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Genetic Polymorphisms and Haplotype Structure of the Organic Cation Transporter 1 Gene in the Zulu Population of South Africa

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***Abstract*—**Organic cation transporter (OCT) 1could influence an individual's response to various treatments and increase their susceptibility to diseases. Genotypic and allelic frequencies of nineteen non-synonymous and one intronic Single Nucleotide Polymorphism (SNP) from the OCT1 gene were determined in 101 unrelated healthy Zulu participants, using a SNaPshot® multiplex assay. Minor allele frequencies (MAF) were compared to representative populations of Africa, Asia and Europe, from Ensembl. MAFs for S14F, V519F, rs622342 and P341L were 2.0%, 6.0%, 6.0% and 1.0%, respectively. Sixteen of nineteen investigated non-synonymous SNPs were monomorphic. No study participant harbored variant alleles for S189L, G220V, P283L, G401S, M420V, M440I, G465R, I542V, R61C, R287G, C88S, A306T, A413V, I421F, C436F and V501E. Haplotype, CGTCGCCGCGCAAGAGGTGA, was most frequently observed (81.23%). Further investigations are encouraged to evaluate potential roles these SNPs could play in the therapeutic efficacy of clinically important drugs and in the development of various diseases in the Zulu population.

***Keywords*—**OCT1, PCR, SNaPshot assay, Zulu population.

I. Introduction

THE *SLC22A1* gene, encoding the human organic cation transporter 1 (OCT1), is 37 kb in size and is located within a cluster on chromosome 6.q26-7 consisting of 11 exons and 10 introns [1], [3]. OCT1 is predominantly expressed in hepatocytes and enterocytes, mediating the uptake of anti-diabetic, anti-cancer and anti-viral drugs [4]-[11]. Previous studies have shown that OCT1 is highly polymorphic in ethnically diverse populations [12]-[14]. Single nucleotide polymorphisms (SNPs) resulting in a functional change in the *SLC22A1* gene, have been shown to affect the uptake of 1-methyl-4-phenylpyridinium (MPP^+), tetraethylammonium (TEA) bromide, metformin, anti-viral, and anti-cancer drugs compared to the wild-type [10]-[13], [15]-[16]. These genetic variations could also have an impact on complex diseases such as cancer, diabetes mellitus, and hypertension and could explain the patients inter-individual variability in response to drugs used in the treatment of these diseases [3], [15]-[18]. A Dutch study reported on a glucose-lowering effect of metformin, in diabetes mellitus patients, and that metformin therapy was less effective in patients who have the minor C allele for SNP rs622342 [3]. SNP rs622342 (1386A>C), an intronic variant, is located between exons 8 and 9 [3]. This variant has also been shown to be associated with a reduced response to the anti-Parkinson drug, levodopa, and more severe symptoms resulting in a shorter survival period in Caucasian Dutch patients [19]. Furthermore, SNPs rs2282143 and rs622342 variants have been associated with severe progression to primary biliary cirrhosis in Japanese patients [20]. In addition, it has been reported that the expression of OCT1 variants with reduced transport activity may result in the accumulation of various toxic metabolites and enhanced exposure to environmental toxins capable of reaching the brain and trigger the development of neurodegenerative diseases such as Parkinson's disease [21], [22].

South Africa is highly diverse, consisting of many indigenous and immigrant population groups [23], [24]. Amongst these are the Bantu-speaking populations such as the Xhosa, Zulu, and Sotho, which are believed to have originated approximately 3000-5000 years ago in West Africa between the present-day Cameroon and Nigeria [25], [26]. These indigenous populations potentially contain a significant amount of genomic diversity [23], [27]. The Zulu population belongs to the southern branch of the Nguni ethnic group [28], and is the largest ethnic group in South Africa, accounting for more than 11 million people of which 7.9 million reside in the KwaZulu-Natal province [29]. Previous studies have shown that South African populations exhibit unique allele frequencies and novel genetic variation in pharmacogenetically relevant genes [30]. These studies have primarily focused on variations in the drug metabolizing enzyme genes. These studies have shown that South African populations have unique genetic profiles which include novel and rare variants, with allele frequencies differing from each other and other African populations [31].

The aim of the study was to investigate the genotypic and allelic distributions of nineteen non-synonymous and one intronic SNP(s), and to infer the haplotype structure of the

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SLC22A1 gene in the Zulu population. The data was compared to other population data available in literature and relevant databases and the potential implications of these polymorphisms are discussed in this report.

II. Materials and Methods

A. Selection of SLC22A1 variants

Nineteen non-synonymous and one intronic SNP of the *SLC22A1* gene were targeted for this study. Variants were selected based on the previously reported effects on transport efficiency of OCT1, and suggested clinical relevance [3], [12], [13], [15], [16], [29]. Selection was also based on predicted functional effects of variants using the SIFT (Sorting Intolerant from Tolerant) and PolyPhen (Polymorphism Phenotyping) scores [32].

