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An exploratory pharmacogenetic screening of *SLC22A6*, *SLC22A8*, *ABCC4* and *ABCC10* genes in a cohort of Ghanaian HBV patients

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Abstract

Background Organic anion transporters and efflux transporters are involved in the metabolism of drugs such as tenofovir disoproxil fumarate (TDF). Given the important role of organic anions and efflux transporters in drug disposition, genetic variations lead to interindividual differences in drug response. Variations in the SLC and ABC transporters have been associated with drug-induced renal dysfunction. Looking at the prevalence of HBV infection in our population and the use of drugs such as TDF in managing this condition, this study aimed to undertake an exploratory analysis of genetic variation in renal transporters *SLC22A6, SLC22A8, ABCC10* and *ABCC4* in a Ghanaian HBV infected cohort.

Methods We genotyped 160 HBV infected patients for SNPs in *SLC22A6* (rs12293966, rs4149170, rs6591722, rs955434), *SLC22A8* (rs11568487), *ABCC10* (rs700008, rs831311*) and ABCC4* (rs9282570) genes. Clinicodemographic data was taken, and glomerular filtration rate (eGFR) was estimated using the CKD-EPI formula. Genotyping was undertaken using Iplex gold SNP genotyping protocol on the Agena MassARRAY® system. Statistical analysis was undertaken using packages in Stata SE (v17) and GraphPad prism. Hardy–Weinberg equilibrium, haplotype inference, and linkage disequilibrium (LD) were evaluated using web-based tools LDlink and Shesis.

Results The average eGFR was 79.78 ± 33.08 mL/min/1.73 m^2 with 31% classified as stage 1 with normal or high GFR (eGFR > 90 mL/min/1.73 m^2) and 45% with stage 2 CKD (> 60–89.99 mL/min/1.73 m^2). All variants were in HWE except rs4149170, rs9282570 and rs700008 where $p < 0.05$. Strong LD was observed in the variants rs6591722, rs4149170, rs12293966, rs955434 and rs11568487. There was significant association between rs12293966 and eGFR stage under crude dominant inheritance model (OR 0.27, 95% CI 0.08–0.81; $p = 0.019$). Under crude model (OR 0.21, 95% CI 0.07–0.66; $p = 0.008$), adjusted model 1 (OR 76, 95% CI 0.39–7.89; $p = 0.014$) and adjusted model 2 (OR 0.30, 95% CI 0.12–0.78; $p = 0.013$) there was significant association observed between rs12293966 and eGFR stage in a codominant inheritance.

Conclusion The associations observed in this study point to the need for further evaluation with the population of HBV patients on TDF treatment in addition to other factors that would lead to unfavorable outcomes. This exploratory finding may require confirmation in a larger cohort with proper phenotyping to investigate the exact pharmacogenetic mechanisms.

Keywords Tenofovir, *SLC22A6*, HBV, Pharmacogenetics, eGFR, Renal impairment, Transporters

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Introduction

Membrane transporters are important in drug disposition as they are key determinants in the pharmacokinetics of drugs where they affect absorption, distribution, metabolism and elimination (ADME) [1, 2]. Their role in the overall response to drugs and subsequent adverse reactions is well established. Several of these membrane transporters have been elucidated with their physiological roles highlighted. The ATP-binding cassette proteins (ABC) and solute carrier (SLC) transporters are two major superfamilies of membrane transporters [3–5]. ABC transporters use energy from ATP hydrolysis to function as efflux transporters, while SLC transporters are involved in the uptake of molecules into cells. These transporters play an important role in regulating the inflow and outflow of substances across plasma membranes such as organic or inorganic molecules, sterol, metal ions, polypeptides and proteins [6, 7]. In drug metabolism, considerable interest lies in these transporters, especially for drugs that are not metabolized by the cytochrome p450 family of enzymes. These transporters then serve in ADME of such pharmacologically diverse drugs and may serve as sites for interactions that underlie drug toxicities and adverse drug reactions.

In this study, we screened for transporters that are relevant to nephrotoxic drugs that are of clinical and pharmacological importance, such as tenofovir disoproxil fumarate (TDF), which has been recommended in treatment guidelines for human immunodeficiency virus (HIV) and hepatitis B virus [8]. Drug clearance in the proximal tubule of the renal nephron is controlled mostly by membrane transport proteins [9] and its uptake in the epithelial cells of the kidney tubules is mediated through anion transporters coded for by solute carrier family 22 member (SLCs) through basolateral membranes [10, 11]. There are other transporters involved in active efflux through the apical membrane responsible for excreting drugs. The anion transporters are encoded by *SLC22A6* and *SLC22A8* which act in the uptake of drugs into the renal proximal tubule while *ABCC10, ABCC2* and *ABCC4* control efflux across the apical membrane [11–14]. The genes that code for transporters have genetic variations which affect their accumulation, function, and efficiency.

