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

N. Hoosain, S. Nene, B. Pearce, C. Jacobs, M. Du Plessis, M. Benjeddou

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 glucoselowering 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

N. Hoosain is with the Pharmacogenetics Unit, Biotechnology Department, University of the Western Cape, Cape Town, South Africa (phone: 2721-959-2065; fax: 2721-959-3505; e-mail: nisreenhoosain@gmail.com).

S. Nene, B. Pearce, C. Jacobs and M. Du Plessis are with the Pharmacogenetics Unit, Biotechnology Department, University of the Western Cape, Cape Town, South Africa (e-mail: snene@uwc.ac.za, brendon.biff@gmail.com, cjacobs@uwc.ac.za, duplessismg@gmail.com, respectivelv).

M. Benjeddou is with the Pharmacogenetics Unit, Biotechnology Department, University of the Western Cape, Cape Town, South Africa (phone: 2721-959-2080; fax: 2721-959-3505; e-mail: mbenjeddou@uwc.ac.za).

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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 μ l reaction

volume using 3 μ l of the purified PCR products, 0.2 μ M of primers, and 5 μ l of SNaPshot[®] ready reaction mix. Sequence cycling consisted of 25 cycles of 96°C for 10s, 50°C for 5s and 60°C for 30s, followed by a hold at 4°C. Post extension products were purified using 1 U FastAP, incubated at 37°C for 15 min and inactivated at 75°C for 15 min.

E. Electrophoresis of the Mini-Sequencing Products

The purified mini-sequencing products $(1 \ \mu l)$ were mixed with 8.7 μl of HiDiTM 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.

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		(JCT I MULTIPLEX PCR PRIMERS		
Primer Name	Length	T _m	Nucleotide Sequence	Amplicon Size	
F 1	24	65.9	5' - TGCTGAGCCATCATGCCCACCGTG - 3'	255	
Exon 1	21	62.8	5' - GGACACAGCCAGACACCCACG - 3'		
E	24	59.6	5' - CTCTTGCCGTGGTATGACTGGCAG - 3'	1/2	
Exon 2	23	57.9	5' - CAGAGGGGCTTACCTGGACTGG - 3'	162	
E	25	58.1	5'- CCTCCATGTCTCCTTCTCTCTGAAG - 3'	207	
Exon 3	25	57.2	5'- CTGGCCTCATCCCCATGATAATTAC - 3'	207	
	24	61.3	5' - CCCGCATAACGTCCACACCTCCTG - 5'	222	
Exon 4	23	60.3	5' - GTAGGCAGGAGGAAGGGCCTCAC -3'	222	
E	24	57.4	5' - GATAGTGATGAGTGGTGTTCGCAG - 3'	502	
Exons 5 and 6	21	62.7	5' - GCGAGCGTGCTGATTCTGCCT - 3'	503	
F	25	59.3	5' - GACTTGAAACCTCCTCTTGGCTCAG - 3'	200	
Exon /	25	64.2	5' - TTCCCCACACTTCGATTGCCTGGGA - 3'	298	
F 0	25	67.6	5' - GAAGCCCCCATCCACCACCACACC - 3'	101	
Exon 8	25	63.4	5' - GGCTACCCCTGTTCCATGCACTCAC - 3'	181	
ΕΟ	22	62.4	5' - ATTGCATGGGCAACGGATGGCT - 3'	(15	
Exon 9	25	67.8	5' - CCATGCTGAGCCCACTGCCGAGCTG - 3'	615	
E 10	23	60.6	5' - TTCCTCTCTTTGGCTGGCTGTGA - 3'	(21	
Exon 10	24	60.5	5' - ACTCCAGCAAACCTTGCTCTCTGT - 3'	621	
F	25	58.9	5' - TGCCCTTTTCTTCTTTGCTGTTTGC - 3'	460	
Exon 11	25	60.8	5' - AGCACCAACAGCTTTCCCTAGATCG - 3'	400	
Tertain a O	25	58.8	5' - GAGTAGGAGGGGTTAATAGAGAGAG - 3'	226	
Intron 9	27 65.7 5' -	5' - GTAGCTGAGACTACATGCATGCACCAC - 3'	236		

TABLE I Oct 1 Multiplex PCR Prime

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' - CATAATCATGTGTGTGTGGCCGAAT - 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' - AGGAGGCAACTTCCCATTCTTTGAG - 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 wildtype [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

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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 SNPs: rs142448543 (M420V, 1258A>G); data for 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.