B. Study Participants

One hundred and one (n=101) unrelated healthy individuals from the Zulu population of South Africa were recruited to participate in this study. Eligibility criteria were determined by survey indicating that both parents of the donor were of Zulu descent. The distributions of donors were 42.6% with ages ranging from 22-57 for females and 57.4% with ages ranging from 19-43 for males. Ethics approval was obtained by the Senate's Research Committee of the University of the Western Cape and is in accordance with the guidelines of the Helsinki Declaration of 1964.

C. Multiplex Polymerase Chain Reaction (PCR)

Genomic DNA was extracted using the phenol-chloroform method [33]. All of the *SLC22A1* exons and the portion of intron 9 spanning rs622342 were simultaneously amplified using the primers listed in Table I. A negative control was included to confirm that no contamination was present during PCR. PCR primers were synthesized by Integrated DNA Technologies (Germany). The PCR reactions consisted of 1X Multiplex PCR Reaction Mix (Qiagen, U.S.A), 0.2 µM primer mix and 25 ng of genomic DNA in a final reaction volume of 20 µl. The PCR conditions consisted of an initial denaturation step at 95°C for 15 min; 39 cycles of denaturation at 94°C for 30s, annealing at 60°C for 90s and extension at 72°C for 90s; final extension at 72°C for 10 min and hold at 4°C in a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, U.S.A.). Amplifications were confirmed by gel electrophoresis. PCR products were purified using a Thermosensitive Alkaline Phosphatase (FastAP) (Thermoscientific, U.S.A.) and Exonuclease 1 (Exo 1) (Thermoscientific, U.S.A.) according to manufacturer's instructions.

D. Mini-Sequencing Reactions

The mini-sequencing was performed using the SNaPshot® kit (Applied Biosytems, U.S.A.). Two SNaPshot® Multiplex systems were specifically designed for the study, successfully optimized and used for genotyping. The single base extension primer sets for multiplex 1 and 2 are listed in Table II. Multiplex mini-sequencing was performed in a 10 μl reaction volume using 3 μl of the purified PCR products, 0.2 μM of primers, and 5 μl of SNaPshot® ready reaction mix. Sequence cycling consisted of 25 cycles of 96ºC for 10s, 50ºC for 5s and 60ºC for 30s, followed by a hold at 4ºC. Post extension products were purified using 1 U FastAP, incubated at 37ºC for 15 min and inactivated at 75ºC for 15 min.

E. Electrophoresis of the Mini-Sequencing Products

The purified mini-sequencing products (1 μl) were mixed with 8.7 μl of HiDi™ formamide and 0.3 μl of GeneScan-120 Liz size-standard (Applied Biosystems) and denatured at 95°C for 5 minutes. The fluorescently labeled fragments were separated on 36 cm-long capillaries in POP4 polymer on an ABI Prism 3500 Genetic Analyzer (Applied Biosystems). Data analyses were performed using GeneMapper® IDX Software Version 1.2.

F. Statistical Analysis

Allele and genotype frequencies as well as the deviation from the Hardy-Weinberg Equilibrium were determined using the GeneAlEx version 6.5 software [34]. The haplotypes were determined using the online software, SHEsis [35], [36]. Statistical significance was defined as $P < 0.05$.

TABLE I
OCT 1 MULTIPLEX PCR PRIMERS

Primer Name	Length	T_m	Nucleotide Sequence	Amplicon Size
Exon 1	24	65.9	5' - TGCTGAGCCATCATGCCCACCGTG - 3'	255
	21	62.8	5' - GGACACAGCCAGACACCCACG - 3'	
Exon 2	24	59.6	5' - CTCTTGCCGTGGTATGACTGGCAG - 3'	162
	23	57.9	5' - CAGAGGGGCTTACCTGGACTGG - 3'	
Exon 3	25	58.1	5'- CCTCCATGTCTCCTTCTCTCTGAAG - 3'	207
	25	57.2	5'- CTGGCCTCATCCCCATGATAATTAC - 3'	
Exon 4	24	61.3	5' - CCCGCATAACGTCCACACCTCCTG - 5'	222
	23	60.3	5' - GTAGGCAGGAGGAAGGGCCTCAC -3'	
Exons 5 and 6	24	57.4	5' - GATAGTGATGAGTGGTGTTCGCAG - 3'	503
	21	62.7	5' - GCGAGCGTGCTGATTCTGCCT - 3'	
Exon 7	25	59.3	5' - GACTTGAAACCTCCTCTTGGCTCAG - 3'	298
	25	64.2	5' - TTCCCCACACTTCGATTGCCTGGGA - 3'	
Exon 8	25	67.6	5' - GAAGCCCCCATCCACCACCCACACC - 3'	181
	25	63.4	5' - GGCTACCCCTGTTCCATGCACTCAC - 3'	
Exon 9	22	62.4	5' - ATTGCATGGGCAACGGATGGCT - 3'	615
	25	67.8	5' - CCATGCTGAGCCCACTGCCGAGCTG - 3'	
Exon 10	23	60.6	5' - TTCCTCTCTTTGGCTGGCTGTGA - 3'	621
	24	60.5	5' - ACTCCAGCAAACCTTGCTCTCTGT - 3'	
Exon 11	25	58.9	5' - TGCCCTTTTCTTCTTTGCTGTTTGC - 3'	460
	25	60.8	5' - AGCACCAACAGCTTTCCCTAGATCG - 3'	
Intron 9	25	58.8	5' - GAGTAGGAGGGGTTAATAGAGAGAG - 3'	236
	27	65.7	5' - GTAGCTGAGACTACATGCATGCACCAC - 3'	

