Genetic polymorphisms of the organic cation transporter 1 gene (*SLC22A1*) within the Cape Admixed population of South Africa

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Abstract Human organic cation transporter 1 (hOCT1) is expressed primarily in hepatocytes and mediate the electrogenic transport of various endogenous and exogenous compounds, including clinically important drugs. Genetic polymorphisms in the gene coding for hOCT1, *SLC22A1*, are increasingly being recognized as a possible mechanism explaining the variable response of individual patients to clinical drugs which are substrates for this transporter. The aim of this study was to investigate the allele and genotype frequencies of single-nucleotide polymorphisms (SNPs) of *SLC22A1* in the Cape Admixed population of South Africa. The genotypic and allelic distributions of nineteen non-synonomous and one intronic *SLC22A1* SNPs were determined in 100 healthy Cape Admixed participants, using a SNaPshot® multiplex assay. In addition, haplotype structure for *SLC22A1* was inferred from the genotypic data. The minor allele frequencies for S14F, P341L, S189L, G220V, V519F, M440I, G465R and the rs622342 intronic variant were 1.0, 0.5, 1.0, 1.0, 1.5, 0.5, 0.5 and 18.0 %, respectively. None of the participants carried the variant allele for R61C, C88R, P283L, R287G and G401S. In addition, no variant alleles were observed for A306T, A413V, M420V, I421F, C436F, V501E, and I542V in the population. Twelve haplotypes were inferred from the genotypic data. The frequencies for most common haplotypes CCTCGGCGCGCTAGAGCTGA, CCTCGGCGCGCTAGCGCTGA and CCTCGGCGCGCGAGCGCTGA were 80, 9.9, and 3.5 %, respectively.

Introduction

The human organic cation transporters, also designated as hOCT1, hOCT2, and hOCT3, mediate electrogenic transport of small organic cations with different molecular structures, independent of sodium gradient. These organic cation substrates include clinically important therapeutics (e.g., metformin, procainamide, and cimetidine), endogenous compounds (e.g., dopamine and norepinephrine), as well as toxic substances [e.g., tetraethylammonium bromide (TEA)] [1]. The genes encoding the three organic cation transporter isoforms (hOCT1, hOCT 2, and hOCT 3) are clustered together on the long arm of chromosome 6. Population genetics analyses identified numerous single-nucleotide polymorphisms (SNPs) in the three genes [2]. These polymorphisms may be the reason for inter-individual variability in pharmacokinetic disposition, efficacy, and toxicity of therapeutic drugs. Recent studies suggest that inter-patient variability in response to metformin therapy, for intense, could be related to some of the genetic polymorphisms in the OCT genes and/or the MATE genes [3].

Human organic cation transporter 1 (*SLC22A1*), expressed predominantly in the liver, plays a critical role in cellular uptake and elimination of numerous cationic substrates, including therapeutically important compounds [4, 5]. Numerous functionally relevant SNPs have been reported within *SLC22A1*. Kerb et al. [6] have reported that 25 SNPs have been identified within 57 Caucasian individuals in human *SLC22A1*. Furthermore, three non-synonymous SNPs (R61C, C88R, and G401S) showed reduced transport

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activities. A total of 15 protein-altering variants from *SLC22A1* from diverse ethnic backgrounds were reported by Shu et al. [7]. Further findings indicated that four SNPs (R61C, G220V, G401S, and G465R) showed reduced transport function, whereas S14F exhibited increased transport function [7]. Two non-synonymous SNPs found in Korean and Japanese populations (P283L and P341L) showed reduced transport activity [8, 9]. Furthermore, it has been established that polymorphic variations exist within the SLC22A superfamily from different ethnic groups.

Genomic diversity within sub-Saharan Africa, and for that matter the entire African continent, is relatively understudied, despite being home to significant portion of human genomic diversity [10]. There is thus much to be learnt from characterizing human genomic variation in this part of Africa, especially with regards to health applications [10]. South Africa in particular contains a wealth of different population groups. It is home to the indigenous Khoisan, Xhosa, Zulu, Venda, and Sotho Pedi groups, the Afrikaners and the Cape Coloured, the latter being a uniquely admixed population of immigrant Europeans, Asians and the indigenous populations [10]. Admixed groups, such as Latinos, African Americans, or Cape Coloureds from South Africa, share varying proportions of different ancestral populations and their genetic complexity can potentially complicate biomedical research studies [11]. Their mixed ancestry, however, can provide the intrinsic variability needed to untangle complex gene–environment interactions, which may help to explain the population differences in the epidemiology of complex diseases [11].

