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Research article

# Development and validation of methodology to study the genetic polymorphism of organic cation transporter 1 (OCT1) - rs622342: A Prospective open-label study

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## ABSTRACT

The main objective is to develop and validate the methodology to study the genetic polymorphism of Organic cation transporter (OCT1) rs622342. The Reverse Transcriptase-PCR method for identifying the OCT1 rs622342 genetic polymorphism was developed and validated. This study is a prospective open-label study conducted over a period of six months with 61 South Indian healthy male volunteers. Phenol chloroform method was used for the DNA isolation, which was subjected to PCR for identifying the polymorphism. The method was successfully developed. After the addition of restriction enzyme (PsiI) in the ten samples, none of the samples showed fragmentation, which shows that there is a lack of rs622342 genetic polymorphism in the selected ten samples.

**Keywords:** Genetic polymorphism, Organic cation transporter, Methodology & Validation, Healthy volunteers.

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## INTRODUCTION

Genetic polymorphism ranges in different species and within genomes, and has significant implications for the evolution and conservation of species. The degree of genetic polymorphism determines how the body responds to the drug. This is because a minute genetic alteration can bring a significant change in the drug target or enzymes that are responsible for the metabolism of the drug [1]. Not every individual respond to drugs in a uniform and predictable manner. An individual's response to a drug can be affected by various other factors like physiological, environmental factors, health factors, and not completely on basis of genetic variance [2]. The most common variations seen in the human genome are single nucleotide polymorphisms (SNPs). Approximately, it is estimated that there are 11 million SNPs in the human population, with an average of one every 1,300 base pairs. Single nucleotide polymorphism occurs when there is an alteration in the single nucleotide (A, T, C, or G) in the genome sequence [3].

Genetic polymorphism is defined as a variation in the DNA sequence with a 1% allelic frequency or greater in a population. Gene mutations and polymorphisms code for the enzymes characterized by different metabolic activity or receptors with different affinities for the drug. These causes modified pharmacological responses in individuals [4]. Pharmacogenomics helps in refining focus on treatment and makes drugs more effective and with less adverse effects which result in more individualized treatment [3] and hence pharmacogenomics is an indicator of personalized medicine. Thus, the concept of one-drug fits for all experienced paradigm to 'the right drug for the right patient at the right dose and time [5]. The need for personalized medicine becomes a necessity because polymorphic drug-metabolizing enzymes produce severe adverse drug effects that are fatal in nature [6]. However, differences in the drug response among patients are common, and this leads to challenges in optimizing a dosage regimen for an individual patient [5].

Poly-specific organic cation transporters (OCTs) belong to the solute carrier SLC22 superfamily of transporters that translocate various exogenous and endogenous substances of cationic nature. Currently, there are three known isoforms of OCT, namely OCT1, OC2, OCT3. All three isoforms share a similar membrane topology that consists of 12 domains. Out of these three isoforms, OCT1 is one of the abundantly expressed transporters in the liver, and plays a major role in the hepatic uptake and renal transport of several drugs.

Genetic variants of the organic cation transporter (OCT1) gene could influence interindividual variation in clinical response to drug therapy. The gene encoding human OCT1, also termed SLC22A1, is mapped onto chromosome 6q25.3 and consists of 11

exons spanning 37 kb. The Human OCT1 gene is highly polymorphic, and numerous polymorphisms have been described in various populations leading to differences in transporter function [7]. Since there are no evident articles that describe the validation of the Real-time PCR method for identifying the OCT1 rs622342 group, our study has developed a clear-cut method for recognizing the rs622342 group, which would be helpful for supporting further studies.

The objective of the study was to develop and validate the methodology to study the genetic polymorphism of Organic cation transporter OCT1 rs622342.

## MATERIALS AND METHODS

This study is a prospective open-label study conducted over a period of six months with 61 South Indian healthy male volunteers.

**Ethical Considerations**

Informed consent form has been obtained from all the volunteers and the study has been approved by the Institutional Ethics committee, JSS College of Pharmacy, Rocklands, Ooty.

**Inclusion criteria**

Adult male volunteers between the age group of 18 and 35 years in good health based on medical history, physical examination, ECG, and routine laboratory tests (CBC, blood glucose, liver function tests, renal function tests, and urinalysis).