The organic anion transporter *SLC22A6,* located on 11q12.3, has been shown to transport several substances including xenobiotics with its important role in renal function been greatly elucidated in literature [15]. Variants in *SLC22A6* have been previously associated with pharmacokinetic differences in nucleoside analogues such as adefovir, cidofovir and tenofovir [16–18]. The *SLC22A8* gene which is located on 11q12.3 encoding a protein is involved in Na^+ -independent transport and excretion of organic anions. This protein appears to be localized to the basolateral membrane of the kidney and therefore genetic variations may influence renal function [19]. *SLC22A8* has also been implicated in TDF-induced Falconi syndrome [20]. The *ABCC4* gene, located on 13q.32.1 is a member of the ABC transporters which plays an important role in cellular detoxification as a pump for substrates and may also function in prostaglandin-mediated cAMP signaling [21, 22]. Variations in *ABCC4* have been highlighted in studies involving tenofovir [13, 23]. *ABCC10* is located on 6p21.1 and is a transporter of various molecules across extra- and intra-cellular membranes. This transporter is a member of the multidrug resistance protein family that has been involved in multi-drug resistance [24]. *ABCC10* plays a significant role in gefitinib transport and its mechanism of action has been implicated in gefitinib drug resistance [25]. Genetic variations in *ABCC10* have been found to influence tenofovir renal tubular transport and may potentially contribute to kidney dysfunction [26].

Due to reports of genetic variations that are associated with impaired transport function, which may influence drug disposition, it is important to identify SNPs that may confer susceptibility to individuals.

In this study, we selected HBV patients being treated with tenofovir and those not on treatment and screened them for variations in *SLC22A6*, *SLC22A8*, *ABCC4* and *ABCC10.* Also, since these genes are involved in drug metabolism with variations affecting renal function, we explored any potential association between the variants and renal function using eGFR.

Methods

Study design and subjects

This study is a single-center cross-sectional study to explore genetic variations in genes encoding tubular transporters and the potential for renal dysfunction in HBV patients in Ghana. The study cohort consisted of 160 unrelated participants. We recruited participants from March 2021 to April 2022. To be considered eligible, participants, male or female had to be at least 18 years and diagnosed with HBV infection. HBV infection was diagnosed using Wondfo One step HBsAg whole blood/serum/plasma rapid immunochromatographic assay (Guangzhou Wondfo Biotech Co., Ltd, China) using blood plasma. Eligible participants were approached and informed of the study verbally and those willing to enroll consented. The participants were recruited at the hepatitis clinic at the Cape Coast Teaching Hospital in Cape Coast, Ghana. To maintain confidentiality, all participants were given unique codes as a means of deidentification and assigned study numbers. Participants were recruited irrespective of whether they were at the acute

stage or chronic stage of infection. Clinical data was collected, including their medication history. The following people were excluded from the study: pregnant or lactating women; patients with hypertension, diabetes mellitus or known renal dysfunction; or any other medication known to affect kidney function. Whole blood was collected into vacutainer tubes for renal function tests and DNA extraction. Aliquoted samples were stored at − 20 °C until ready for DNA extraction.

Sample size

The prevalence of HBV in Ghana is estimated to be 8.36% [27]. Based on the data at the hepatitis clinic at the Cape Coast Teaching Hospital, the average population is 1000. With a confidence interval of 95%, 5% margin of error and a 5% provision for contingency, the required sample size for this exploratory study is 160. The Raosoft software was used to calculate the sample size.

Ethics statement

This study obtained ethical clearance from the Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC/EC/2021/005). All research protocols were in accordance with the Helsinki declaration. Participants provided both written and verbal consent to participate in this study. Participants were provided with study numbers and unique codes to deidentify and anonymize them.

Biochemical measurement

Blood was collected on the day of recruitment along with other body measurements. Serum creatinine (sCr) and urea were estimated using Selectra Pro XL autoanalyzer (ElitechGroup, Puteaux, France). The estimated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI formula [28].