TABLE II
SLC22A1 MULTIPLEX 1 AND 2 SINGLE BASE EXTENSION PRIMERS

NCBI (dbSNP)	Amino Acid Change	Nucleotide Change	Single Base Extension Primers	dGACT	Size bp
Multiplex 1					
rs34447885	S14F	C/T	5' - TGACTATTCTGGAGCAGGTTGGGGAGT - 3'	13	40
rs34104736	S189L	C/T	5' - GAACTGTGCTGGTCAACGCGGTGT - 3'	21	45
rs36103319	G220V	G/T	5' - GGTCAGCAAGGGCAACTGGATGGCTG -3'	24	50
rs4646277	P283L	C/T	5' - GATAACAGCCACCGGGGGACACC - 3'	32	55
rs34130495	G401S	G/A	5' - AGCCCTCATCACCATTGACCGCGTG - 3'	35	60
rs142448543	M420V	A/G	5' - AACTTACCAGGTGAGATAAAAATCA - 3'	40	65
rs35956182	M440I	G/A	5' - CATAATCATGTGTGTTGGCCGAAT - 3'	46	70
rs34059508	G465R	G/A	5' - CCACAGGGAGGAACACACCATCACTC - 3'	49	75
rs78899680	V519F	G/T	5' - CTACTTCTTCCAGAGACCAAGGGG - 3'	56	80
rs137928512	I542V	A/G	5' - CAGAGGTTTGGACCTTAAGGTAAA - 3'	61	85
Multiplex 2					
rs622342	Intron	A/C	5' - ATTTCTTCAAATTTGATGAAAACTTC - 3'	14	40
rs12208357	R61C	C/T	5' - TCCTGGGGTGGCTGAGCTGAGCCAG - 3'	20	45
rs4646278	R287G	C/G	5' - CAGTGTTTCTTTTTTGTGATAACAGCCACC - 3'	20	50
rs55918055	C88S	T/A	5' - TCCAGTCCACTTCATAGCGCCTGC - 3'	31	55
COSM164365	A306T	G/A	5' - AGGAGGCAACTTCCCATTCTTTTGAG - 3'	34	60
rs2282143	P341L	C/T	5' - CTTCATTTGCAGACCTGTTCCGCACGC - 3'	38	65
rs144322387	A413V	C/T	5' - CCCCATGGCCATGTCAAATTTGTTGG - 3'	44	70
rs151333280	I421F	A/T	5' - CCAACTTACCAGGTGAGATAAAAA - 3'	51	75
rs139512541	C436F	G/T	5' - GCACTGGTTAAACATCATAATCATGT - 3'	54	80
rs143175763	V501E	T/A	5' - CACTCCCGCGGCAAGCAGGCCCAAC - 3'	60	85

III. RESULTS AND DISCUSSION

The genotype and allele frequencies for the 20 *SLC22A1* variants, observed in the Zulu population, are reported in Table III. The minor allele frequency (MAF) for the 20 SNPs of the Zulu population was compared to six ethnic populations, globally, in Table IV. The populations used for comparison were: LWK (Luhya in Webuye, Kenya); YRI (Yoruba in Ibadan, Nigeria); PUR (Puerto Ricans from Puerto Rica); GBR (British in England and Scotland); CHS (Southern Han Chinese) and JPT (Japanese). LWK and YRI served as representative Sub-Saharan African groups; PUR for the Admixed population of America; GBR for the Caucasian group of Europe; and CHS and JPT for Asia. The allelic frequency of each SNP was in HWE ($p>0.05$), except for rs622342. Sixteen out of the nineteen investigated non-synonymous SNPs were monomorphic in the Zulu population.

Heterozygosity was only observed for SNP variants S14F (rs34447885), V519F (rs78899680), rs622342 and P341L (rs2282143), shown in Table III. The S14F variant genotype frequencies for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 96%, 4% and 0%, respectively. The MAF observed for S14F was 2%. The genotype frequencies for variant V519F were 88.1% (homozygote wild-type, GG) and 11.9% (heterozygote, GT). The frequency for the intronic SNP was determined to be 89.1% homozygous (AA) for the ancestral allele, 9.9% heterozygous (AC) and 1.0% homozygous (CC) for the minor allele. The P341L variant genotype frequencies, on the other hand, for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 98%, 2% and 0%, respectively.