**Exclusion criteria**

Smokers, alcoholics.

**History and Examination**

The Potential volunteers were identified and explained about the study in the following aspects Asked about the social habits of the volunteers such as smoking, alcohol, and any other medication that the volunteer consumed in the past 1 month. Explained to the volunteers about the importance of the study and were requested to participate in the study verbally.

**Methodology**

Written informed consent was taken from the interested volunteers, screened and fitness was obtained from the physician. Among those, 61volunteerswho were healthy based on the routine laboratory tests were included in the study.

**Sampling methods**

2ml of blood sample was collected from each volunteer. The blood samples were collected using 5 ml disposable syringe from the venflon and transferred into a K3 EDTA tube which contains 20 µl of EDTA as an anticoagulant. The tube was then closed and labelled with the volunteer identity and then centrifuged at, 12000 rpm for 1 minute to separate the plasma. The supernatant layer of the plasma was transferred from the centrifuged tube to the plasma collection tube using an adjustable micropipette and labelled appropriately with the volunteer identity.

**Materials**

**Chemicals and Reagents**

1X SSC buffer; 0.2M Sodium acetate; 10% SDS; Phenol-Chloroform-Isoamyl alcohol; Cold 100% Ethanol; 6. 1X TE buffer

**Identification of Genetic Polymorphism from Blood**

**Isolation of DNA from whole blood**

The DNA extraction from the whole blood of the volunteers was carried out by the phenol-chloroform method [8]. Phenol chloroform method involves digesting Eukaryotic cells or tissues with proteinase K in the presence of EDTA (to sequester divalent cations and thereby inhibit DNAase) and solubilizing membrane and denaturing proteins with the detergent such as Sodium Dodecyl Sulphate (SDS), the nucleic acids are then purified by the phase extraction with organic solvents. This method can be scaled to yield amounts of DNA ranging from less than ten to more than hundreds of microorganisms of DNA.30

**Chemicals and reagents**

(All the chemicals used are molecular biology grade /HPLC grade/AR grade) 1X SSC buffer (100ml): 3M NaCl - 8.765 g 0.3M Sodium Citrate - 4.410 g. The above chemicals were weighed and dissolved in 80ml distilled water. The pH was adjusted to 7.0 with concentrated HCl and made up to 100 ml. The solution was then at room temperature.

0.2M Sodium acetate (100ml): 2.74g of Sodium acetate was weighed and dissolved in 80 ml distilled water. This was then adjusted to pH 5.2 with glacial acetic acid and made up to 100 ml. The solution was then at room temperature.

10% SDS (50ml): 5g of SDS was weighed and dissolved in 50ml distilled water. The solution was then at room temperature.

Proteinase K: 20 mg of Proteinase K was weighed and dissolved in 1ml distilled water. The solution was then at room temperature.

Phenol -Chloroform - Isoamyl alcohol (50ml) Buffered Phenol: 25ml Chloroform: 24ml

1X TE Buffer (100ml) 40mM Tris acetate pH 8.0: 4ml of 1M stock 1mM EDTA pH 8.0: 0.2ml of 0.5M stock Distilled water: 95.8ml Equipment required are Cooling centrifuge C24; Incubator 37° C; Deep freezer -85° C; Vortex; 15ml PP Centrifuge tubes;

0.5 ml Micro Centrifuge tubes

**Extraction procedure**

Added 1.6ml of 1X SSC buffer to 2 ml of sample and mix. Centrifuged for 1 min at 12,000 rpm in a microfuge and discard 2 ml of supernatant. Added 2ml of 1X SSC buffer again, vortex, and centrifuge at 12,000 rpm for 1 minute, and removed all the supernatants. Added 740µl of 0.2M Sodium acetate to each pellet and mixed by inverting the tubes briefly. Added 50µl of 10% SDS and 10µl of proteinase K (20 mg/ml), mixed by inverting the tubes briefly, and incubated for 1 hour at 55℃. Added 200µl phenol – chloroform-isoamyl alcohol and mixed for 30 seconds. Centrifuged for 2 minutes at 12,000 rpm in a microcentrifuge tube. Collected the aqueous layer (top layer) carefully in a new centrifuge tube, added 2ml of cold 100%