In this report, we examine the genetic diversity of the *SLC22A1* gene and discuss its possible pharmacogenetic implications within the Cape Admixed population of South Africa. The genotypic and allelic frequencies of 20 SNPs in the OCT1 gene was determined from 100 healthy individuals in the Cape Admixed population, and the data was compared to other population data available in literature and relevant databases. The haplotype structure defined by the 20 SNPs in the *SLC22A1* gene was also inferred for this population.

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 100 unrelated healthy volunteers from the Cape Admixed population. This population is known in South Africa as the "Cape Coloured" population [10]. The term Cape Coloured is used to define a

uniquely admixed population which was established with contributions from Asian, European and indigenous populations [11, 12]. This population is known to have the highest rate of admixture in the world and comprises approximately 8.9 % (~4 million) of the South African population [12]. Ethnicity of volunteers was determined by self-report as obtained from their completed anonymous questionnaires. To confirm the volunteers' mixed ancestry, the questionnaires included a short genealogy of two generations for maternal and paternal family members, as well as place of birth, home language and religious affiliation.

SNP selection

A total of 20 OCT1 gene SNPs (19 nonsynonomous and 1 intronic) were selected for this study. SNPs were selected from the literature and the Ensembl database (http://www.ensebl.org) [13]. Variants A306T; A413V; M420V, C436F; I421F: V501E, V519F and I542V were included in this study based on predicted effect on function, using the sorting intolerant from tolerant (SIFT) program [14]. 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 and 60 °C using Primer3 software [15]. To test for possible non-specific amplification, primers were aligned with the NCBI sequence databases using Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/blast/blast-cgi). Two SNaPshot® Multiplex systems were designed, 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 amplification

All of the OCT1 exons and the portion of intron 9 spanning rs622342 were simultaneously amplified using the primers listed in Table 1. Isolated DNA was diluted to a final concentration of 20 ng μl^{-1} to be used for multiplex PCR amplification. PCR amplifications were performed using the Quiagen Multiplex kit in a final volume of 10 µl according to manufacturer's instructions. Amplification was performed on a GeneAmp 2700 (Life Technologies) under cycling conditions: 95 °C for 15 min, 94 °C for 30 s, 58 °C for 60 s, 72 °C for 90 s, for a total of 39 cycles, followed by 72 °C for 10 min and a final hold at 4 °C. PCR products were confirmed by agarose gel electrophoresis. Amplified targets were enzymatically purified using SAP (Amersham) and Exo I (USB, Cleveland, OH, USA) according to the manufacturer's instructions.

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 & 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′-TGCCCTTTTTCTTCTTTGCTGTTTGC-3′	460
	25	60.8	5′-AGCACCAACAGCTTTCCCTAGATCG-3′	
Intron 9	25	58.8	5′-GAGTAGGAGGGGTTAATAGAGAGAG-3′	236
	27	65.7	5′-GTAGCTGAGACTACATGCATGCACCAC-3′	

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

Multiplex mini-sequencing reaction

Multiplex mini-sequencing was performed using the SNaPshot® kit (Life Technologies), according to manufacturer's instructions as well as in-house design and optimization. Cycling conditions were as follows: 30 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 30 s, followed by a 4 °C hold. Post-extension treatment was performed 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 and 0.3 μl of GeneScan-120 Liz size standard (Life Technologies) 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 (Life Technologies). Data analyses were performed using GeneMapper® IDX Software Version 1.2. Typical electropherograms for multiplexes I and II are shown in Fig. 1.