The various stages of renal dysfunction were therefore classified based on the eGFR as follows:

- Stage 1 with normal or high GFR (GFR > 90 mL/min/1.73 m^2)
- Stage 2 Mild CKD (GFR = 60–89 mL/min/1.73 m^2)
- Stage 3A Moderate CKD (GFR = 45–59 mL/min/1.73 m^2)
- Stage 3B Moderate CKD (GFR = 30–44 mL/min/1.73 m^2)
- Stage 4 Severe CKD (GFR = 15–29 mL/min/1.73 m^2)
- Stage 5 End Stage CKD (GFR < 15 mL/min/1.73 m^2)

DNA extraction and pharmacogenetic analysis/genotyping

DNA was extracted from 200uL of whole blood for each patient using the E.Z.N.A ® blood DNA mini kit (Omega Bio-tek, Inc. Norcross, USA) according to the manufacturer's instructions. Extracted DNA was diluted to a concentration of 20 ng/uL for genotyping procedures. A candidate gene approach was utilized to select 8 SNPs in total. The SNPs selected were rs12293966, rs4149170, rs6591722, rs955434 rs11568487, rs700008, rs831311 and rs9282570. Selection criteria for these SNPs included those that had been previously reported in African populations. A total of SNPs were genotyped using the Iplex GOLD SNP genotyping protocol on the Agena MassARRAY ®system (Agena Bioscience™, San Diego, CA, USA) [29].

Statistical analysis

Data for this study was collected using the KoBo toolbox [30]. Statistical analysis was undertaken using STATA, version 17.0 (StataCorp, College Station, Texas, USA) and Graphpad v9 (Prisma, San Diego, California) statistical software packages. Continuous variables were expressed as mean ± standard deviation or median (inter-quartile range), with categorical variables being expressed as absolute values and or frequencies. Baseline characteristics were compared between patients who had eGFR < 60 mL/min/1.73 m^2 and those that had > 60 mL/min/1.73 m^2 by z-test and either chi-square test or Fisher's exact test for categorical variables. The Wilcoxon signed test was used to test for non-parametric data. Linkage disequilibrium, haplotype genotype and allele frequencies were calculated using a web based tool LDlink [31] and Shesis Plus [32]. SNPs were tested for departure from Hardy–Weinberg Equilibrium (HWE) using a chi-square goodness of fit test. To test for an association between eGFR/CKD stages and SNPs, logistic regression models were fitted. Each SNP was presented as a predictor variable in the presence of a minimum of one (1) copy of the minor allele in a dominant model, two (2) copies of the minor allele in a recessive model and a codominant model. Age, duration of diagnosis, medication use, and gender were included in the model as covariates. Statistical significance was considered at $p < 0.05$.

Results

Our study included 160 patients who had been diagnosed with HBV infection with or without TDF treatment. The characteristics of our study population are listed in Table 1. Of these 160, only 17 (10.62%) were on TDF treatment. The HBV cases were made up of acute and chronic forms, and only 13% of patients who had chronic HBV were on treatment. HBV DNA was low in 35% of the

Table 1 Clinical and demographic data of patient population (N = 160)

Gender	N (%)	*p* value
Male	76 (47.50)	0.528
Female	84 (52.50)	
Age		
Male median age (IQR)	31 (24–40)	0.054
Female median age (IQR)	35 (28–43)	
Herbal medicine usage		
Yes	12 (7.64)	0.001*
No	140 (92.36)	
Treatment		
TDF	17 (10.62)	0.001*
Without treatment	143 (89.38)	
Creatinine (mg/dL) median (IQR)		
TDF	0.32 (0.29–0.36)	0.305
Without treatment	0.34 (0.27–0.47)	
Classification		
Acute	47 (29.38)	0.001*
Chronic	113 (70.62)	
Duration versus treatment		
< 6 months + TDF	3 (5.67)	0.001*
< 6 months + without treatment	44 (94.33)	
> 6 month + TDF	14 (9.62)	0.001*
> 6 month + without treatment	99 (90.38)	
VL (copies/mL)		
0–299	57 (35.62)	0.001*
300–9999	84 (52.50)	
10,000–999991	15 (9.38)	
> 100,000	4 (2.50)	
eGFR, mL/min/1.73 m²		
Stage 1 (> 90 mL/min/1.73 m²)	50 (31.25)	0.001*
Stage 2 (> 60–89.99 mL/min/1.73 m²)	72 (45.00)	
Stage 3a (> 45–59.99 mL/min/1.73 m²)	23 (14.38)	
Stage 3b (> 30– 44.99 mL/min/1.73 m²)	10 (6.25)	
Stage 4(15–29.99 mL/min/1.73 m²)	3 (1.88)	
Stage 5 (< 15 mL/min/1.73 m²)	2 (1.25)	

Continuous variables are expressed as median (IQR) while categorical variables are expressed as absolute values and or frequencies

TDF Tenofovir disoproxil fumarate, *eGFR* Estimated glomerular filtration rate, *IQR* Interquartile range

*statistically significant

study population, while 53% had HBV DNA of more than 300 copies/mL. The average eGFR was 79.78 ± 33.08 mL/min/1.73 m^2 with 31% of our study population classified stage 1 with normal or high GFR (eGFR > 90 mL/min/1.73 m^2) and 45% with stage 2 CKD (> 60–89.99 mL/min/1.73 m^2). Some patients were on herbal medicine to treat their HBV infection and out of our cohort 7.6% indicated using some form of herbal medicine. There was no effect shown on renal function.