Population data from this study as well as from databases suggest that rs78899680 (V519F, 1555G>T) and rs34447885

(S14F, 41C>T) may be variants of African descent. The MAFs for variant V519F for the Zulu, LWK and YRI populations were 6%, 2.1% and 5.1%, respectively (Table IV). S14F was also observed in these populations (Zulu: 2%; LWK: 2.6% and YRI: 1.7%) (Table IV). MAF for S14F of 3.1% was also reported for the African American population [12]. S14F has been shown to reduce transport of metformin by 56% as compared to the reference sequence in stably transfected HEK293 cells [16]. This variant has also been shown to be associated with increased uptake of MPP^+ [12] but unaltered transport activity for sorafenib, a cationic drug used to treat hepatocellular carcinoma and cholangiocarcinoma, compared to the reference OCT1 sequence in *Xenopus laevis* oocytes [11]. However, no clinical data is available to determine the effect of this variant on the transport of OCT1 substrates, *in vivo*. It would be interesting to investigate the possible clinical implications and drug response profiles for these variants on patients from Zulu and Sub-Saharan African populations.

SNP rs622342 (1386A>C), an intronic variant, is located between exons 8 and 9 [3]. The MAF for the Zulu population (6%) was much lower than those for the Caucasian (37.6%); Admixed (34.5%); Asian (14.5-19.1%); and other African populations (15.0-22.0%) (Table IV). It was also lower than those previously reported for the Dutch (37%) [3] and Tamilian (24.5%) [18] populations. A Dutch study reported on a glucose-lowering effect of metformin, in diabetes mellitus patients, and that metformin therapy was less effective in patients who have the minor C allele [3]. However, the homozygous ancestral allele (A) for the intronic SNP of the MATE1 gene (rs2289669) has been shown to increase the metformin response in the same patients [37]. A report from a population-cohort study suggested that patients with the minor C allele would have a reduced response to anti-Parkinson drugs, possibly due to a decreased efficacy in drug transportation to the brain, and that these patients would experience more severe symptoms resulting in a shorter survival with this disease [19]. Furthermore, the minor C allele has also been associated with an increased susceptibility to jaundice-type progression of primary biliary cirrhosis (PBC) in Japanese patients [20]. Based on the MAF (Table IV), the Zulu population would be more responsive to anti-Parkinson drugs compared to other global populations. It also suggests that the Zulu population would be less susceptible to PBC compared to Caucasian, Admixed, Asian and other African populations. The effect of metformin therapy cannot be predicted at this time as MATE 1 SNPs have not been investigated for the Zulu population, as yet.

The MAF for the non-synonymous variant rs2282143 in exon 6 (P341L, 1022C>T) for the Zulu, Sub-Saharan African, Admixed, Caucasian and Asian populations were 1.0%, 7.7-9.1%, 2.7%, 2.2% and 11.0-15.7%, respectively (Table IV). Interestingly, the MAF for the Zulu population is closer to those reported for the Admixed and Caucasian populations compared to the two African populations (Table IV). P341L is among the most intensively studied SNPs in relation to metformin response and has been associated with decreased transporter activity in an Indian population [18]. Other studies, however, have reported on an unaltered uptake of metformin compared to the wild-type sequence in the Korean population [10], [16]. Reduced uptakes of TEA; MPP^+ [10], [13], [29]; and lamivudine [10] have been reported in *in vitro* studies. Furthermore, the minor allele T has been associated with increased susceptibility to severe progression of primary biliary cirrhosis (PBC) in Japanese patients [20]. The data suggests that lamivudine therapy could be more effective in the Zulu population compared to other populations. It also suggests that the Zulu population would be less at risk of developing PBC than other populations.

SNP variant rs4646277 (P283L, 848C>T) has been associated with decreased transport activity of OCT1 in Korean subjects [14]. It has been shown to prevent the uptake of TEA and MPP^+ [13] and decrease the uptake of lamivudine by 85.1% compared to the wild-type in the *Xenopus laevis* oocyte expression system [10]. Lamivudine is used as a combination therapy with other anti-HIV agents and monotherapy in the treatment of hepatitis B virus infection [38]. This suggests that lamivudine therapy could be effective in Zulu patients. Another research group also reported on a reduced uptake of TEA in HEK293 cells as a result of the "complete diminished" transport activity of OCT1 brought on by this polymorphism [29].

