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Research Article

Genetic polymorphisms and haplotypes of the organic cation transporter 1 gene (*SLC22A1*) in the Xhosa population of South Africa

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Abstract

Human organic cation transporter 1 is primarily expressed in hepatocytes and mediates the electrogenic transport of various endogenous and exogenous compounds, including clinically important drugs. Genetic polymorphisms in the gene coding for human organic cation transporter 1, *SLC22A1*, are increasingly being recognized as a possible mechanism explaining the variable response to clinical drugs, which are substrates for this transporter. The genotypic and allelic distributions of 19 nonsynonymous and one intronic *SLC22A1* single nucleotide polymorphisms were determined in 148 healthy Xhosa participants from South Africa, using a SNAPshot® multiplex assay. In addition, haplotype structure for *SLC22A1* was inferred from the genotypic data. The minor allele frequencies for S14F (rs34447885), P341L (rs2282143), V519F (rs78899680), and the intronic variant rs622342 were 1.7%, 8.4%, 3.0%, and 21.6%, respectively. None of the participants carried the variant allele for R61C (rs12208357), C88R (rs55918055), S189L (rs34104736), G220V (rs36103319), P283L (rs4646277), R287G (rs4646278), G401S (rs34130495), M440I (rs35956182), or G465R (rs34059508). In addition, no variant alleles were observed for A306T (COSM164365), A413V (rs144322387), M420V (rs142448543), I421F (rs139512541), C436F (rs139512541), V501E (rs143175763), or I542V (rs137928512) in the population. Eight haplotypes were inferred from the genotypic data. This study reports important genetic data that could be useful for future pharmacogenetic studies of drug transporters in the indigenous Sub-Saharan African populations.

Keywords: polymorphism, haplotype, population genetic structure, genotyping, genetic variability.

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Introduction

Membrane transporters play an important role in the metabolism of clinical drugs and endogenous compounds. Single nucleotide polymorphisms (SNPs) in ATP-binding cassette (*ABC*) and solute carrier transporter (*SLC*) genes have been increasingly recognized as a possible mechanism accounting for altered transport activity, which may have profound clinical implications (Leabman *et al.*, 2003). In general, genetic factors are estimated to account for 15-20% of inter-individual variations in drug disposition and responses (Evans and Relling, 1999; Eichelbaum *et al.*, 2006; Choi and Song, 2008). However, for certain drugs genetic factors can account for up to 95% of inter-individual variability in drug disposition and effect (Evans and Relling, 2004; Eichelbaum *et al.*, 2006).

Polyspecifc organic cation transporters (OCTs) are involved in the sodium-independent electrogenic transport of small organic cations with different molecular structures (Koepsell *et al.*, 2007). These organic cations include clinically important drugs (*e.g.*, metformin, cimetidine, procaindamide), endogenous compounds (*e.g.*, dopamine, norephinephrine, and toxic substrates (*e.g.*, tetraethyl ammonium, haloperidol-derived pyridinium metabolite, 1-methyl-4-phenylpyridinium) (Gorboulev *et al.*, 1997). Based on their substrate properties and tissue distributions, OCT1-3 are thought to play important roles in the biliary and renal excretion of their substrates and the distribution of organic cationic drugs in the liver, kidney, heart, and brain (Jonker and Schinkel, 2004).

Human OCT1 is encoded by the *SLC22A1* gene which is located on chromosome 6q26, and consists of 11 exons spanning approximately 37 kb (Koehler *et al.*, 1997; Koepsell *et al.*, 2007). OCT1 is primarily expressed in the sinusoidal or basolateral membrane of hepatocytes and is thought to play an important role in the hepatic uptake, distribution and excretion of clinically important drugs (Gorboulev *et al.*, 1997; Zhang *et al.*, 1997). Previous studies have shown that hOCT1 is highly polymorphic in ethnically diverse populations (Shu *et al.*, 2003; Sakata *et al.*, 2004; Kang *et al.*, 2007).