Inferred haplotype analysis also showed five (5) haplotype blocks that existed between these variants on chromosome 11 (Table 2) with haplotype frequencies < 0.03 being ignored. The most common haplotype was TCAGG with a frequency of 0.551.

Genotype data for the study population is shown in Table 3. All polymorphisms were in Hardy–Weinberg equilibrium (HWE) except *SLC22A6 rs4149170 C > T, ABCC4 rs9282570 T > C* and *ABCC10 rs700008 A > G* that displayed a departure ($p < 0.05$) (Table 3). There was no significant statistical difference in the distribution of the genotypes among the patients that had eGFR < 60 mL/min/1.73 m^2 or not.

D' values for linkage disequilibrium (LD) analysis showed strong LD for the *SLC22A6* and *SLC22A8* variants (Fig. 1). High LD was observed for rs12293966, rs4149170, rs955434, rs11568487 and rs6591722.

The variant allele frequencies (VAF) of genotyped SNPs were compared to data from different populations, as shown in Table 4. Comparison was between VAF from this study, Africans, East Asians, Americans, South Asians, and Europeans with data as reported in dbSNP (www.ncbi.nlm.nih.gov/snp/). All our frequencies were similar to what has generally been reported in Africans.

Table 5 summarizes the genotypic distribution of transporters with eGFR. A combined percentage of the patients had stage 1/normal eGFR (> 90 mL/min/1.73 m^2) and stage 2 (60–89.99 mL/min/1.73 m^2) eGFR distributed across the various genotypes. There was no significant association between transporter genotype and CKD stage. Thirty-three percent (33.03%) of participants had eGFR lower than 60 mL/min/1.73 m^2.

Further analysis was undertaken for the association between transporter SNPs and renal dysfunction using

Table 2 Inferred haplotypes in *SLC22A6* and *SLC22A8* in overall study cohort

Gene	rs6591722	rs4149170	rs12293966	rs955434	rs11568487	Freq.
SLC22A	T	C	A	G	G	0.551
	T	T	G	G	G	0.207
	T	T	A	A	G	0.140
	A	C	A	G	G	0.046
	T	T	G	G	T	0.038

Table 3 Distribution of different genotypes in overall population and patients based on non-graded CKD categories

Genotype	Overall population, N = 160; n (%)	^{o}eGFR > 60 mL/min/1.73 m^2, N = 122; n (%)	^{o}eGFR < 60 mL/min/1.73 m^2 N = 38; n (%)	*P**
SLC22A6 rs12293966 A > G				
AA	76 (47.50)	52 (42.62)	24 (63.57)	0.209
GA	64 (40.00)	56 (45.90)	8 (21.05)	
GG	20 (12.50)	14 (11.48)	6 (15.79)	
HWE-p$^{\Delta}$	0.264			
SLC22A6 rs4149170 C > T				
CC	63 (39.38)	46 (37.70)	17 (44.74)	0.446
TC	40 (25.00)	31 (25.41)	9 (23.68)	
TT	57 (35.62)	45 (36.89)	12 (31.57)	
HWE-p$^{\Delta}$	0.001			
SLC22A6 rs6591722 T > A				
TT	140 (87.50)	107 (87.71)	33 (27.05)	0.889
AT	20 (12.50)	15 (12.29)	5 (13.15)	
AA	00(0.00)	0 (0.00)	0 (0.00)	
HWE-p$^{\Delta}$	0.399			
SLC22A6 rs955434 G > A				
GG	132 (82.50)	102 (83.61)	30 (78.95)	0.512
GA	28 (17.50)	20 (16.39)	8 (21.05)	
AA	0 (00)	0 (0.00)	0 (0.00)	
HWE-p$^{\Delta}$	0.225			
ABCC10 rs700008 A > G				
AA	28 (17.50)	23 (18.85)	5 (13.16)	0.423
GA	0 (00)	0 (0.00)	0	
GG	132 (82.50)	99 (81.15)	33 (86.84)	
HWE-p$^{\Delta}$	0.001			
ABCC10 rs831311 C > T				
CC	105 (65.62)	83 (68.03)	22 (57.89)	0.245
TC	49 (30.62)	35 (28.69)	14 (36.84)	
TT	6 (3.75)	4 (3.25)	2 (5.26)	
HWE-p$^{\Delta}$	0.145			
ABCC4 rs9282570 T > C				
TT	145 (90.62)	110 (90.16)	35 (92.10)	0.581
TC	13 (8.12)	10 (8.20)	3 (7.90)	
CC	2 (1.25)			
HWE-p$^{\Delta}$	0.015			
SLC22A8 rs11568487 G > T				
GG	149 (93.12)	113 (92.62)	36 (94.74)	0.655
GT	11 (6.88)	9 (7.37)	2 (5.26)	
TT	0 (00)	0 (0.00)	0 (0.00)	
HWE-p$^{\Delta}$	0.652			