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
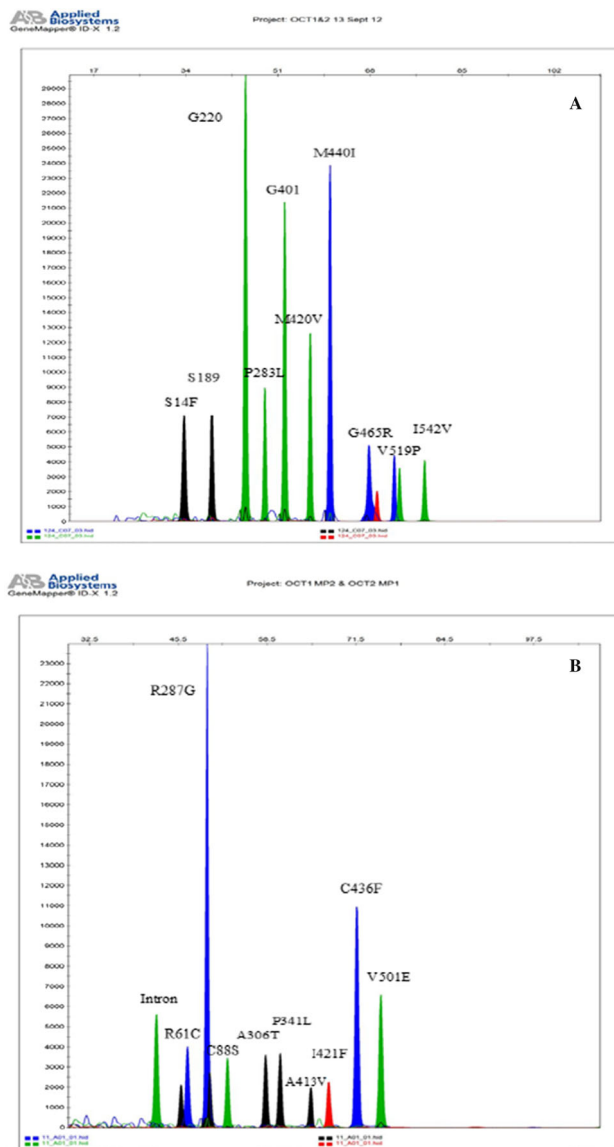
Fig. 1 Typical electropherograms for multiplex I (**a**) and II (**b**). *Green* peaks indicate the A nucleotide, *blue* peaks indicate the G nucleotide, *black* peaks indicate a C nucleotide and *red* peaks indicate the T nucleotide. (Color figure online)

Statistical analysis

Genotype and allele frequencies as well as the deviation from the Hardy–Weinberg equilibrium were calculated using GenAlEx 6.4.1. [16]. The SHEsis analysis platform was used to infer the haplotypes and determine their frequencies [17, 18]. Statistical significance was defined as having a p value greater than 0.05 ($p > 0.05$).

Results

The genotype and allele frequencies of the 20 investigated SNPs of the OCT1 gene within a group of 100 Cape Admixed subjects are summarized in Table 4. The allelic frequency of each SNP was in HWE ($p > 0.05$). Twelve out of the twenty investigated nonsynonomous SNPs were monomorphic in the Cape Admixed population (Table 4). Heterozygosity was only observed for the nonsynonomous SNP variants S14F (rs34447885), P341L (rs2282143), S189L (rs34104736), G220V (rs36103319), M440I (rs35956182), G465R (rs34059508) and V519F (rs78899680). The S14F variant genotype frequencies for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 98, 2 and 0.0 %, respectively. The MAF observed for S14F was 1 %. The P341L variant genotype frequencies, on the other hand, for homozygote wild-type (CC), heterozygote (CT) and homozygote (TT) were 96, 1 and 0.0 %, respectively. The S189L and G220V variants displayed the same genotype frequencies for their respective genotypes; 98, 2 and 0.0 %, respectively. Additionally, the M440I and G465R variants displayed the same genotype frequencies for homozygote wild-type (GG), heterozygote (GA) and homozygote (AA); 99, 1 and 0.0 %, respectively for each. Whereas the genotype frequencies for variant V519F were 97 % [homozygote wild-type (GG)] and 3 % [heterozygote (GT)].