These aforementioned reduced-function genetic variants were however primarily found in studies with European participants and have not been consistently replicated

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for other ethnic groups (Leabman *et al.*, 2003; Chen *et al.*, 2010). Recent reports using genome-wide polymorphisms suggested that: (i) genetic variation seen outside of Africa is generally a subset of the total genetic variation that exists within Africa, (ii) genetic diversity decreases with increased geographic distance from Africa, and (iii) linkage disequilibrium (LD) patterns increase proportionally to the distance from Africa (Jakobsson *et al.*, 2008; Li *et al.*, 2008; Tishkoff *et al.*, 2009). Moreover, Rosenberg *et al.* (2002) found that there is greater genetic diversity among African populations compared to Caucasian or Asian populations (Rosenberg *et al.*, 2002). However, despite Africa harboring a significant proportion of human genomic diversity, this genomic diversity is unfortunately relatively under-studied (Hardy *et al.*, 2008; Tishkoff *et al.*, 2009).

South Africa is home to a large number of indigenous and immigrant population groups (Hardy *et al.*, 2008; Benjeddou, 2010). 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 (Lane *et al.*, 2002; Berniell-Lee *et al.*, 2009). The indigenous populations potentially contain a significant amount of genomic diversity (Hardy *et al.*, 2008; Tishkoff *et al.*, 2009). These populations include the Xhosa, historically indigenous to the Eastern Cape Province of South Africa, and the second largest ethnic grouping in the country making up an estimated 8 million or 17.6% of the South African population (Drögemöller *et al.*, 2010; Warnich *et al.*, 2011).

This genomic diversity could provide a wealth of information and knowledge, which could eventually be applied to aid our understanding of the impact of genetic variation on complex diseases such as cancer, diabetes mellitus, hypertension and the inter-individual variability in response of patients to drugs used in the treatment of these diseases. Although limited, studies that have been conducted in South Africa suggest 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 (Warnich *et al.*, 2011).

Previous studies have shown that South African populations exhibit unique allele frequencies and novel genetic variation in pharmacogenetically relevant genes (Ikediobi *et al.*, 2011). Nyakutira *et al.* (2008), for example, showed that the *CYP2B6*6* allele occurs at a higher frequency in people of African origin compared to other population groups, and is associated with high blood concentrations of the anti-HIV drug efavirenz in this group (Nyakutira *et al.*, 2008). In addition, African populations also have higher allele frequencies for two *CYP2D6* variant alleles, *CYP2D6*17* and *29, which in part explain the high incidence of intermediate metabolizers (IMs) of substrate drugs in these populations (Matimba *et al.*, 2009). In another study, Chigutsa *et al.* (2011) investigated the distribution of the organic anion transporter (*OATP*) gene *SLCO1B1* rs4149032 polymorphism, and found that the variant allele occurred at a higher frequency in African populations than in Caucasians or Asians (Chigutsa *et al.*, 2011). The rs4149032 polymorphism is associated with low blood concentrations of the anti-tuberculosis drug rifampicin, which requires the prescription of a higher dosage for people of African origin in order to reach the concentration target. These studies have primarily focused on variants in the drug metabolizing enzyme genes. Information on variants in drug transporter genes for South African populations is however limited or non-existent. Therefore, the aim of the study was to investigate the genotypic and allelic distributions of 19 nonsynonymous and one intronic SNP(s), and to infer the haplotype structure of the *SLC22A1* gene in the Xhosa population. These SNPs include A306T (COSM164365), A413V (rs144322387), M420V (rs72552763), I421F (rs151333280), C436F (rs139512541), V501E (rs143175763), V519F (rs78899680), and I542V (rs137928512) for which, to our knowledge, no population data exist in the public domain.

Materials and Methods

Subjects

Samples were obtained from the participants with informed consent. This study was approved by the Senate Research Ethics Committee of the University of the Western Cape, South Africa. Biological samples were collected in the form of buccal swabs from 148 unrelated healthy volunteers from the Xhosa population. Ethnicity of volunteers was determined by self-report.

DNA Extraction and SNP selection

Genomic DNA was isolated from buccal swab samples using a standard salt-lysis protocol and stored frozen at -20 °C until the time of genotyping (Leat *et al.*, 2004a). A total of 20 OCT1 gene SNPs (19 nonsynonymous and 1 intronic) were selected for this study. SNPs were selected from the literature and the Ensembl database (Flicek *et al.*, 2012). Variants A306T, A413V, M420V, C436F, I421F, V501E, V519F, and I542V were included in this study based on predicted effect on function, using the SIFT (Sorting Intolerant From Tolerant) program (Ng and Henikoff, 2003; Kumar *et al.*, 2009; Flanagan *et al.*, 2010). To our knowledge no population data exist in the public domain for these variants.