*HWE-p$^{\Delta}$—Hardy Weinberg p values, p$^{\Delta}$ represents the chi square test to compare genotype frequencies. If the $p < 0.05$ depicts inconsistency with HWE

^{o} The two categories were based on the NKF/KDOQI guidelines[33]

eGFR/CKD stage under three genetic models dominant, recessive, and codominant. Table 6 shows all the genotypes of the SNPs under the 3 models. It was observed that under dominant and codominant models rs12293966 showed a significant association to reduced eGFR. The remaining polymorphisms were not significantly associated with eGFR or medication usage (Table 6).

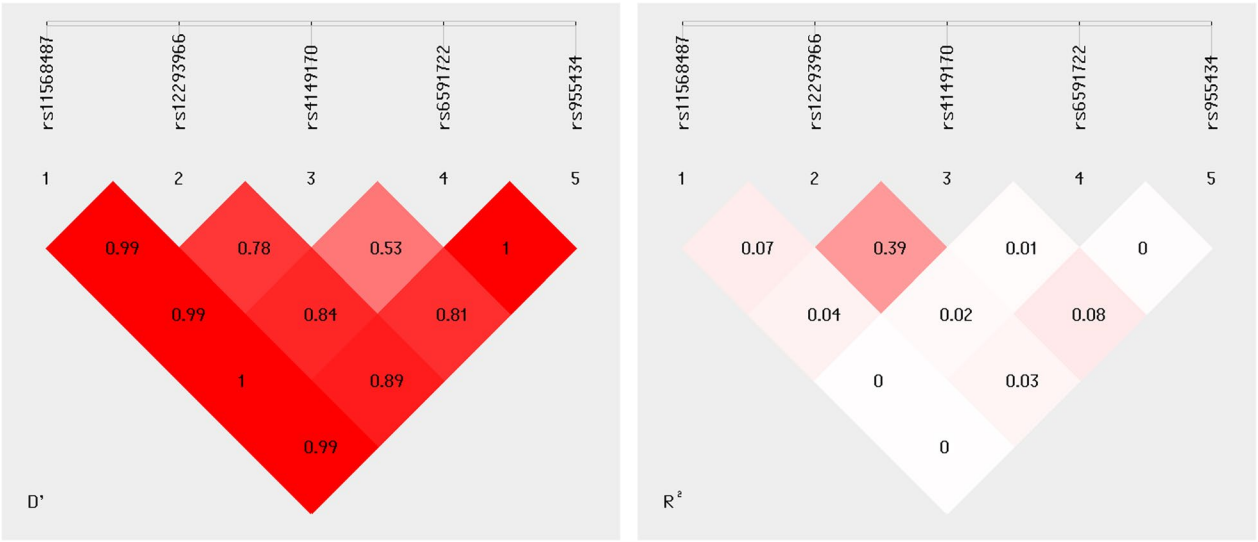

Fig. 1 Linkage disequilibrium for five SNPs on the SLC22A6 and SLC22A8. The LD shows the D' and R^2 values between each pair of SNPS

Table 4 Compares the frequency of variant alleles in different populations

Gene	SNP	Variant allele	Frequency in %					
			This study	Africans	East Asians	Americans	South Asians	EUR
SLC22A6	rs12293966	G	0.33	0.27	0.01	0.02	0.00	0.00
	rs4149170	T	0.48	0.377	0.23	0.12	0.16	0.08
	rs6591722	A	0.06	0.06	0.15	0.26	0.18	0.32
	rs955434	A	0.09	0.13	0.41	0.18	0.20	0.25
ABCC10	rs700008	G	0.83	0.86	0.95	0.81	0.92	0.87
	rs831311	T	0.19	0.15	0.00	0.00	0.00	0.00
ABCC4	rs9282570	C	0.09	0.06	0.00	0.01	0.00	0.00
SLC22A8	rs11568487	T	0.03	0.04	0.00	0.00	0.00	0.00

Discussion

Pharmacogenetic variations have implications for drug disposition in Africa, and therefore screening of such genetic variants is meaningful for bridging studies and precision medicine.