The MAF of a selected number of the investigated OCT1 gene SNPs in different ethnic groups are

Table 4 Allele and genotype frequencies of 20 SNPs of the OCT1 gene (*SLC22A1*) in 100 healthy individuals from the Cape Admixed population

dbSNP ID	Genotype counts[a] (%)			MAF counts (%)	HWE (*p*)
rs34447885	CC 98	CT 2	TT	T 2 (1)	0.920
rs34104736	CC 98	CT 2	TT	T 2 (1)	0.920
rs36103319	GG 98	GT 2	TT	T 2 (1)	0.920
rs4646277	CC 100	CT	TT	T 0	
rs34130495	GG 100	GA	AA	A 0	
rs72552763	AA 92	AG 8	GG	G 8 (4)	0.677
rs35956182	GG 99	GA 1	AA	A 1 (0.5)	0.960
rs34059508	GG 99	GA 1	AA	A 1 (0.5)	0.960
rs78899680	GG 97	GT 3	TT	T 3 (1.5)	0.879
rs139928512	AA 100	AG	GG	G 0	
rs622342	AA 67	AC 30	CC 3	C 36 (18)	0.871
rs12208357	CC 100	CT	TT	T 0	
rs4646278	CC 100	CG	GG	G 0	
rs55918055	TT 100	TC	CC	C 0	
COSM164365	GG 100	GA	AA	A 0	
rs2282143	CC 97	CT 3	TT	T 3 (1.5)	0.879
rs144322387	CC 100	CT	TT	T 0	
rs151333280	AA 100	AT	TT	T 0	
rs139512541	GG 100	GT	TT	T 0	
rs143175763	TT 100	TA	AA	A 0	

[a] $n = 100$

summarized in Table 5. Variants R61C (rs12208357), C88R (rs55918055), P283L (rs4646277) and G401S (rs34130495) were not observed in the Cape Admixed nor the Native American/Hispanic Admixed populations. The S14F variant was observed in the Cape Admixed and other Sub-Saharan populations, but not in all other populations. The S189L and G220V variants were only observed in the Cape Admixed population at a frequency of 1 %.

The inferred haplotypes for investigated population are listed in Table 6. The most frequently observed haplotypes were CCTCGGCGCGCTAGAGCTGA (80 %), CCTCGG CGCGCTAGCGCTGA (9.9 %) and CCTCGGCGCGCGA GCGCTGA (3.5 %).

Discussion

Single nucleotide polymorphisms in OCT1 gene have been recognized as a possible mechanism explaining inter-individual variation in drug response [19]. In this study, we determined the genotypic and allelic frequencies of 20 SNPs in the OCT1 gene from 100 healthy individuals in the Cape Admixed population, and the data was compared to other population data available in literature and relevant databases.

In a study conducted by Leabman et al. [19] numerous polymorphisms within the *SLC22A1* gene were identified, and have constituted the focus of many subsequent studies. Furthermore, it was determined that these variant might predict functional changes of the transporter protein [19]. Several SNPs were found to be protein-altering variants. This became important as these inherited differences may contribute to the inter-individual variability in drug response [19, 20]. Fifteen of these protein-altering SNPs were also characterized and evaluated with respect to MPP^+ uptake [20]. It was determined that the variant S14F increased function of the transporter protein, whereas variants S189L, M420V and M440I were shown to exhibit the same functionality as the reference transporter. Furthermore, R61C and P341L were identified as reduced function variants, whereas variants G220V, G401S and G465R exhibited a loss of function compared to the reference sequence [20]. In subsequent studies, by Shu et al. [21] and Tzvetkov et al. [2], evaluating the effect OCT1 variants have on metformin uptake, it was shown that the R61C, G401S, M420V and G465R variants all exhibited a reduction in transporter function. In a prior study by Kerb et al. [6], the uptake of 0.1 [3H]1-methyl-4-phenylpyridinium ([3H]MPP) by R61C, C88R and G401S were reduced to 30, 1.4 and 0.9 % compared to wild-type, respectively. The transport of 1 μM [3H] serotonin by C88R and G401S, on the other hand, was reduced to only 13 and 12 % of wild-type respectively [6]. From the data obtained, Kerb et al. [6] suggested that mutants R61C, C88R and G401S could affect the disposition of OCT1 substrates in terms of affinity and selectivity. This has led them to conclude that the duration and intensity of effects of drugs and neurotransmitters may be altered for these substrates [6].