Primer design

Multiplex PCR primers, listed in Table 1, were designed to have an annealing temperature between 55 °C and 60 °C using Primer3 software. To test for possible non-specific amplification, primers were aligned with the NCBI sequence databases using Basic Local Alignment Search

Table 1 - 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′	

Tool. 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 Tables 2 and 3.

Multiplex PCR

All the OCT1 exons and the portion of intron 9 spanning rs622342 were simultaneously amplified using the primers listed in Table 1. The PCR reactions were performed in a 20 µL volume, containing 20-50 ng of genomic DNA, 1 x Qiagen multiplex PCR master mix (Qiagen, Courtaboeuf, France) and 0.2 µM of each primer. Cycling consisted of an initial 15 min activation step for HotStar *Taq* polymerase (Qiagen) at 95 °C, followed by a total of 35 cycles using the following conditions: 94 °C denaturation for 30 s, primer annealing at 60 °C for 90 s, and primer extension at 72 °C for 30 s, and 15 min of final extension at 72 °C and a 4 °C holding step. PCR products were purified

Table 2 - OCT1 multiplex 1 single base extension primers.

NCBI (dbSNP)	Amino acid change	Nucleotide change	Single base extension primers	dGACT	Size bp
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
rs72552763	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	V519P	G/T	5′ - CTACTTCTTCCAGAGACCAAGGGG - 3′	56	80
rs137928512	I542V	A/G	5′ - CAGAGGTTTGGACCTTAAGGTAAA - 3′	61	85

Table 3 - OCT1 multiplex 2 single base extension primers.

NCBI (dbSNP)	Amino acid change	Nucleotide change	Single base extension primers	dGACT	Size bp
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

to remove excess primers and un-incorporated dNTPs using an Exo/SAP protocol. The entire 20 μL of PCR products were incubated with 0.5 μL of *Exo*1 (20 U/μL) enzyme (Thermo Scientific, Waltham, MA, USA) and 1 μL of FastAP (1 U/μL) (Thermo Scientific) for 30 min at 37 °C followed by 15 min at 80 °C for enzyme inactivation. PCR quality and yield were checked using NanoDrop spectrophotometer.

Multiplex minisequencing reactions

Multiplex minisequencing was performed in a 10 μL reaction volume using 3 μL of a 1/10 dilution of purified PCR products, 0.1-0.2 μM of primers, and 5 μL of SNaPshot® ready reaction mix (Applied Biosystems, Foster City, CA, USA). Sequence cycling consisted of 25 cycles of denaturation at 96 °C for 10 s, primer annealing at 50 °C for 5 s, and primer extension at 60 °C for 30 s. Post-extension treatment was done by adding 1 U of FastAP to the 10 μL reaction volume and incubation at 37 °C for 30 min followed by 15 min at 80 °C to deactivate the enzyme.

Electrophoresis of the minisequencing products

The purified minisequencing products (1 μL) were mixed with 8.7 μL of HiDi formamide (Applied Biosystems) and 0.3 μL of GeneScan-120 Liz size standard (Applied Biosystems) and denatured at 95 °C for 5 min. The fluorescently labelled 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 (Applied Biosystems).

Statistical analysis

Genotype and allele frequencies as well as the deviation from the Hardy-Weinberg Equilibrium were calculated using GenAlEx 6.5 software (Wigginton *et al.*, 2005; Peakall and Smouse, 2012). Allele and genotype frequencies are given with binomial proportion 95% confidence intervals (CI) calculated according to the method of Wilson. The SHEsis analysis platform was used to infer the haplotype frequencies (Yong and Lin, 2005; Li *et al.*, 2009). Statistical significance was defined as $p < 0.05$.

Results

The population studied consisted of 148 healthy Xhosa individuals between the ages of 18 and 61 years. There were 80 (54%) female and 68 (46%) male participants. The mean age of female participants was 25.3 ± 9.0 years, whereas male participants had a mean age of 24.8 ± 7.7 years.

