parallel-group longitudinal phase 2 study[9] undertaken in anti-diabetic drug-naïve patients with type 2 diabetes mellitus (T2DM) who voluntarily provided informed consent for genetic analysis. The dataset included patients that had a valid genotype result and an apparent oral clearance value estimated by a population pharmacokinetic model[10] using dapagliflozin plasma concentrations assayed from sparse samples. Only subjects who voluntarily signed the informed consent and provided DNA samples for the pharmacogenetic analysis were included in the genetic analysis ($n = 187$ with dapagliflozin apparent oral clearance values and UGT1A9 genotype data, out of a total of 279 patients randomized to dapagliflozin). These data were deidentified and utilized to create a model that incorporated appropriate clinical covariates known to affect dapagliflozin pharmacokinetics[10] before estimating apparent oral clearance differences by genotype. The analysis used the dataset to explore associations among genetic variation and estimated dapagliflozin apparent oral clearance. The Hardy–Weinberg equilibrium (HWE) test for potential genotyping error was conducted before analysing for association.[11]

An analysis of covariance (ANCOVA) model was used to estimate the effect of eight different SNPs of the UGT1A9 gene (UGT1A9*2, UGT1A9*3, I.399C>T, rs2011404, rs1105880, rs6759892, rs7577677 and rs4148323) on dapagliflozin apparent oral clearance. The initial model covariates consisted of baseline body weight and estimated glomerular filtration rate (eGFR) values, treatment regimen and demographic factors such as race, age and gender. The model was reduced to include only covariates significantly explaining the variability in dapagliflozin apparent oral clearance. The model selection procedure took place before any SNPs were introduced to select only the SNPs that contributed additional variability in the dapagliflozin apparent oral clearance. The standard errors, covariate-adjusted genotype least square means (LSMEAN) and 95% confidence interval (CI) for each SNP were calculated. The control of the false discovery rate (FDR) proposed by Benjamini and Hochberg was used in this analysis.[12] The adjustment is called FDR-adjusted *P*-value, hereafter.

A logarithmic transformation was required for the dapagliflozin apparent oral clearance to have a linear relationship with the covariates. Several demographic/laboratory baseline characteristics that might affect pharmacokinetics were tested when building the ANCOVA model. The covariates that were significantly associated with the dapagliflozin apparent oral clearance were baseline values of weight and eGFR. These two baseline characteristics were also found in the population PK model.[10] Equation 1 shows the ANCOVA model used to test the significance of the genotype for each SNP (GT1 and GT2) on dapagliflozin apparent oral clearance:

$$\mathrm{Log}\,(\mathrm{clearance}) = \beta_0 + \beta_1\,\mathrm{baseline\_weight} + \beta_2\,\mathrm{baseline\_GFR} + \beta_3\,\mathrm{GT1} + \beta_4\,\mathrm{GT2} + \varepsilon \quad (1)$$

The FDR-adjusted and raw (unadjusted) *P*-values for SNP effects and covariates in the ANCOVA model were arranged in tabular form. The

LSMEAN of the genotypes for each SNP was tabulated separately, and the contrasts of mean clearance by genotype (geometric mean ratio) were calculated.

To evaluate the possibility of a genotype effect between heterozygotes and common homozygotes (Contrast 1 [C01]), as well as between common and rare homozygous genotypes (Contrast 2 [C02]), two contrasts were used. C01 was the primary contrast of interest. These contrasts were specified as follows, where μ_i is the mean final endpoint value for the genotype of interest (GT*i*):

C01: μ_0 - μ_1.
C02: μ_0 - μ_2.

These contrasts are equivalent to estimating the geometric mean ratios for clearance between genotypes. Exponentiating the C01 value yields the geometric mean ratio of common homozygotes to heterozygotes. Likewise, exponentiating the C02 value produces the geometric mean ratio of common to rare homozygotes. If C01 is equal to zero, both the C01 *P*-values obtained from testing and the common homozygous to heterozygous geometric mean ratio will be equal to one. Similarly, where C02 is equal to zero, both the C02 *P*-values obtained from testing and the common to rare homozygous geometric mean ratio will be equal to one.

3 | RESULTS

Using a goodness of fit test, it was determined that one SNP (rs6431625) was out of HWE due to its abundance of heterozygotes ($n = 128$) compared with common homozygotes ($n = 31$) and rare homozygotes ($n = 2$). Thus, rs6431625 was not included in the analysis.