The effects of variants rs35956182 (M440I, 1320G>A) and rs34130495 (G401S, 1201G>A) on the *in vitro* uptake of MPP^+ have previously been investigated by two independent research groups [12], [15]. The M440I mutation resulted in an unaltered uptake; while a reduction of 0.9% was reported in the G401S variant [12], [15]. M440I and G401S variants were not detected in the Zulu and Sub-Saharan African populations (Table IV); however, low frequencies of 0.5% and 0.7% were reported [12] for these SNPs. The G401S polymorphism was shown to reduce the uptake of serotonin compared to the wild-type [15]. Kerb and co-workers [15] suggested that this variant could affect the disposition of OCT1 substrates resulting in an altered duration and intensity of the effects of various drugs and neurotransmitters with specific affinities for the OCT1 transporter. G401S has also been shown to reduce the transport of metformin by 10% in stably transfected HEK293 cells [16] but have unaltered transport activity for sorafenib compared to the wild-type in Alexander and SK-Hep-1 (HCC) and TFK1 (CGC) cell lines [11]. Subjects with the G401S variant have been reported to have increased plasma metformin concentrations, decreased systemic clearance and volume of distribution of metformin [39], [40]. Interestingly, the G401S variant was also found to decrease the uptake of TEA in one study [15] but remained unaltered in another study [11] upon comparison with the wild-type. Discrepancies in the two studies may be a result of the different expression systems used and also attributed to the changes such as reduced localization of the plasma membrane [32].

No polymorphisms were observed for G220V (rs36103319, 659G>T); G465R (rs34059508, 1393G>A); R61C (rs12208357, 181C>T); R287G (rs4646278, 859C>G); C88S (rs55918055, 262T>C); and S189L (rs34104736, 566C>T), which were all homozygous for the ancestral allele for the

Zulu population. Variants G220V and G465R have been shown to reduce the transport of metformin [16], MPP^+ [12] and TEA [11] but have no effect on the transport of sorafenib [11] compared to the wild type in stably transfected HEK293 cells. Other *in vitro* studies reported on reduced uptakes of MPP^+ [12], [13], [15]; TEA [11], [13], [15], [29] and sorafenib [11] for the variants R61C (rs12208357, 181C>T), R287G (rs4646278, 859C>G) and C88S (rs55918055, 262T>C). A reduced uptake of metformin, in stably transfected HEK293 cells, compared to the reference sequence was also reported for R61C [16]. For the S189L variant, reduced uptakes of metformin [16], TEA and sorafenib [11] were reported but no effect was observed on the transport of MPP^+ [12] in stably transfected HEK293 cells compared to the reference sequence.

To our knowledge, this study is the first to report population data for SNPs: rs142448543 (M420V, 1258A>G); rs137928512 (I542V, 1624A>G); COSM164365 (A306T, 916G>A); rs144322387 (A413V, 1238C>T); rs151333280 (I421F, 1261A>T); rs139512541 (C436F, 1307G>T); and rs143175763 (V501E, 1649T>A). Further investigation is needed to explore the genetic variation among these SNPs in global populations. It is also the first study reporting on the genotype and allele frequency distributions for SNPs of the OCT1 gene as well as the inferred haplotype structure for the Zulu population.

The inferred haplotypes, for the Zulu population, was defined by 20 SNPs in the *SLC22A1* gene and are listed in Table V. The most frequently observed haplotypes were CGTCGCCGCGCAAGAGGTGA (81.23%), CGTCGCCGCGCAAGCGGTGA (6.89%) and CGTCGCCGCGCAAGAGGTTA (5.90%). It is known that an individual's variation in drug response could be attributed to specific genetic variants. Thus the incorporation of haplotypes in pharmacogenetic studies would provide a more holistic view of relevant loci in the practice of "genetic medicine" at an individual and population level [41].

Findings from this study would be valuable to motivate for *in vitro* and *in vivo* pharmacogenetic studies to determine the effect these SNPs have on a population's response to various drugs used in treatment of various diseases such as HIV, TB, cancer, diabetes, hypertension, etc. It may also shed light on a population's susceptibility to various diseases and disorders.

TABLE III
GENOTYPE AND ALLELE FREQUENCIES FOR 20 SNPS OF SLC22A1 IN 101 HEALTHY PARTICIPANTS FROM THE ZULU POPULATION

SNP	Polymorphism	Genotype frequency (%)			Allele Frequency (%)		HWE (P)
rs34447885	41 C>T	CC	CT	TT	C	T	
		96.0	4.0	0	98.0	2.0	0.839
rs34104736	566 C>T	CC	CT	TT	C	T	
		100	0	0	100	0	
rs36103319	659 G>T	GG	GT	TT	G	T	
		100	0	0	100	0	
rs4646277	848 C>T	CC	CT	TT	C	T	
		100	0	0	100	0	
rs34130495	1201 G>A	GG	GA	AA	G	A	
		100	0	0	100	0	
rs72552763	1258 A>G	AA	AG	GG	A	G	
		100	0	0	100	0	
rs35956182	1320 G>A	GG	GA	AA	G	A	
		100	0	0	100	0	
rs34059508	1393 G>A	GG	GA	AA	G	A	
		100	0	0	100	0	
rs78899680	1555 G>T	GG	GT	TT	G	T	
		88.1	11.9	0	94.0	6.0	0.526
rs137928512	1624 A>G	AA	AG	GG	G	A	
		100	0	0	0	100	
rs622342	1386 A>C	AA	AC	CC	A	C	
		89.1	9.9	1.0	94.0	6.0	0.019
rs12208357	181 C>T	CC	CT	TT	C	T	
		100	0	0	100	0	
rs4646278	859 C>G	CC	CG	GG	C	G	
		100	0	0	100	0	
rs55918055	262 T>C	TT	TC	CC	T	C	
		100	0	0	100	0	
COSM164365	916 G>A	GG	GA	AA	G	A	
		100	0	0	100	0	
rs2282143	1022 C>T	CC	CT	TT	C	T	
		98.0	2.0	0	99	1.0	0.920
rs144322387	1238 C>T	CC	CT	TT	C	T	
		100	0	0	100	0	
rs151333280	1261 A>T	AA	AT	TT	A	T	
		100	0	0	100	0	
rs139512541	1307 G>T	GG	GT	TT	G	T	
		100	0	0	100	0	
rs143175763	1649 T>A	TT	TA	AA	T	A	
		100	0	0	100	0	