The genotype and allele frequencies of the 20 OCT1 gene SNPs investigated in the 148 Xhosa subjects are summarized in Table 4. The allelic frequency of each SNP was in HWE ($p > 0.05$), except for rs622342. The genotype frequencies for rs622342 for homozygote wild-type (AA), heterozygote (AC) and homozygote (CC) were 63.5%, 29.1%, and 7.4%, respectively. The MAF observed for rs622342 was 23%. Sixteen out of the 19 investigated nonsynonymous SNPs were monomorphic in the Xhosa population. None of the participants were homozygous for the variant allele for S14F (rs34447885), P341L (rs2282143), and V519F (rs78899680). The S14F (rs34447885) variant genotype frequencies for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 96.6%, 3.4% and 0.0%, respectively. The MAF observed for S14F (rs34447885) was 1.7%. The P341L (rs2282143) variant genotype frequencies, on the other hand, for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 83.1%, 16.9% and 0.0%, respectively. The V519F (rs78899680) variant genotype frequencies for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 93.9%, 6.1% and 0.0%, respectively.

The minor allele frequency (MAF) of a selected number of the investigated OCT1 gene SNPs in different ethnic groups are summarized in Table 5. *SLC22A1* SNP variants

Table 4 - Genotype and allele frequencies of OCT1 (*SLC22A1*) gene SNPs in 148 healthy Xhosa individuals.

Amino acid substitution	Observed genotype frequency				Allele frequency			
	dbSNP ID	Genotype	%	95% CI	Allele	%	95% CI	HWE (p)
S14F	rs34447885	CC	96.6	92.0 - 98.8	C	98.3	96.3 - 99.1	0.834
		CT	3.4	1.2 - 8.0	T	1.7	0.9 - 3.7	
		TT	0.0	0.0 - 3.1				
R61C	rs12208357	CC	100.0	96.9 - 100.0	C	100.0	98.4 - 100.0	
		CT	0.0	0.0 - 1.3	T	0.0	0.0 - 1.6	
		TT	0.0	0.0 - 1.3				
C88R	rs55918055	TT	100.0	96.9 - 100.0	T	100.0	98.4 - 100.0	
		TA	0.0	0.0 - 1.3	A	0.0	0.0 - 1.6	
		AA	0.0	0.0 - 1.3				
S189L	rs34104736	CC	100.0	96.9 - 100.0	C	100.0	98.4 - 100.0	
		CT	0.0	0.0 - 1.3	T	0.0	0.0 -1.6	
		TT	0.0	0.0 - 1.3				
G220V	rs36103319	GG	100.0	96.9 - 100.0	G	100.0	98.4 - 100.0	
		GT	0.0	0.0 - 1.3	T	0.0	0.0 -1.6	
		TT	0.0	0.0 - 1.3				
P283L	rs4646277	CC	100.0	96.9 - 100.0	C	100.0	98.4 - 100.0	
		CT	0.0	0.0 - 1.3		0.0	0.0 - 1.6	
		TT	0.0	0.0 - 1.3				
P341L	rs2282143	CC	83.1	74.6 - 80.9	C	91.6	87.0 - 93.7	0.261
		CT	16.9	12.8 - 19.1	T	8.4	6.3 -13.0	
		TT	0.0	0.0 - 3.1				
G401S	rs34130495	GG	100.0	96.9 - 100.0	G	100.0	98.4 - 100.0	
		GA	0.0	0.0 - 1.3	A	0.0	0.0 -1.6	
		AA	0.0	0.0 - 1.3				
M440I	rs35956182	GG	100.0	96.9 - 100.0	G	100.0	98.4 - 100.0	
		GA	0.0	0.0 - 1.3	A	0.0	0.0 -1.6	
		AA	0.0	0.0 - 1.3				
G465R	rs34059508	GG	100.0	96.9 - 100.0	G	100.0	98.4 - 100.0	
		GA	0.0	0.0 - 1.3	A	0.0	0.0 - 1.6	
		AA	0.0	0.0 - 1.3				
V519F	rs78899680	GG	93.9	88.6 - 96.9	G	97.0	94.2 - 98.5	0.703
		GT	6.1	3.1 - 11.4	T	3.0	1.5 - 3.7	
		TT	0.0	0.0 - 3.1				
Intronic SNP	rs622342	AA	64.2	54.7 - 70.3	A	78.4	72.2 - 81.8	0.048
		AC	28.4	22.3 - 36.9	C	21.6	18.2 - 23.0	
		CC	7.4	6.1 - 16.2				
R287G	rs4646278	CC	100.0	96.9 - 100.0	C	100.0	98.4 - 100.0	
		CG	0.0	0.0 - 1.3	G	0.0	0.0 - 1.6	
		GG	0.0	0.0 - 1.3				
I542V	rs137928512	AA	100.0	96.9 - 100.0	A	100.0	98.4 - 100.0	
		AG	0.0	0.0 - 1.3	G	0.0	0.0 - 1.6	
		GG	0.0	0.0 - 1.3				
M420V	rs142448543	AA	100.0	96.9 - 100.0	A	100.0	98.4 - 100.0	
		AG	0.0	0.0 - 1.3	G	0.0	0.0 - 1.6	
		GG	0.0	0.0 - 1.3				

Table 4 (cont.)