This exploratory pharmacogenetics study therefore investigated genetic variation in *SLC22A6*, *SLC22A8*, *ABCC4* and *ABCC10* genes. These genes are drug transporters involved in the metabolism of drugs such as acyclovir, atenolol, cisplatin, lamivudine, metformin and tenofovir (TDF). In this exploratory study, our participants were HBV patients. HBV patients are prescribed with TDF monotherapy or TDF-based combination therapies that have proven effective in achieving viral suppression and viral progression to liver complications. Despite the effectiveness of TDF, renal safety remains one of the greatest concerns with respect to long-term administration or usage in HBV-infected patients though debatable in everyday reported clinical data [34, 35]. Our study did not focus on comparing patients on TDF and those that were not on treatment as recruitment was not tailored towards that. We anticipated that this study would provide a basis for cohort studies to establish clinical effects of these variants on renal function in patients being managed with TDF-based therapy as obtaining pharmacogenetic-phenotype data would be useful for monitoring renal function in such patients.

One pathway that mediates active renal secretion and reabsorption of organic anions is the renal organic anion transporter (OAT, SLC22A) family [36]. Renal excretion of drugs such as TDF is mediated through multidrug resistance protein 2 (MRP2), 4 (MRP4) and MRP10 encoded by *ABCC2*, *ABCC4* and *ABCC10* [9, 13]. We explored variations in these transporters and the risk of renal dysfunction in HBV-infected patients. We observed that all polymorphisms studied were in HWE except

Table 5 SNP genotypes among patients and eGFR

	Total number in category	Stage 1/normal (> 90 mL/min/1.73 m^2)	Stage 2 (> 60–89.99 mL/min/1.73 m^2)	Stage 3a (> 45–59.99 mL/min/1.73 m^2)	Stage 3b (> 30–44.99 mL/min/1.73 m^2)	Stage 4 (15–29.99 mL/min/1.73 m^2)	Stage 5 (< 15 mL/min/1.73 m^2)	*P* value
Genotype								
ABCC10 rs831311								
CC	105	32	51	14	6	1	1	0.565
TC	49	15	20	7	4	2	1	
TT	6	3	1	2	0	0	0	
Total	160	50	72	23	10	3	2	
SLC22A6 rs12293966								
AA	76	18	34	14	5	3	2	0.104
GA	64	23	33	6	2	0	0	
GG	20	9	5	3	3	0	0	
Total	160	50	72	23				
SLC22A6 rs4149170								
CC	63	15	31	11	3	2	1	0.904
TC	40	17	14	5	4	0	0	
TT	57	18	27	7	3	1	1	
Total								
ABCC10 rs700008								
GG	132	39	60	21	8	2	0	0.715
GA								
AA	28	11	12	2	2	1	2	
Total								
ABCC4 rs9282570								
TT	145	44	66	21	10	2	2	0.772
TC	13	6	4	2	0	1	0	
CC	2	0	2	0	0	0	0	
Total								
SLC22A6 rs955434								
GG	132	44	58	20	8	1	1	0.153
GA	28	6	14	3	2	2	1	
AA	0	0	0	0	0	0	0	
Total								
SLC22A8 rs11568487								
GG	149	47	66	22	9	3	2	0.961
GT	11	3	6	1	1	0	0	
TT	0	0	0	0	0	0	0	
Total								
SLC22A6 rs6591722								
TT	140	45	62	20	8	3	2	0.900
AT	20	5	10	3	2	0	0	
AA	0	0	0	0	0	0	0	
Total								

for rs9282570 T > C, rs4149170 C > T and rs700008 A > G which showed a departure (Table 3). This departure could have resulted from several reasons including population substructure or genotyping error. Renal dysfunction in this study was evaluated with eGFR. In our study, 31.25% and 45% of our participants had stage 1 and stage 2 CKD, which, according to NKF/KDOQI is kidney damage with normal or increased GFR and kidney damage with mild reduced GFR [37, 38]. A combined 23.75% of our participants had eGFR of < 60 mL/min/1.73 m^2.

