

Genetic analysis of *OCT1* gene polymorphisms in an Indian population

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BACKGROUND: Genetic variants of the organic cation transporter (*OCT1*) gene could influence interindividual variation in clinical response to metformin therapy. The genetic basis for the single-nucleotide polymorphism (SNP) of *OCT1* gene has been established in other populations, but it remains to be elucidated in the Indian population. This study is focused on *OCT1* gene variants rs2282143 (P341L, 1022C>T), rs628031 (M408V, 1222A>G) and rs622342 (1386C>A) frequency distributions in the South Indian Tamilian population.

MATERIALS AND METHODS: A total of 112 unrelated healthy subjects of South Indian Tamilian origin, aged 18–60 years, of either sex were recruited for the study. Genotyping was determined using the quantitative real time-polymerase chain reaction and polymerase chain reaction followed by restriction fragment length polymorphism methods.

RESULTS: Allele frequencies of rs2282143, rs628031 and rs622342 polymorphisms were 8.9%, 80.3% and 24.5%, respectively. Interethnic differences in the genotype and allele frequencies of *OCT1* gene polymorphism were observed when compared with other major populations. The SNPs rs2282143, T allele and rs628031, G allele were more common in Asians (5.5–16.8% and 76.2–81%) and African Americans (8.2% and 73.5%) than in Caucasians (0–2% and 57.4–60%).

CONCLUSION: This is the first time the frequency of *OCT1* gene polymorphism was determined in the Indian population, and is similar to the frequencies observed in African-Americans and other Asian populations but different from those in Caucasians. The data observed in

this study would justify further pharmacogenetic studies to potentially evaluate the role of *OCT1* gene polymorphism in the therapeutic efficacy of metformin.

Key words: Diabetes, metformin, *OCT1*, polymorphism

Introduction

Polyspecific organic cation transporters (OCTs) belong to the solute carrier SLC22 superfamily of transporters, and they translocate a wide variety of endogenous and exogenous substances of cationic nature. There are three isoforms of OCTs, namely *OCT1*, *OCT2* and *OCT3*, with similar membrane topology consisting of 12 transmembrane domains.^[1] Of these, OCT1 is one of the most abundantly expressed transporters in the liver and plays a major role in the hepatic uptake and renal transport of the biguanide agent, metformin.^[2] Metformin is an oral antidiabetic drug, widely used for treating type 2 diabetes mellitus as first-line monotherapy. The main action of metformin is the suppression of hepatic gluconeogenesis.^[3] *In vivo* studies have shown that the concentration of metformin in liver was greatly decreased in *OCT1* gene knockout mice than in mice with normal OCT1 transporter activity.^[4]

The gene encoding human OCT1, also termed *SLC22A1*, is mapped onto chromosome 6q25.3 and consists of 11 exons spanning 37 kb. Human *OCT1* gene is highly polymorphic, and numerous polymorphisms have been described in various populations leading

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to differences in transporter function.^[2,4] Among the many variants, nonsynonymous variants rs2282143 in exon 6 (Pro341Leu, 1022C>T) and rs628031 in exon 7 (Met408Val, 1222A>G) were the most extensively explored in relation to metformin response. More recently, it has been reported that a novel polymorphism rs622342 (1386C>A) located in an intron between exon 8 and exon 9 was associated with the glucose-lowering effect of metformin.^[5] Several studies have evaluated the relationship between genetic variations of *OCT1* gene and the pharmacokinetics and clinical consequences of metformin with conflicting results.^[5-7] Further available data indicate substantial differences in the allele frequencies of the *OCT1* gene polymorphisms between the different ethnic groups.

Tamilians are Dravidians living in the southern parts of India and north eastern part of Sri Lanka. They are ethnically, linguistically and culturally related to the other Dravidian people of the subcontinent. An estimated 77 million Tamilians reside in India and around the world (Tamil people available at <http://en.wikipedia.org>, accessed on December 10, 2010). There are studies reporting the allele and genotype frequency of *OCT1* gene polymorphism in other populations, but so far there is no data available for Indians. Hence, the present study was aimed to determine the genotype and allele frequency of *OCT1* gene polymorphism in the South Indian Tamilian population.