In the present study, 19 protein-altering SNPs were evaluated within the Cape Admixed population. The intronic SNP (rs622342) was also included in the study because it was previously suggested to be associated with the blood glucose-lowering effect of metformin [22].

Variant S14F was observed in the Cape Admixed, Luhya and Yoruba populations at a MAF of 1, 2.5 and 1.5 % respectively. This variant was not observed in any of the other populations included in the analysis (Table 5). Therefore, it may be suggested that OCT1 substrate drugs might have different response profiles in the Cape Admixed and African populations compared to Caucasian and Asian populations.

The G220V variant was observed in the Cape Admixed population with MAF of 1 %, whereas, the other reduced-function variants G401S and G465R were not observed in the population. This may suggest an increased therapeutic response to metformin for our population [21]. The G401S and G465R variants were only observed in the Caucasian

Table 5 Comparison of MAF of OCT1 gene (*SLC22A1*) SNPs of the Cape Admixed population to other ethnic groups

dbSNP ID	Amino acid change	Minor Allele	Minor allele frequency (%)					
			Cape Admixed[a]	Luhya[b]	Yoruba[b]	Native American/ Hispanic Admixed[b]	Caucasian England/ Scotland[b]	Chinese-Han[b]
rs34447885	S14F	T	1.0	2.5	1.5	0.0	0.0	0.0
rs12208357	R61C	T	0.0	0.0	0.0	0.0	5.0	0.0
rs55918055	C88R	A	0.0	0.0	0.0	0.0	0.5	0.0
rs34104736	S189L	T	1.0	0.0	0.0	ND	0.0	0.0
rs36103319	G220V	T	1.0	0.0	0.0	ND	0.0	0.0
rs4646277	P283L	T	0.0	0.0	0.0	ND	0.0	0.5
rs2282143	P341L	T	0.5	8.0	9.0	2.0	2.3	12.4
rs34130495	G401S	A	0.0	0.0	0.0	ND	2.3	0.0
rs35956182	M440I	A	0.5	0.0	0.0	0.0	0.6	0.0
rs34059508	G465R	A	0.5	0.0	0.0	0.0	3.4	0.0
rs78899680	V519F	T	1.5	2.0	6.0	ND	0.0	0.0
rs622342	Intronic	C	18.0	22.1	15.3	ND	37.6	13.2

ND No data

[a] This study

[b] Data from 1,000_Genomes database

Table 6 Haplotype structure defined by 20 SNPs in the *SLC22A1* gene in the Cape Admixed population

Haplotype no.	Haplotype[a]	Frequency (%)
1	CCTCGGCGCGCTAGAGCTGA	80.0
2	CCTCGGCGCGCTAGCGCTGA	9.9
3	CCTCGGCGCGCGAGCGCTGA	3.5
4	CCTCGGCGGGCTAGAGCTGA	1.5
5	CCTCGGCGCGCTAGCGCTTA	1.1
6	TCTTTGCGCGCTAGCGCTGA	1.0
7	CCTCGGCGCGCTAGCGTTGA	0.6
8	CCTCGGAGCGCTAGAGCTGA	0.5
9	CCTCGGCGCGCGAGCACTGA	0.5
10	CCTCGGCGCGCTAGCGATGA	0.5
11	CCTCGGCGTGCTAGCGCTGA	0.5
12	CCTCGGCGCGCTAGCGTTTA	0.4
	TOTAL	100

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

population at a MAF of 2.3 and 3.4 % respectively. These variants were not observed in the African and Asian populations (Table 5).

The MAF of P341L observed in the Cape Admixed population (0.5 %) was lower than that of other sub-Saharan African, Asian and Caucasian populations (Table 5). Functional in vitro transport assays have shown that the P341L variant results in a decrease rate of MPP+ transport, and has no effect on the transport of the anti-diabetic drug metformin [21, 23].

The observed MAF at loci S189L, M440I and M420V was 1, 0.5 and 4 % respectively. However, S189L was only observed in the Cape Admixed population whereas M440I was observed in the Cape Admixed and Native American/ Hispanic Admixed populations at a similar frequency (Table 5). Although at low frequencies, these variants are suggested to have a reduced transporter function with respect to metformin [21].