Table 1 contains the model-adjusted geometric mean apparent oral dapagliflozin clearance ratios and the corresponding 95% CIs. These ratios compare the model-estimated dapagliflozin apparent oral clearance of common homozygous subjects to subjects with other genotypes.

Table 2 summarizes the *P*-values for testing the significance of the genotype for SNPs and for testing if each contrast is significantly different from zero. None of the *P*-values for genotype or the primary contrast of interest (C01) are less than 0.05. FDR-corrected *P*-values are also provided and none are less than 0.05.

Table 3 contains the model-adjusted geometric mean clearance ratios and corresponding 95% CIs by genotype for each SNP. The ratios compare the model-adjusted dapagliflozin apparent oral clearance values of common homozygous subjects to individuals with other genotypes.

Supplementary Table S1 contains population allele frequencies of UGT1A9 variants analysed in the study. Supplementary Tables S2–S5 contain UGT1A9 variant annotations in the Pharmacogenomics Knowledgebase.[13]

4 | DISCUSSION

The pharmacodynamic effect of urinary glucose excretion for dapagliflozin is driven mainly by area under the curve (AUC), and it has been shown that dapagliflozin AUC is determined primarily by clearance, the majority of which is via UGT1A9.[14] Based on the pharmacokinetic, pharmacodynamic and relatively dose-independent safety/tolerability profile of dapagliflozin, population AUC/apparent oral clearance differences of less than two-fold relative to a reference population (ratio range of 0.5–2) are not considered as clinically meaningful and do not need a dose adjustment.[15] The objective of this analysis was to determine whether SNPs within the UGT1A9 gene affect the apparent oral clearance of dapagliflozin beyond these magnitudes.

TABLE 1 Model-adjusted geometric mean ratios comparing clearance between genotypes, with 95% CIs

SNP ID	Common/Het (C01)			Common/rare (C02)		
	Geometric ratio estimate	95% CI	N″	Geometric ratio estimate	95% CI	N″
UGT1A9*2+, C3Y (rs145084767)	–	–	183/0	–	–	183/0
UGT1A9*3+, M33T (rs72551330)	–	–	182/3	–	–	182/0
I.399C>T (rs2741049)	0.93	0.79, 1.10	53/93	0.87	0.71, 1.08	53/37
rs2011404	0.87	0.72, 1.04	118/42	–	–	118/1
rs1105880	1.17	0.99, 1.39	80/61	1.27	0.98, 1.63	80/20
rs6759892	1.08	0.91, 1.29	65/72	1.29	1.01, 1.64	65/24
rs7577677	1.13	0.96, 1.34	71/72	1.31	0.99, 1.73	71/16
rs4148323+	–	–	156/4	–	–	156/0

Ratios were not estimated for SNPs indicated by (+) that contained fewer than five subjects with non-wildtype genotypes. N″ shows counts for common homozygotes/heterozygotes or common homozygotes/rare homozygotes. C01 *P*-values are equal to testing if common/heterozygote geometric mean ratio = 1, and C02 *P*-values are equivalent to testing if common/rare geometric mean ratio = 1.
C01, Contrast 1; C02, Contrast 2; CI, confidence interval; Het, heterozygote; SNP, single nucleotide polymorphism; UGT1A9, uridine diphosphate glucuronosyltransferase 1A9.

TABLE 2 *P*-values and FDR corrections of genotype and contrasts/ratios

SNP ID	Overall genotype *P*-value	Overall genotype FDR corrected	Common/Het (C01) *P*-value	C01 FDR corrected	Common/rare (C02) *P*-value	C02 FDR corrected
*2, C3Y+	–	–	–	–	–	–
*3, M33T+	–	–	–	–	–	–
rs2741049 (I.399C>T)	0.4373	0.4373	0.4004	0.4004	0.2059	0.2059
rs2011404	0.1253	0.1585	0.1253	0.2300	–	–
rs1105880	0.0802	0.1585	0.0732	0.2300	0.0677	0.0903
rs6759892	0.1268	0.1585	0.3746	0.4004	0.0428	0.0903
rs7577677	0.1062	0.1585	0.1380	0.2300	0.0579	0.0903
rs4148323+	–	–	–	–	–	–

SNPs indicated by (+) were not analysed as there were fewer than five individuals with non-wildtype genotypes.
C01, Contrast 1; C02, Contrast 2; FDR, false discovery rate; Het, heterozygote; SNP, single nucleotide polymorphism.