Materials and Methods

Subjects: The study was carried out in 112 unrelated healthy volunteers of South Indian Tamilian origin, aged between 18 and 60 years, of either sex. This includes 62 (55.4%) women and 50 (44.6%) men. All of them were residents of South India (Tamilnadu and Pondicherry) for at least three generations. Written informed consent was obtained from all the volunteers and this protocol was approved by the Institute Ethics Committee, JIPMER. For all subjects, 5 ml of venous blood was collected using EDTA as an anticoagulant. Genomic DNA was extracted from peripheral blood leucocytes using the standard phenol-chloroform method, and the samples were stored at 4°C.

Genotyping

rs2282143 and rs622342

Genotyping for rs2282143 and rs622342 was carried out using a Real Time Thermocycler (ABI Prism 7300, Foster city, CA, USA) using the TaqMan SNP genotyping assay method. C__11764545_20 was used as the SNP genotyping assay ID (Applied Biosystems, Foster City, CA, USA) for rs2282143 and TaqMan custom genotyping assay was used for rs622342 polymorphism. The primers 5'-GGGTTAATAGAGAGAGAAATAAT-GCTCAAAA-3' (forward) and 5'-GCCTTTCTTGAT-GTTTTTTTGTGGTTAGTATT-3' (reverse) and probes VIC-TGATGAAAACCTTCAAATACA-MGB and FAM-TGATGAAAACCTTCCAATACA-MGB were used to detect *OCT1* rs622342 SNP. The polymerase chain reaction (PCR) reaction was carried out in duplicates using 20 µl final volume that contained 10 µl of TaqMan Universal PCR master mix (2X), 0.75 µl of 20 X working stock of SNP genotyping assay and 4.5 µl of genomic DNA diluted in DNase-free water and 4.75 µl of deionised water. The thermocycler conditions included one cycle at 50°C for 2 min; one cycle at 95°C for 10 min to activate the AmpliTaq Gold polymerase followed by 40 cycles of denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min. The allelic discrimination analysis was performed using 7300 SDS software version 1.3.1.

rs628031

Genotyping of rs628031 was determined by PCR followed by restriction fragment length polymorphism. PCR was performed using the following primers: 5'-TTTCTTCAGTCTCTGACTCATGCC-3' and 5'-AAAAAACTTTGTAGACAAAGGTAGCACC-3', respectively. The PCR conditions used for amplification were as follows. An initial denaturation at 94°C for 5 min followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 45 s and extension at 72°C for 1 min, with a final extension at 72°C for 5 min.

The amplified products (397 bp) were analyzed on 1% agarose gel followed by restriction digestion using *MscI*, incubated overnight at 37°C. The digested PCR products were analyzed on 8% polyacrylamide gel and identified by ethidium bromide staining. Digestion of the

397 bp amplicon resulted in either the retention of the 397 bp amplicon or complete digestion to 210 bp and 187 bp fragments, corresponding to individuals homozygous for Val/Val or Met/Met genotypes, respectively. The presence of all three fragments (397 bp, 210 bp and 187 bp) corresponded to heterozygous for Met/Val genotypes [Figure 1]. The accuracy and reliability of all the genotyping methods were further confirmed by direct DNA sequencing (Macrogen, South Korea).

Statistical analysis: Data analysis was performed using InStat Graph pad 3. The direct gene counting method was used to determine the frequency of genotypes and alleles. The genotype data was analyzed using Chi-square and Fischer's exact test. The observed frequencies were compared with the expected frequencies and tested for the Hardy-Weinberg equilibrium. Differences in allele frequencies between Tamilian and other ethnic populations were measured using the Chi-square test and Fisher's exact test. Linkage disequilibrium (LD) was calculated using Haploview software version 4.1. A *P*-value <0.05 was considered statistically significant.