The observed MAF for variant V519P in our population was 1.5 %. This value was lower than that of the Luhya and Yoruba, with respective values of 2 and 6 % (Table 5). However, this variant was not observed in any of the other populations, indicating that it might 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.

The intronic SNP (rs622342) was observed in the Cape Admixed population at lower MAF (18 %) compared to previously reported frequencies for the European population (Table 5). This includes the Dutch diabetic patients cohort where the variant was initially reported [22]. In this population-based cohort study, Becker et al. [22] 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.

Twelve out of the nineteen investigated nonsynonomous SNPs were monomorphic in the study population. No polymorphisms were observed for these SNPs and they were all homozygous for the ancestral allele. This includes variants R61C, C88S, P283L, R287G, A306T, G401S, A413V, M420V, I421F, C436F, V501E and I542V. The population data available in the databases and the literature

for variants R61C, C88S, P283L and G401S is limited, and low MAFs are usually reported for these SNPs. To our knowledge, this study is the first to report population data for A306T; A413V; M420V, C436F; I421F: V501E, V519F and I542V. Further investigations are needed to explore the genetic variations among these SNPs in world populations.

The haplotype structure defined by 20 SNPs in the *SLC22A1* gene was also inferred for the Cape Admixed population. A total of 12 haplotypes were observed in the study population (Table 6). The most frequently observed haplotypes were CCTCGGCGCGCTAGAGCTGA (80 %), CCTCGGCGCGCTAGCGCTGA (9.9 %) and CCTCGGCGCGCGAGCGCTGA (3.5 %). Haplotype structure could provide a more complete picture of genetic variations at multiple loci important in drug response at the individual and population level [24]. In our study population, it could be interesting to investigate the overall effect of the observed rare haplotype on the transport efficiency of the OCT1 protein, as well as possible clinical implications related to therapeutic drugs including metformin. This rare haplotype includes variants S14F, S189L, G220V and the intronic SNP rs622342, where further investigations are required to determine the cumulative effect of these variants on substrate transport efficiency and glucose-lowering effect of metformin.

Conclusion

To our knowledge, this is the first study that investigated the allele and genotype frequency distributions of SNPs in the OCT1 gene of the Cape Admixed population from South Africa. This uniquely admixed population is known to have the highest rate of admixture in the world. This study reports also the observed haplotypes in the investigated population. This is the first world population data report for variants A306T, A413V, M420V, C436F, I421F, V501E, V519F and I542V. The reduced-function variants G401S and G465R were not observed in the population, nor have been previously reported in other African populations. This may suggest an increased therapeutic response to metformin for these populations. However, it should be noted that this was only a descriptive study and that no associations are made between any diseases or treatment outcomes. It could be interesting to investigate the overall effect of the rare haplotypes observed in this admixed population on the transport efficiency of the OCT1 protein, as well as possible clinical implications related to therapeutic drugs.