TABLE 3 Model-adjusted geometric mean clearance and 95% CIs

SNP ID	Common homozygote (AA)		Heterozygote (AB)		Rare homozygote (BB)	
	Geometric mean CL	95% CI	Geometric mean CL	95% CI	Geometric mean CL	95% CI
*2, C3Y+	–	–	–	–	–	–
*3, M33T+	–	–	–	–	–	–
rs2741049	19.2	16.7, 21.9	20.6	18.6, 22.8	22.0	18.7, 25.8
rs2011404	19.7	17.9, 21.6	22.8	19.4, 26.7	–	–
rs1105880	22.4	20.0, 25.1	19.1	16.8, 21.8	17.7	14.1, 22.2
rs6759892	22.0	19.4, 25.0	20.4	18.1, 22.9	17.1	13.9, 21.1
rs7577677	22.1	19.7, 24.9	19.5	17.3, 21.9	16.9	13.2, 21.7
rs4148323+	–	–	–	–	–	–

Ratios were not estimated for SNPs indicated by (+) that contained fewer than five subjects with non-wildtype genotypes.
CI, confidence interval; CL, clearance; SNP, single nucleotide polymorphism.

Model-based results considering baseline weight and baseline eGFR did not indicate that the primary comparison of interest was clinically meaningful. The wide CIs shown in Table 3 indicate that the model-based results do not identify clinically different estimates of clearance across genotypes for any SNPs. For some SNPs, however, there were insufficient heterozygotes and/or rare homozygotes to reliably estimate the mean clearance values.

Table 1 contains values derived from the reference value divided by the experimental clearance for the different SNPs. A ratio of two in this table would indicate that clearance has decreased by half. Similarly, a value of 0.5 would indicate that clearance has increased two-fold. All SNPs show a 95% CI within these two values, and thus the results do not indicate a clinically meaningful variation. The model analysis did not display widely varying estimates of the apparent oral clearance of dapagliflozin across all SNPs.

As observed in Table 3, the geometric mean clearances for common homozygotes with UGT1A9*3 or I.399C>T that have been shown to affect the pharmacokinetics of other UGT1A9 substrates were not clinically different from those of other SNPs on the clearance of dapagliflozin. These results, in conjunction with those previously observed in other studies,[7,8,16] suggest that variations in clearance due to polymorphisms in UGT1A9 are substrate dependent. Other frequent risk-prone SNPs such as T-275A and C-2152T, which lower the exposure to mycophenolic acid,[16] were not a part of this study; therefore, a conclusion on their potential effect on dapagliflozin clearance cannot be drawn from this analysis.

A limitation of this analysis is the small amount of viable data for non-wildtype subjects. Small clearance values may also be unreliable due to the paucity of data in the extremes of the distribution of clearance values used to predict the clearance via a noncompartmental analysis. For example, as observed in Tables 1 and 3, the marginally different mean clearance values for the rare homozygous subjects (rs6759892 and rs7577677) may have occurred by chance due to the small number of tested individuals. Similarly, in Table 2, the small number of subjects tested may have caused the rs6759892 common/rare (C02) *P*-value to be less than 0.05 and occur by chance.

In conclusion, the UGT1A9 results and the distribution of predicted dapagliflozin apparent oral clearance values in the analysis indicate that UGT1A9 genetic variation in the SNPs assessed do not result in clinically meaningful effects on the pharmacokinetics of dapagliflozin.

4.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.[17]

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M.D.N. and R.C. have no conflicts of interest to report. M.N., W.T. and D.W.B. are employees and shareholders of AstraZeneca.

CONTRIBUTORS

All authors contributed to the conception or design of the work, the acquisition, analysis, or interpretation of data for the work, and drafting the work or revising it critically for important intellectual content. All authors provided final approval of the version of the manuscript to be published and agree to be accountable for all aspects of the work. The authors meet the criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE). The authors received no direct compensation related to the development of the manuscript. Dr Mats Någård takes responsibility for (is the guarantor of) all the content in the manuscript, including the data and analysis.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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