Results

Table 1 illustrates the genotype and allele frequencies of *OCT1* gene obtained in the study population. The

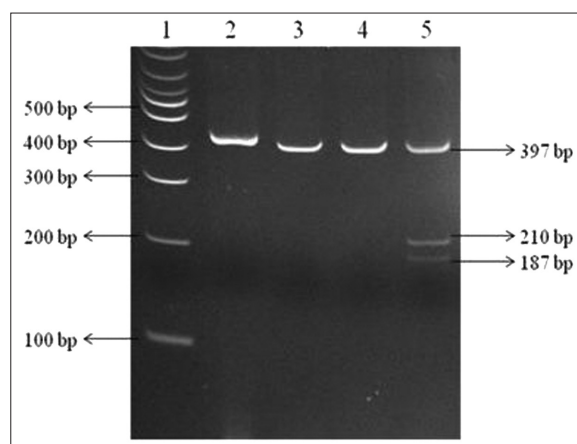


Figure 1: Representative polyacrylamide gel electrophoresis picture of *OCT1* rs628031 (M408V, 1222A>G) polymorphism. Lane 1 is the 100 bp DNA ladder, Lane 2 is the undigested polymerase chain reaction product, Lanes 3 and 4 show restriction patterns corresponding to the GG homozygous variant and Lane 5 shows the heterozygous variant for AG

genotype frequencies of rs2282143 homozygote wild type (C/C), heterozygote (C/T) and homozygote variant (T/T) were 83.0%, 16.1% and 0.9%, respectively. Forty-four individuals (39.3%) were found to be heterozygous (A/G) carriers and 68 individuals (60.7%) were homozygous for mutant type (GG), whereas no homozygous wild type (A/A) was found in the studied population regarding rs628031, while the distribution of the rs622342 genotypes was 59.0% for AA, 33.0% for AC and 8.0% for CC. The variant allele frequencies for rs2282143, rs628031 and rs622342 were observed at 8.9%, 80.3% and 24.5%, respectively. The studied polymorphisms were in Hardy-Weinberg equilibrium except the SNP rs628031. LD analysis showed that the *OCT1* gene polymorphisms were not in LD. The comparisons among *OCT1* allele frequencies in worldwide populations show different patterns between Asians, Africans and Europeans [Table 2].

Discussion

In recent years, *OCT1* gene polymorphisms have gained considerable attention due to their role in the

Table 1: Observed allele and genotype frequency of *OCT1* gene polymorphisms in Tamilian population

Polymorphism	Genotype frequency (%)			Allele frequency (%)	
	CC	CT	TT	C	T
rs2282143 (1022 C>T)	83.0	16.1	0.9	91.1	8.9
rs628031 (1222 A>G)	0	39.3	60.7	19.7	80.3
rs622342 (1386 C>A)	59.0	33.0	8.0	75.5	24.5

Table 2: Frequency of *OCT1* gene variants in different ethnicities as compared with the Tamilian population

Population	n	Allele frequency (%)			Ref
		rs2282143	rs628031	rs622342	
Chinese, Hans	100	11.0	nd	nd	8
Germany	100	2.0 ^{**}	nd	nd	8
Germany	102	nd	57.4 ^{***}	nd	10
India, Tamilian	112	8.9	80.3	24.5	Present study
Japan [#]	116	16.8 [*]	81.0	nd	9
Korea	150	16.7 [*]	74.0	nd	8
Netherlands [#]	102	nd	60.0 ^{***}	37.0 ^{**}	5
USA, African	100	8.2	73.5	nd	7
USA, Asian	30	11.7	76.2	nd	7
USA, Caucasian	100	0	59.8 ^{***}	nd	7
Vietnam	100	5.5	nd	nd	8