Table 6 Association between eGFR and SNPs under multiple models of inheritance

Inheritance MODEL	Alleles	CRUDE MODEL		MODEL 1		MODEL2	
SNP		OR (95%)	*p* value	OR (95%)	*p* value	OR (95%)	*p* value
SLC22A6 rs955434							
Dominant	GA-AA	1.54 (0.35–6.82)	0.570	1.50 (0.33–6.74)	0.53	1.13 (0.33–3.80)	0.211
Recessive	AA	–		–		–	
Codominant	GA	2.05 (0.57–7.39)	0.271	1.78 (0.51–6.19)	0.366	1.14 (0.41–3.20)	0.800
SLC22A6 rs12293966							
Dominant	GA-GG	0.27 (0.08–0.81)	**0.019**	0.33 (0.08–1.23)	0.099	0.35(0.11–1.09)	0.070
Recessive	GG	1.45 (0.51–4.07)	0.48	0.64 (0.09–4.16)	0.641	1.69 (0.49–5.85)	0.41
Codominant	GA	0.21 (0.07–0.66)	**0.008**	1.76 (0.39–7.89)	**0.014**	0.30 (0.12–0.78)	**0.013**
SLC22A6 rs4149170							
Dominant	TC-TT	3.90 (0.94–16.16)	0.061	1.58 (0.41–6.06)	0.507	1.46 (0.46–4.64)	0.52
Recessive	TT	0.91(0.38–2.13)	0.830	1.49(0.40–5.61)	0.55	0.82 (0.29–2.32)	0.71
Codominant	TC	0.87 (0.29–2.5)	0.806	0.91 (0.31–2.67)	0.863	1.19 (0.48–2.96)	0.700
SLC22A6 rs6591722							
Dominant	AT-AA	0.52 (0.12–2.22)	0.380	0.81 (0.23–2.86)	0.747	0.95 (0.29–3.07)	0.93
Recessive	TT	–		–		–	
Codominant	AT	0.69 (0.18–2.59)	0.580	0.76 (0.21–2.73)	0.674	1.15 (0.37–3.67)	0.802
SLC22A8 rs11568487							
Dominant	GT-TT	0.92 (0.10–8.11)	0.701	1.40 (0.20–9.82)	0.734	1.08 (0.20–5.77)	0.92
Recessive	TT	–		–		–	
Codominant	GT	1.01 (0.13–7.36)	0.989	0.96 (0.13–6.87)	0.967	0.93 (0.18–4.87)	0.936
ABCC10 rs831311							
Dominant	TC-TT	4.53 (0.38–54.25)	0.234	1.06 (0.41–2.69)	0.22	1.96 (0.30–12.93)	0.48
Recessive	TT	1.64(0.28–9.32)	0.58	2.05(0.26–16.26)	0.5	1.76 (0.30–10.27)	0.53
Codominant	TC	0.83 (0.30–2.31)	0.723	0.88 (0.32–2.37)	0.801	1.30 (0.57–2.94)	0.535
ABCC10 rs700008							
Dominant	GA-GG	2.17 (0.51–9.18)	0.941	2.12 (0.52–8.57)	0.293	1.58 (0.53–4.72)	0.41
Recessive	GG	1.53 (0.54–4.36)	0.42	2.10(0.60–7.51)	0.25	1.45 (0.79–0.50)	0.5
Codominant	GA	–		–		–	
ABCC4 rs9282570							
Dominant	TC-CC	0.25 (0.04–1.49)	0.125	0.37 (0.06–2.05)	0.256	0.64 (0.15–2.66)	0.52
Recessive	CC	–		–		–	
Codominant	TC	0.27 (0.04–1.70)	0.164	0.33 (0.06–1.94)	0.224	0.93 (0.18–4.87)	0.936

CI Confidence interval, bolded values are statistically significant

Model 1 Adjusted for duration of diagnosis, herbal medicine usage and medication usage

Model 2 Adjusted model 1 + age, gender and BMI

According to KDIGO definition of CKD, abnormalities of kidney structure or function that persists for more than 3 months and requires of one of two criteria for diagnosis. There should be either a reduced eGFR of 60 mL/min/1.73 m^2 (Stages 3a–5) or kidney damage markers such as albuminuria [33, 38]. This therefore implies that our participants were moving to a stage of renal dysfunction, which may require clinical management. Some patients acknowledge the use of herbal medicine to self-manage their condition, which may potentially affect renal function if continuously used indiscriminately. Herbal medicine being multi-phytoconstituent may have components that may be substrates of these genes, and they, therefore still stand a risk of renal dysfunction if the use of these herbal medicines is not monitored for these patients [39, 40]. The combined 23.75% participants who had eGFR < 60 mL/min/1.73 m^2 (Stage 3a to 5) constituted patients who were either on TDF or not. However, apart from self-reported usage of other nephrotoxic medications or foods such as herbal medicine, these patients could have been on other forms of "treatment" for their condition which has implications for renal function.

ABCC10 rs700008 A > G had a genotypic frequency of 127 for homozygous mutant (GG) and 24 for homozygous wildtype (AA) with departure from HWE (χ^2, $P \leq 0.05$). *ABCC10* variants have been associated with kidney tubular dysfunction [26]. Variations in *ABCC10* may influence how drug substrates are metabolized and may influence renal function. This therefore makes our study very important as there was 84% representation of the variant allele in our cohort, implying a possibility of influence on renal function. The variant allele frequency (VAF) for this SNP (G) was comparable to what has been reported in other African populations (Table 4). For instance, in designing TDF pharmacogenomics, a proposed list of transporters that includes the *SLC22A6* group of families, the variant rs4149170 *C > T* has been highlighted as one of the key variants to evaluate [41]. Our study showed a departure from HWE for rs4149170 with a VAF of 0.48, which was comparable to other populations. The VAF detected, however, was higher than a study that assessed transporters and chronic kidney disease, where they obtained a VAF of 0.09 among a group of chronic renal insufficiency cohort with variable ancestry [42]. The *SLC22A6* rs4149170 has been linked to antiviral drug metabolism, which causes renal toxicity [43].