TABLE IV
A COMPARISON OF THE MINOR ALLELE FREQUENCY FOR THE SLC22A1 SNPS OF THE ZULU POPULATION TO OTHER ETHNIC GROUPS

			Minor Allele Frequency (%)						
SNP	Amino acid change	Minor Allele	Zulu[a]	LWK[b]	YRI[b]	PUR[b]	GBR[b]	CHS[b]	JPT[b]
rs34447885	S14F	T	2.0	2.6	1.7	0.0	0.0	0.0	0.0
rs34104736	S189L	T	0.0	0.0	0.0	1.0	0.0	0.0	0.0
rs4646277	P283L	T	0.0	0.0	0.0	0.0	0.0	0.5	1.1
rs34130495	G401S	A	0.0	0.0	0.0	0.0	2.2	0.0	0.0
rs35956182	M440I	A	0.0	0.0	0.0	1.0	1.0	0.0	0.0
rs34059508	G465R	A	0.0	0.0	0.0	2.0	3.0	0.0	0.0
rs78899680	V519F	T	6.0	2.1	5.1	0.0	0.0	0.0	0.0
rs622342	Intronic	C	6.0	15.0	22.0	34.5	37.6	14.5	19.1
rs12208357	R61C	T	0.0	0.0	0.0	1.0	5.1	0.0	0.0
rs55918055	C88S	C	0.0	0.0	0.0	0.0	0.6	0.0	0.0
rs2282143	P341L	T	1.0	7.7	9.1	2.7	2.2	11.0	15.7

[a]Population of this study; [b]Population data from the 1000 Genomes database; LWK (Luhya in Webuye, Kenya); YRI (Yoruba in Ibadan, Nigeria); PUR (Puerto Ricans from Puerto Rica); GBR (British in England and Scotland); CHS (Southern Han Chinese) and JPT (Japanese).

TABLE V
HAPLOTYPE STRUCTURE DEFINED BY THE 20 SNPS IN THE SLC22A1 GENE OF THE ZULU POPULATION

Haplotype Number	Haplotype[a]	Frequency (%)
1	CGTCGCCGCGCAAGAGGTGA	81.23
2	CGTCGCCGCGCAAGCGGTGA	6.89
3	CGTCGCCGCGCAAGAGGTTA	5.90
4	TGTCGCCGCGCAAGAGGTGA	1.98
5	CGTCGCCGCGCAAGAGGAGA	1.98
6	CCTCGCCGCGCAAGAGGTGA	0.99
7	CGTCGCCGTGCAAGAGGTGA	0.99
8	CGTCGCCGCGCAAGCGGTTA	0.04
	Total	100

[a]Haplotype sequences are based on the position of SNPs on chromosome 6.

REFERENCES

[1] M. Hayer, H. Bönisch, and M. Brüss, "Molecular cloning, functional characterization and genomic organization of four alternatively spliced isoforms of the human organic cation transporter 1 (hOCT1/SLC22A1)," *Ann Hum Genet*, vol. 63, Nov. 1999, pp. 473-482.

[2] S. Verhaagh, N. Schweifer, D. P. Barlow, and R. Zwart, "Cloning of the mouse and human solute carrier 22a3 (Slc22a3/SLC22A3) identifies a conserved cluster of three organic cation transporters on mouse chromosome 17 and human 6q26-q27," *Genomics*, vol. 55, Jan 1999, pp. 209-218.

[3] M.L. Becker, L. E. Visser, R. H. van Shaik, A. Hofman, A. G. Uitterlinden, and B. H. Stricker, "Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus," *Pharmacogenomics J*, vol. 9, Aug. 2009, pp. 242-247.

[4] M. Takeda, S. Khamdang, S. Narikawa, H. Kimura, Y. Kobayashi, T. Yamamoto *et al.*, "Human organic anion transporters and human organic cation transporters mediate antiviral transport," *J Pharmacol Exp Ther*, vol. 300, Mar. 2002, pp. 918-924.