References

1. Kang HJ, Song IS, Shin HJ, Kim WY, Lee CH, Shim JC, Zhou HH, Lee SS, Shin JG (2007) Identification and functional characterization of genetic variants of human organic cation transporters in a Korean population. Drug Metab Dispos 35(4):667–675
2. Tzvetkov MV, Vormfelde SV, Balen D, Meineke I, Schmidt T, Sehrt D, Sabolic I, Koepsell H, Brockmoller J (2009) The effects of genetic polymorphisms in the organic cation transporters OCT1 OCT2 and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther 86:299–306
3. Avery P, Mousa SS (2009) Pharmacogenomics in type II diabetes mellitus management: steps toward personalized medicine. Pharmacogenomics Personalized Med 2:79–91
4. Zhang L, Dresser MJ, Gray AT, Yost SC, Terashita S, Giacomini KM (1997) Cloning and functional expression of a human liver organic cation transporter. Mol Pharmacol 51(6):913–921
5. Gorboulev V, Ulzheimer JC, Akhoundova A, Ulzheimer-Teuber I, Karbach U, Quester S, Baumann C, Lang F, Busch AE, Koepsell H (1997) Cloning and characterization of two human polyspecific organic cation transporters. DNA Cell Biol 16(7):871–881
6. Kerb R, Brinkmann U, Chatskaia N, Gorbunov D, Gorboulev V, Mornhinweg E, Keil A, Eichelbaum M, Koepsell H (2002) Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. Pharmacogenetics 12(8):591–595
7. Shu Y, Leabman MK, Feng B, Mangravite LM, Huang CC, Stryke D, Kawamoto M, Johns SJ, DeYoung J, Carlson E, Ferrin TE, Herskowitz I, Giacomini KM (2003) Evolutionary conservation predicts function of variants of the human organic cation transporter OCT1. Proc Natl Acad Sci USA 100(10):5902–5907
8. Sakata T, Anzai N, Shin HJ, Noshiro R, Hirata T, Yokoyama H, Kanai Y, Endou H (2004) Novel single nucleotide polymorphisms of organic cation transporter 1 (*SLC22A1*) affecting transport functions. Biochem Biophys Res Commun 313(3):789–793
9. Takeuchi A, Motohashi H, Okuda M, Inui K (2003) Decreased function of genetic variants Pro283Leu and Arg287Gly in human organic cation transporter hOCT1. Drug Metab Pharmacokinet 18(6):409–412
10. Hardy BJ, Seguin B, Goodsaid F, Jimenez-Sanchez G, Singer PA, Daar AS (2008) The next steps for genomic medicine: challenges and opportunities for the developing world. Nat Rev Genet 9(1):23–27
11. Via M, Ziv E, Burchard EG (2009) Recent advances of genetic ancestry testing in biomedical research and direct to consumer testing. Clin Genet 76:225–235
12. Abrahams Z, D'Amato ME, Davison S, Benjeddou M (2010) Allele frequencies of six non-CODIS miniSTR loci (D1S1627 D3S4529 D5S2500 D6S1017 D8S1115 and D9S2157) in three South African populations. Forensic Sci Int Genet. doi:10.1016/j.fsigen.2010.1001.1019
13. Flicek P, Amode MR, Barrell D, Beal K, Brent S, Carvalho-Silva D et al (2012) Ensembl 2012. Nucleic Acids Res 40(D1):D84–D90
14. Flanagan SE, Patch A-M, Ellard S (2010) Using SIFT and PolyPhen to predict loss-of-function and gain-of-function mutations. Genet Test Mol Biomark 14(4):533–537

15. Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, pp 365–386

16. Peakall R, Smouse P (2006) Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6(1):288–295

17. Yong Y, Lin H (2005) SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 15(2):97–98

18. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z et al (2009) A partition–ligation–combination–subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis. Cell Res 19((4):519–523. http://analysis.bio-x.cn

19. Leabman MK, Huang CC, DeYoung J, Carlson EJ, Taylor TR, De la Cruz M, Johns SJ, Stryke D, Kawamoto M, Urban TJ, Kroetz DL, Ferrin TE, Clark AG, Risch N, Herskowitz I, Giocomini KM (2003) Natural variation in human membrane transporter genes reveals evolutionary and functional constraints. Proc Natl Acad Sci USA 100:5896–5901

20. Leabman MK, Huang CC, Kawamoto M, Johns SJ, Stryke D, Ferrin TE, DeYoung J, Taylor T, Clark AG, Herskowitz I, Giacomini KM (2002) Polymorphisms in a human kidney xenobiotic transporter OCT2 exhibit altered function. Pharmacogenetics 12(5):395–405

21. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, Sheardown SA, Yue L, Burchard EG, Brett CM, Giacomini KM (2008) Effect of genetic variation in the organic cation transporter 1 OCT1 on metformin pharmacokinetics. Clin Pharmacol Ther 83(2):273–280

22. Becker ML, Visser LE, van Schaik RHN, Hofman A, Uitterlinden AG, Stricker BHC (2009) Genetic variation in the organic cation transporter 1 is associated with metformin response in patients with diabetes mellitus: genetic variation in OCT1 and metformin. Pharmacogenomics J 9:242–247

23. Fujita T, Urban TJ, Leabman MK, Fujita K, Giacomini KM (2006) Transport of drugs in the kidney by the human organic cation transporter OCT2 and its genetic variants. J Pharm Sci 95(1):25–36

24. Crawford DC, Nickerson DA (2005) Definition and clinical importance of haplotypes. Annu Rev Med 56:303–320