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SNP	Polymorphism	Genotype fr	equency (%)	Allele Frequency (%)			HWE (P)	
		CC	CT	TT	C	Т		
rs34447885	41 C>T	96.0	4.0	0	98.0	2.0	0.839	
	5((O T	CC	СТ	TT	С	Т		
rs34104/36	566 C>1	100	0	0	100	0		
2(102210	(10 G T	GG	GT	TT	G	Т		
rs36103319	659 G>T	100	0	0	100	0		
1(1(277	040 C T	CC	СТ	TT	С	Т		
rs4646277	848 C>1	100	0	0	100	0		
24120405	1201 Ct 4	GG	GA	AA	G	Α		
rs34130495	1201 G>A	100	0	0	100	0		
	1050 AS C	AA	AG	GG	Α	G		
IS/2552/05	1258 A>G	100	0	0	100	0		
	1220 C> A	GG	GA	AA	G	А		
1833930182	1320 G>A	100	0	0	100	0		
ra24050508	1202 C>A	GG	GA	AA	G	А		
1834039308	1393 G>A	100	0	0	100	0		
m79900690	1555 CNT	GG	GT	TT	G	Т		
18/0099000	1555 0-1	88.1	11.9	0	94.0	6.0	0.526	
ra127029512	1624 450	AA	AG	GG	G	А		
1813/920312	1024 A-0	100	0	0	0	100		
*******	1296 450	AA	AC	CC	А	С		
15022542	1300 A-C	89.1	9.9	1.0	94.0	6.0	0.019	
re12208257	181 C>T	CC	CT	TT	С	Т		
1812208557	101 0-1	100	0	0	100	0		
rs/6/6278	850 C>G	CC	CG	GG	С	G		
154040278	357 0- 0	100	0	0	100	0		
rs55918055	262 T>C	TT	TC	CC	Т	С		
1355710055		100	0	0	100	0		
COSM164365	916 G>A	GG	GA	AA	G	Α		
00511101505	910 G. H	100	0	0	100	0		
rs2282143	1022 C>T	CC	CT	TT	С	Т		
152202115	1022 C- 1	98.0	2.0	0	99	1.0	0.920	
rs144322387	1238 C>T	CC	СТ	TT	С	Т		
10111022007	1250 0 1	100	0	0	100	0		
rs151333280	1261 A>T	AA	AT	TT	Α	Т		
10101000200	120171-1	100	0	0	100	0		
rs139512541	1307 G>T	GG	GT	TT	G	Т		
		100	0	0	100	0		
rs143175763	1649 T>A	TT	TA	AA	Т	A		
	1077 IZA	100	0	0	100	0		

GENOTVE AND ALLETE EDECHENCIES FOR 20 SNIPS OF SUC22A1 IN 101 HEALTRY PARTICIDANTS FROM THE 7111 II PODILI ATION

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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	Т	2.0	2.6	1.7	0.0	0.0	0.0	0.0
rs34104736	S189L	Т	0.0	0.0	0.0	1.0	0.0	0.0	0.0
rs4646277	P283L	Т	0.0	0.0	0.0	0.0	0.0	0.5	1.1
rs34130495	G401S	А	0.0	0.0	0.0	0.0	2.2	0.0	0.0
rs35956182	M440I	А	0.0	0.0	0.0	1.0	1.0	0.0	0.0
rs34059508	G465R	Α	0.0	0.0	0.0	2.0	3.0	0.0	0.0
rs78899680	V519F	Т	6.0	2.1	5.1	0.0	0.0	0.0	0.0
rs622342	Intronic	С	6.0	15.0	22.0	34.5	37.6	14.5	19.1
rs12208357	R61C	Т	0.0	0.0	0.0	1.0	5.1	0.0	0.0
rs55918055	C88S	С	0.0	0.0	0.0	0.0	0.6	0.0	0.0
rs2282143	P341L	Т	1.0	7.7	9.1	2.7	2.2	11.0	15.7

TABLE IV

^aPopulation of this study; ^bPopulation 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)

IABLE 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			

TIDITY

^aHaplotype sequences are based on the position of SNPs on chromosome 6.

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