[5] N. Kimura, S. Masuda, Y. Tanihara, H. Ueo, M. Okuda, T. Katsura *et al.*, "Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1," *Drug Metab Pharmacokinet*, vol 20, Oct. 2005, pp. 379-386.

[6] A. Yonezawa, S. Masuda, S. Yokoo, T. Katsura, and K. L. Inui, "Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and MATE family)," *J Pharmacol Exp Ther*, vol. 319, Aug. 2006, pp. 879-886.

[7] M. L. Reitman and E. E. Schadt, "Pharmacogenetics of metformin response: a step in the path toward personalized medicine," *J Clin. Invest*, vol. 117, May 2007, pp. 1226-1229.

[8] N. Jung, C. Lehmann, A. Rubbert, M. Knispel, P. Hartmann, J. van Lunzen *et al*, "Relevance of the organic cation transporters 1 and 2 for antiretroviral therapy in HIV infection," *Drug Metab Dispos*, vol. 36, Aug. 2008, pp. 1616-1623.

[9] G. Minuesa, C. Volk, M. Molina-Areas, V. Gorboulev, I. Erkizia, P. Arndt *et al.*, "Transport of lamivudine (3TC) and high-affinity interaction of nucleoside reverse transcriptase inhibitors with human organic cation transporters 1, 2 and 3," *J Pharmacol Exp Ther*, vol. 329, Jan. 2009, 252-261.

[10] M. K. Choi and I. S. Song, "Organic cation transporters and their pharmacokinetic and pharmacodynamic consequences," *Drug Metab Pharmacokinet*,vol. 23, Sep. 2008, pp. 243-253.

[11] E. Herraez, E. Lozano, R. I. Macias, J. Vaquero, L. Bujanda, J. M. Banales *et al*, "Expression of *SLC22A1* variants may affect the response of hepatocellular carcinoma and cholangiocarcinoma to sorafenib," *Hepatology*, vol. 58, Sep. 2013, pp. 1065-1073.

[12] Y. Shu, M. K. Leabman, B. Feng, L. M. Mangravite, C. C. Huang, D. Stryke *et al.*, "Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1," *Proc Natl Acad Sci USA*, vol. 100, May 2003, pp. 5902-5907.

[13] T. Sakata, N. Anzai, H. J. Shin, R. Noshiro, T. Hirata, H. Yokoyama *et al.*, "Novel single nucleotide polymorphisms of organic cation transporter 1 (*SLC22A1*) affecting transport functions," *Biochem Biophys Res Commun*, vol. 313, Jan. 2004, pp. 789-793.

[14] H. J. Kang, I. S. Song, H. J. Shin, W. Y. Kim, C. H. Lee, J. C. Shim *et al.*, "Identification and functional characterization of genetic variants of human organic cation transporters in a Korean population," *Drug Metab Dispos*, vol 35, Apr. 2007, pp. 667-675.

[15] R. Kerb, U. Brinkmann, N. Chatskaia, D. Gorbunov, V. Gorboulev, E. Mornhinweg *et al.*, "Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences," *Pharmacogenetics*, vol. 12, Nov. 2002, pp. 591-595.

[16] Y. Shu, S. A. Sheardown, C. Brown, R. P. Owen, S. Zhang, R. A. Castro *et al.*, "Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action," *J Clin Invest*, vol 117, May 2007, pp. 1422-1431.

[17] B. Chowbay, S. Zhou, and E. J. Lee, "An interethnic comparison of polymorphisms of the genes encoding drug-metabolizing enzymes and