Amino acid substitution	Observed genotype frequency				Allele frequency			
	dbSNP ID	Genotype	%	95% CI	Allele	%	95% CI	HWE (p)
A413V	rs144322387	CC	100.0	96.9 - 100.0	C	100.0	98.4 - 100.0	
		CT	0.0	0.0 - 1.3	T	0.0	0.0 - 1.6	
		TT	0.0	0.0 - 1.3				
I421F	rs151333280	AA	100.0	96.9 - 100.0	A	100.0	98.4 - 100.0	
		AT	0.0	0.0 - 1.3	T	0.0	0.0 - 1.6	
		TT	0.0	0.0 - 1.3				
C436F	rs139512541	GG	100.0	96.9 - 100.0	G	100.0	98.4 - 100.0	
		GT	0.0	0.0 - 1.3	T	0.0	0.0 - 1.6	
		TT	0.0	0.0 - 1.3				
V501E	rs143175763	TT	100.0	96.9 - 100.0	T	100.0	98.4 - 100.0	
		TA	0.0	0.0 - 1.3	A	0.0	0.0 - 1.6	
		AA	0.0	0.0 - 1.3				
A306T	COSM164365	GG	100.0	96.9 - 100.0	G	100.0	98.4 - 100.0	
		GA	0.0	0.0 - 1.3	A	0.0	0.0 - 1.6	
		AA	0.0	0.0 - 1.3				

R61C (rs12208357), C88R (rs55918055), S189L (rs34104736), G401S (rs34130495), and G465R (rs34059508) were not observed in the Xhosa, Sub-Saharan or Asian populations. However, it was observed in Caucasian populations. Two *SLC22A1* SNP variants, S14F (rs34447885) and V519F (rs78899680), were only observed in the Xhosa and the other Sub-Saharan populations, but not in the Asian or Caucasian populations. Inferred haplotypes are listed in Table 6.

Discussion

Single nucleotide polymorphisms in OCT1 have been increasingly recognized as a possible mechanism explaining inter-individual variation in drug response (Leabman *et al.*, 2003). In this study we determined the allelic frequency of 20 SNPs in the OCT1 gene of 148 healthy individuals of the Xhosa population of South Africa, and the data was compared with other published studies. No polymorphisms were observed for the Xhosa population for 16 out of the 20 SNPs investigated in this study. In a previous study aimed at the development of male specific genotyping systems for use in sexual assault cases in South Africa, low levels of polymorphism were also observed for the Xhosa population (Leat *et al.*, 2004a,b, 2007).

hOCT1 carrying the S14F (rs34447885) substitution was previously shown to exhibit an increased uptake of the prototypical organic cation MPP^+ (Shu *et al.*, 2003). However, in a subsequent study by Shu *et al.* (2007) it was shown that the S14F variant displayed a reduced uptake of the anti-diabetic drug metformin which, was attributed to a reduction in the transporter's V_{max} for metformin (Shu *et al.*, 2007). The MAF of S14F (rs34447885) for the Xhosa population (2.0%) was similar to that of African-Americans (3%) (Shu *et al.*, 2003) and of two other sub-Saharan African populations, the Luhya in Webuye, Kenya (3%) and the Yoruba in Ibadan. Nigeria (2.0%). However, the MAF was significantly higher than that observed in Caucasians (0.0%) and Asians (0%). Therefore, it is possible to expect that drugs which are substrates of OCT1 could have different response profiles in the Xhosa population compared to Caucasian and Asian populations.

Previous studies have found that hOCT1 R61C (rs12208357) and G401S (rs34130495) variants showed reduced transport of the prototypical organic cation MPP^+ (Shu *et al.*, 2003). In addition, it was shown that these variants exhibited reduced transport of metformin (Shu *et al.*, 2007). Furthermore, the R61C (rs12208357) variant has been reported to be strongly correlated with low OCT1 protein expression in liver tissues of a 150 Caucasian subjects (Nies *et al.*, 2009). Moreover, these reduced-function variants were associated with an increase in the renal clearance of metformin (Tzvetkov *et al.*, 2009). Both these variants are frequently observed in Caucasian populations with MAF of 7.2% and 4%, respectively (Shu *et al.*, 2003). In contrast, none of these variants were observed for the Xhosa, Luhya, Yoruba, African-Americans or any of the Asian populations.