There were only seventeen patients in our total cohort that had been initiated on TDF treatment for their condition. The natural history and management of HBV infection follow a protocol that looks at HBeAg status, viral loads (HBV DNA), co-infections and liver enzymes levels before deciding on providing medication [44, 45]. The implementation of these protocols explains why only 11% percent of patients were on treatment which is corroborated by a meta-analysis which showed approximately 10% of patients who have been infected with HBV globally are prioritized for treatment [46, 47].

The distribution of the genotypes of *SLC22A6*, *SLC22A8*, *ABCC4* and *ABCC10* among the various categories of renal output measured with eGFR showed no significant variations among genotypes and stages of renal function. However, there is high variability in the pharmacokinetics of drugs in individuals, so identifying factors that will contribute to this variability will inform dosing. We therefore modeled inheritance patterns to look for associations and we observed that dominant (OR 0.27, 0.08–0.81; $p=0.019$) and codominant (OR 0.21, 0.07–0.66; $p=0.008$) models for rs12293966 were significantly associated with renal function measured with eGFR. This variant has been reported in South African Xhosa and Cape mixed Ancestry group in a similar exploratory study [48]. Looking at the use of TDF in HBV treatment, it is important that patients be evaluated for renal dysfunction over time once they initiate TDF treatment. In patients who have used nephrotoxic drugs for a long time, including TDF, the rs12293966 gene should be tested in addition to other susceptibility variants.

The aspect of our study that is important is that this exploratory study has provided genotype information on patients who are following the natural management history of HBV, and based on prevailing factors, they are prescribed with TDF. Our study is the first to report these SNPs in the Ghanaian population. Further studies need to be conducted, especially on these SNPs for their impact on long-term use of their substrate drugs, including TDF.

Key limitations in this study include the fact that we did not recruit patients with renal dysfunction for comparison and that was because we wanted to follow the natural management of HBV at these health facilities. Also, we did not measure albuminuria and proteinuria markers of renal function which could also have informed further classifications. Again, not all genes of the targeted transporter proteins were examined, and we might have therefore missed other important SNPs. Sample size does not match well among the various groups that were used in the classifications which could have potential implications for the outcome. The genotype frequencies for homozygous variant alleles were few which implies further investigations would have to be conducted to affirm the findings of this study.

Conclusion

In conclusion, the findings of this study provide an overview of genetic variations in four important pharmacogenetic transporters. We show that rs12293966 may be associated with renal function measured with eGFR in HBV patients. Therefore, in managing patients with HBV infection, when the decision is made to put them on TDF, metabolite concentrations and genotypes should be evaluated to prevent renal dysfunction. These exploratory results would require further evaluation and confirmation in a cohort that would be followed up after tenofovir dosing for renal functional effects in our population.

Abbreviations

TDF	Tenofovir disoproxil fumarate
LD	Linkage disequilibrium
HBV	Hepatitis B virus
eGFR	Estimated glomerular filtration rate
CKD	Chronic kidney disease
VAF	Variant allele frequencies
OR	Odds ratio
CI	Confidence interval
BMI	Body mass index
MRP2	Multidrug resistance protein 2
MRP4	Multidrug resistance protein 4
MRP10	Multidrug resistance protein 10
CKD-EPI	Chronic kidney disease epidemiology collaboration
ADME	Absorption, distribution, metabolism and elimination
HIV	Human immunodeficiency virus

sCr	Serum creatinine
HWE	Hardy-Weinberg equilibrium
NKF/KDOQI	*National Kidney Foundation* (*NKF*) Kidney Disease Outcome Quality Initiative

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Author contributions
NET conceived the idea, supervised and secured funding for the project. NET, FA and SBN were involved in recruitment, data retrieval and laboratory analysis. NET, CK, AA, PN, RDE and GA the performed analysis. All authors contributed to the writing of the manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article. Any other dataset used and/or analysed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained for this study and patients who consented were recruited. This study obtained ethical clearance from the Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC/EC/2021/005). All research protocols were in accordance with the Helsinki declaration. Participants provided both written and verbal consent to participate in this study. Participants were provided with study numbers and unique codes to deidentify and anonymize them.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

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