drug transporters: experience in Singapore," *Drug Metab Rev*, vol. 37, 2005, pp. 327-378.
[18] G. Umamaheswaran, R. G. Praveen, A. S. Arunkumar, A. K. Das, D. G. Shewade, and C. Adithan C, "Genetic analysis of OCT1 gene polymorphisms in an Indian population," *Indian J Hum Genet*, vol. 17, Sep. 2011, pp. 164-168.
[19] M. L. Becker, L. E. Visser, R. H. van Shaik, A. Hofman, A. G. Uitterlinden, and B. H. Stricker, "OCT1 polymorphism is associated with response and survival time in anti-Parkinsonian drug users," *Neurogenetics*, vol. 12, Feb. 2011, pp. 79-82.
[20] Y. Ohishi, M. Nakamuta, N. Ishikawa, O. Saitoh, H. Nakamura, Y. Aiba *et al*., "Genetic polymorphisms of OCT-1 confer susceptibility to severe progression of primary biliary cirrhosis in Japanese patients," *J Gastroenterol*, vol. 49, Apr. 2013 , pp. 332-342.
[21] J. W. Langston, P. Ballard, J. W. Tetrud, and I. Irwin, "Chronic parkinsonism in humans due to a product of meperidine-analog synthesis," *Science*, vol. 219, Feb. 1983, pp. 979-980.
[22] M. C. Yang, A. J. McLean, and D. G. Le Couteur, "Cell membrane transport of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahy-dropyridine (MPTP) in the liver and systemic bioavailability," *Biochem Biophys Res Commun*, vol. 289, Nov. 2001, pp. 130-136.
[23] B. J. Hardy, B. Seguin, F. Goodsaid, G. Jimenez-Sanchez, P. A. Singer, and A. S. Daar, "The next steps for genomic medicine: challenges and opportunities for the developing world," *Nat Rev Genet*, vol. 9, Oct. 2008, pp. S23-S27.
[24] M. Benjeddou, "Solute carrier transporters: Pharmacogenomics research opportunities in Africa," *Afr. J. Biotechnol,* vol. 9, Dec. 2010, pp. 9191-9195.
[25] A. B. Lane, H. Soodyall, S. Arndt, M. Ratshikhopha, E. Jonker, C. Freeman *et al.*, 2002 "Genetic substructure in South African Bantu-speakers: Evidence from autosomal DNA and Y-chromosome studies," *Am J Phys Anthropol*, vol. 119, Oct. 2002, pp. 175-185.
[26] G. Berniell-Lee, F. Calafell, E. Bosch, E. Heyer, L. Sica, P. Mouguiama-Daouda *et al.* "Genetic and demographic implications of the Bantu expansion: insights from human paternal lineages," *Mol Biol Evol*, vol. 26, Jul. 2009, pp. 1581-1589.
[27] S. A. Tishkoff, F. A. Reed, F. R. Friedlaender, C. Ehret, A. Ranciaro, A. Froment *et al*., "The genetic and history of Africans and African Americans," *Science*, vol. 324, May 2009, pp. 1035-1044.
[28] Encyclopaedia Brittanica, "Zulu," *Encyclopedia Brittanica Online*, Encyclopedia Britannica Inc. Online 03 December 2013. http://www.britannica.com/EBchecked/topic/658352/Zulu.
[29] A. Takeuchi, H. Motohashi, M. Okuda, and K. Inui, "Decreased function of genetic variants, Pro283Leu and Arg287Gly, in human organic cation transporter hOCT1. *Drug Metab*,"*Pharmacokinet*, vol.18, 2003, pp. 409-412.
[30] O. Ikediobi, B. Aouizerat, Y. Xiao, M. Gandhi, S. Gebhardt, and L. Warnich, "Analysis of pharmacogenetic traits in two distinct South African populations," *Hum Genomics*, vol. 5, May 2011, pp. 265-282.
[31] L. Warnich, B. I. Drögemöller, M. S. Pepper, C. Dandra C, and G. E. Wright, "Pharmacogenomic research in South Africa: lessons and future opportunities in the rainbow nation," *Curr Pharmacogenomics Person Med*, vol. 9, Sep. 2011, pp. 191-207.
[32] A. T. Nies, H. Koepsell, S. Winter, O. Burk, K. Klein, R. Kerb *et al*., "Expression of organic cation transporters OCT1 (*SLC22A1*) and OCT3 (*SLC22A3*) is affected by genetic factors and cholestasis in human liver," *Hepatology*, vol. 50, Oct. 2009, pp. 1227-1240.
[33] N. Leat, M. Benjeddou, and S. Davison, "Nine –locus Y-chromosome STR profiling of Caucasian and Xhosa populations from Cape Town, South Africa," *Forensic Sci Int*, vol. 144, Aug. 2004, pp. 73-75.
[34] R. Peakall and P. E. Smouse, "GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research,' *Mol Ecol Notes*, vol. 6, Mar. 2006, 288-295.
[35] Y. Y. Shi and L. He, "SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci," *Cell Res*, vol. 15, Feb. 2005, pp. 97-98.
[36] Z. Li, Z. Zhang, Z. He, W. Tang, T. Li, Z. Zeng *et al*. "A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn)," *Cell Res*, vol, 19, Apr. 2009, pp. 519-523.
[37] M. L. Becker, L. E. Visser, R. H. van Shaik, A. Hofman, A. G. Uitterlinden, and B. H. Stricker, "Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response," *Pharmacogenet Genomics*, vol. 20, Jan. 2010, pp. 38-44.
[38] M. A. Johnson, K. H. Moore, G. J. Yuen, A. Bye, and G. E. Pakes, 1999 "Clinical pharmacokinetics of lamivudine," *Clin Pharmacokinet*, vol. 36, Jan 1999, pp. 41-66.
[39] M. K. Choi and I. S. Song, "Genetic variants of organic cation transport 1 (OCT1) and OCT2 significantly reduce lamivudine uptake," *Biopharm. Drug Dispos,* vol. 33, Apr. 2012, pp. 170-178.
[40] Y. Shu, C. Brown, R. A. Castro, R. J. Shi, E. T. Lin, R. P. Owen *et al*., "Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics," *Clin. Pharmacol Ther*, vol. 83, Feb. 2008, pp. 273-280.
[41] D.C. Crawford and D.A. Nickerson, 2005 "Definition and clinical importance of haplotypes," *Annu Rev Med*, vol. 56, July 2005, pp. 303-320.