In *Xenopus laevis* oocytes expression systems, the uptake of the prototypical organic cation MPP^+ by the C88R (rs55918055) variant transporter was reduced to 1.4% compared with the reference, whereas serotonin uptake was reduced to only 13% of the wild-type (Kerb *et al.*, 2002). The MAF for C88R (rs55918055) in a Caucasian

Table 5 - Comparison of MAF of OCT1 (*SLC22A1*) gene SNPs of the Xhosa population to other ethnic groups.

dbSNP ID	Amino acid change	Minor allele	Minor allele frequency (%)							
			Xhosa[a]	Luhya[b]	Yoruba[b]	African-American[c]	Japanese[d]	Chinese-Han[b]	Caucasian-Finish[b]	Caucasian-American[c]
rs34447885	S14F	T	1.7	2.6	1.7	3.1	0.0	0.0	0.0	0.0
rs12208357	R61C	T	0.0	0.0	0.0	0.0	0.0	0.0	5.4	7.2
rs55918055	C88R	A	0.0	0.0	0.0	ND	ND	0.0	0.0	0.6
rs34104736	S189L	T	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
rs36103319	G220V	T	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
rs4646277	P283L	T	0.0	0.0	0.0	ND	ND	0.5	1.3	ND
rs2282143	P341L	T	8.4	8.0	9.0	8.2	16.8	12.4	16.7	0.0
rs34130495	G401S	A	0.0	0.0	0.0	0.7	0.0	0.0	1.6	1.1
rs35956182	M440I	A	0.0	0.0	0.0	0.5	0.0	0.0	2.7	0.0
rs34059508	G465R	A	0.0	0.0	0.0	0.0	0.0	0.0	1.1	4.0
rs78899680	V519F	T	3.0	2.0	6.0	ND	ND	0.0	0.0	ND
rs622342	Intronic	C	23.0	22.0	15.0	ND	ND	13.2	37.1	ND

[a]This study; b. Data from 1000Genomes; c. Data from (Shu *et al.*, 2003); d. Data from (Itoda *et al.*, 2004).

Table 6 - Haplotype structure defined by 20 SNPs in the *SLC22A1* gene in the Xhosa population.

Haplotype no.	Haplotypes[a]	Frequency %
1	CCTCGCCGCGCAAGGAGTGA	69.4
2	CCTCGCCGCGCAAGGAGTTA	2.8
3	CCTCGCCGCGCAAGGCGTGA	17.6
4	CCTCGCCGTGCAAGGAGTGA	5.0
5	CCTCGCCGTGCAAGGCGTGA	3.2
6	CCTCGCCGTGCAAGGCGTTA	0.2
7	TCTCGCCGCGCAAGGAGTGA	1.1
8	TCTCGCCGCGCAAGGCGTGA	0.6
	Total	99.9

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

population was observed at 6.2% by Kerb *et al.* (2002), compared to 0.0% for the Xhosa population in this study.

The OCT1 variants G220V (rs36103319) and G465R (rs34059508) were first identified as non-functional variants. The G220V (rs36103319) variant has thus far only been observed in the African American population with MAF of 0.5%, whereas, the G465R (rs34059508) was only observed in the Caucasian population at a MAF of 4.0%. Moreover, G465R (rs34059508) was associated with reduced localization at the basolateral membrane (Shu *et al.*, 2007). However, none of these non-functional variants were observed in this study for the Xhosa population. These variants were also not observed in other African populations or in any of the Asian populations.

The allele frequency of P341L (rs2282143) in the Xhosa population (8.4%) was similar to those of other Sub-Saharan African populations, lower than the Asian populations, and significantly higher than that of the Caucasian populations (Table 5). Functional transport assays conducted *in vitro* have shown that the P341L (rs2282143) variant results in a decrease rate of MPP+ transport, and has no effect on the transport of the anti-diabetic drug metformin (Sakata *et al.*, 2004; Shu *et al.*, 2007). Thus, impaired transport activities related to the P341L (rs2282143) SNP may differ between Africans, Asians, and Caucasians, with consequent effects on the pharmacokinetics/pharmacodynamics of certain substrates (Kang *et al.*, 2007).