[#]Patients; n, number; nd, not determined; **P* < 0.05; ***P* < 0.01; ****P* < 0.0001 compared with Tamilians

differential response of metformin. In this study, we addressed the frequency of three previously described polymorphisms of the *OCT1* gene in 112 healthy volunteers of South Indian Tamilian population, and the data was compared with other published studies. *OCT1* gene polymorphisms were found to be common, but the distribution of the variant allele frequencies showed significant interethnic variations [Table 2]. To the best of our knowledge, this is the first study to document the frequencies of *OCT1* gene polymorphisms in any of the Indian populations. The polymorphism associated with decreased transporter activity rs2282143 was detected at 8.9% in this study, which was comparable to the data obtained from Chinese (11%), Vietnamese (5.5%),^[8] Asians residing in USA (11.7%) and African-Americans (8.2%).^[7] On the other hand, it was lower than those observed in North East Asians from Japan (16.8%)^[9] and Korea (16.7%).^[8] Likewise, it was significantly higher than those reported in European Caucasians (0–2%).^[7,8]

In the present study, the frequency of rs628031 genotypes was significantly ($P < 0.001$) lower in the Tamilian population than expected based on the Hardy-Weinberg calculations. The rs628031, Met408Val polymorphism might have accumulated over generations, which perhaps led to substantial changes in Tamilian population. Nevertheless, the frequency of 80.3% obtained for the rs628031 G allele was similar to other Asian populations previously described in Koreans (74%),^[8] Japanese (81%),^[9] Asians living in the USA (76.2%) and African-Americans (73.5%).^[7] However, it was significantly higher than European Caucasians from the USA (59.8%),^[7] Germany (57.4%)^[10] and the Netherlands (60.3%).^[5] The occurrence of relatively low frequency of rs2282143 and rs628031 *OCT1* gene polymorphisms in Caucasians suggests that the impaired *OCT1* transport activity may vary between Asians, Africans and Caucasians.

The SNP rs622342 was first identified by Becker *et al.* in Dutch diabetic patients with a frequency of about 37%, which was higher than the Tamilians (24.5%) observed in the present study. They assessed the association of *OCT1* gene polymorphism with the glucose-lowering effect of metformin, and found that the metformin therapy was less-effective in individuals with heterozygous (AC) and homozygous (CC) mutant carriers of rs622342 *OCT1* gene polymorphism.^[5] Later on, in another study

interaction between *OCT1* (rs622342) and *MATE1* (rs2289669), genes were observed in relation to metformin response.^[11] Furthermore, a study conducted in healthy Caucasian male subjects has shown that the polymorphisms of *OCT1* were associated with increased renal clearance of metformin.^[10] Recently, studies have correlated the expression level of *OCT1* gene with the intracellular concentration of imatinib, an anticancer drug, and have found that chronic myeloid leukemia patients who had higher baseline expression levels of *OCT1* showed greater progression-free survival rates.^[12,13] More recently, a population-based cohort study has suggested that patients with AC and CC genotypes of rs622342 polymorphism will have reduced response to anti-Parkinsonian drugs.^[14] This explains that the polymorphisms of *OCT1* will also show variable response to imatinib and anti-Parkinsonian drugs.

Indians are more prone to diabetes, and its prevalence is becoming an enormous health burden in the country, which has gone beyond epidemic to pandemic proportions. According to the International Diabetes Federation, it has been estimated that the number of diabetic patients in India will increase from 50.8 million to 79.4 million by 2030 (Prevalence of Diabetes available at www.idf.org, assessed on December 23, 2010). In the context of India being the “diabetic capital” of the world and metformin is the most commonly used anti-diabetic drug in India, the pattern and frequency of *OCT1* gene polymorphisms observed in this study becomes important indicating the prevalence of variant alleles in this region. However, it does not reveal a direct evidence for genotype–phenotype correlation. Further, information on the prevalence of *OCT1* gene polymorphisms in populations of different ethnic origin may be essential in explaining the interpatient and interethnic differences in the disposition and distribution of organic cationic drugs such as metformin, imatinib and levodopa. In conclusion, our observation provides a framework for future correlation studies on the relevance of *OCT1* variants in response to drugs that are substrates of *OCT1*.

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