The P283L (rs4646277) variant was first described in a Japanese population and was shown to have reduced transport activity despite similar protein expression levels of the plasma membrane (Takeuchi *et al.*, 2003; Sakata *et al.*, 2004). The P283L (rs4646277) variant was subsequently also found in other Asian populations with an allele frequency of 1.3% in a Korean population and did not differ significantly from those of Chinese and Vietnamese populations (Kang *et al.*, 2007). This variant was however not observed in the Xhosa population or any of the other Afri-

can populations nor in any of the Caucasian populations (Table 5).

The intronic SNP rs622342 was first reported by Becker *et al.* (2009) in a group of Dutch diabetic patients with a MAF of 37.0%. In contrast, the MAF for this variant in the Xhosa population was lower (21.6%) (Becker *et al.*, 2009). Moreover, these authors concluded that an association existed between genetic variation in the gene encoding for the OCT1 transporter protein and the glucose lowering effect of metformin in diabetes mellitus patients, and that metformin therapy was less effective in patients carrying the minor C allele (Becker *et al.*, 2009). In a subsequent study, by the same group, it was shown that the effect of the MATE1 rs2289669 polymorphism on the glucose lowering effect of metformin was larger in patients with the OCT1 rs622342 CC genotype than those with the AA genotype (Becker *et al.*, 2010).

In the present study, the nonsynonymous SNP V519F (rs78899680) was also genotyped and the observed MAF was 3.0%. This value was higher than that of the Luhya (2.0%), a population from Eastern Africa, and lower than that of the Yoruba (6.0%) of Western Africa (Table 5). However, this variant was not observed in any of the Caucasian or Asian populations, indicating that it may be specific to African populations. The impact of this variant on transport function or drug efficacy has not yet been determined and requires further investigation.

It is well known that individual variation in drug response can be attributed to specific genetic variants. Moreover, it is believed that the incorporation of haplotypes in pharmacogenetic studies will provide a more complete picture of loci that are relevant in the practice of "genetic medicine" both at an individual or population level (Crawford and Nickerson, 2005). In this study, the haplotype structure defined by 20 SNPs in the *SLC22A1* gene was inferred for the investigated population. The most frequently observed haplotypes were CCTCGCCGCGCAAGGAGTGA (69.4%), CCTCGCCGCGCAAGGCGTGA (17.6%), and CCTCGCCGTGCAAGGAGTGA (7.0%).

Although Africa is the continent where the burden of disease is the heaviest, research and clinical trials are predominantly performed on Western European and North American Caucasian, and Asian populations. The results of these studies are often extrapolated for use and interpretation in African populations, which contributes to poor treatment response and occurrence of adverse drug reactions in the genetically diverse African populations. Thus, OCT 1 variant alleles which are commonly/only found in African populations will/may have a profound impact on organic cationic drug transport efficacy and toxicity. Given that organic cationic drugs are used in the treatment of diseases such as Type II diabetes mellitus, various cancers, and HIV, these variant alleles may impact profoundly on healthcare provided across the African continent. Therefore, given the aforementioned reasons studies such as this is invaluable in the generation of useful pharmacogenetic information specific for African populations.

Conclusions

To our knowledge, this is the first study that investigated the allele and genotype frequency distributions of SNPs in the OCT1 gene of the Xhosa population. This study also reports the observed haplotypes in the investigated population. It has also been shown that reduced-function nonsynonymous SNPs in the OCT1 gene found in Caucasian and Asian populations are absent from the Xhosa population. We have shown that, although MAF observed for the Xhosa population is largely similar to other African populations, differences exist that may translate into differences in organic cationic drug transport between these ethnic groups. These variations may translate into differences in the transport and efficacy of organic cationic drugs commonly used for the treatment of diseases prevalent in Africa. However, it should be noted that this was only a descriptive study and that no associations are made between any diseases or treatment outcomes. This study contributes towards filling the gap that exists with regards to genetic information about important variations in organic cation transporter genes for the indigenous populations of South Africa.

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Internet Resources

Ensembl database, http://www.ensembl.org (accessed April 2011).

Primer3 software, http://www.genome.wi.mit.edu/cgi-bin/primer/primer3 (accessed April 2011).

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