ethanol, mixed, and incubated for 15 minutes at -20℃ Centrifuged for 2 minutes at 12,000 rpm in a micro centrifuge. Removed the supernatant and drained the tubes Added 360µl 1X TE buffer, vortex, and incubated at 55℃ for 10 minutes. Added 40µl 2M Sodium acetate and mixed. Added 1ml of cold 100% ethanol, mix. Centrifuged for 1 minute at 12,000 rpm in a micro centrifuge. Decanted the supernatant and rinsed the pellet with 2 ml of 70% ethanol. Centrifuged for 1 minute at 12,000 rpm in a micro centrifuge. Decanted the supernatant, and dried the pellet in a speed vac for 10 minutes or until dry at room temperature. Resuspended the pellet by adding 400µl of 1X TE buffer. DNA pellet was dissolved in 200 µl of Tris-EDTA (TE) buffer and to attain optimum dissolution, the tubes were kept in the water bath at 55℃ for 20 to 30 minutes. Dissolved DNA samples were stored at -20℃.

**Qualitative Analysis of DNA samples by Agarose Gel Electrophoresis**

Materials required *are* 1. One Horizontal gel electrophoresis apparatus (GENEI).2. Gel casting platform (GENEI).3. Gel combs (GENEI). 4. DC power supply unit. 5. UV transilluminator (Alpha Innotech corp.) 6. Microwave pipette and tips. 7. Gel scoop. 8. Conical flask.

**Protocol**

Added 0.35 g agarose (electrophoresis grade) to 48ml of Millipore water and 2ml of 1XTE electrophoresis buffer in a 100 ml beaker. Stirred to suspended agarose. Covered beaker with aluminium foil, and heat in boiling water bath until all agarose is dissolved. Swirled the solution and checked the bottom of the beaker to ensure that all agarose has dissolved. Reheat for several minutes until ready to use. Added Ethidium bromide staining solution. The molten agarose was then poured into a pre-set template with a well forming comb.

It was placed horizontally on a levelling table and put on an appropriate well-forming comb. (If the gel plate is not horizontal, a wedge-shaped gel will form and DNA fragments will migrate peculiarly within it. The teeth of the comb should not touch the bottom of the plate, leaving at least 1 mm between the comb and the gel plate while pouring).

The gelation was allowed for 30-40 min. and removed the comb and sealing tapes carefully. (Take care not to damage the wells when the comb is removed, otherwise, DNA fragments are likely to smile when they run on the gel).

Mounted the template in an appropriate electrophoresis tank and filled the tank with 1X TAE buffer, and immersed the gel up to 1 mm. The DNA samples were then loaded into the wells. (While loading the gel be careful to avoid touching the front and back walls of the wells with the tip as this will result in a streaky lane). The electrophoresis apparatus was connected to a power pack and performed electrophoresis at 50 V, using 1X

The electrophoresis was stopped by turning off the power supply when the dye bromophenol blue has migrated a distance judged sufficient for the separation of the DNA fragments.

Visualized DNA bands on a UV Transilluminator. **Caution:** Ethidium bromide is a powerful carcinogen. Wear gloves when handling. For disposal of materials containing ethidium bromide, adopt safely procedure. UV light can burn skin and eyes. Minimize exposure to UV light. TAE as running buffer.

**Selection of OCT1 sequence from NCBI**

The cDNA sequence of OCT1 was obtained from NCBI.25 First go to NCBI website and select Nucleotide; type OCT1then scroll down to NCBI reference sequences (RefSeq) section to find the RefSeq mRNA sequence whose accession number was NCBI Reference Sequence: accession isNC_000006.26The geneOCT1is a part of Homo sapiens chromosome 6q25.3 and consists of 11 exons spanning 37 kb.

**Choosing the regions of mutation to be studied**

The OCT gene consists of 3 isoforms to date. OCT1 mutations are found to be clinically significant. The OCT1mutation refers to an A→C change at 1386 of the gene. The region to be studied to identify polymorphism was selected randomly. The region selected for studying OCT1 polymorphism, which involves a base pair change of A→C at 1386 is as follows:

AGTTATTTAGTAAAGCTCATGAAAATTGTGCCCTCCATTCCCATAT
AATTTATTAATTGTCTAGGAACTT
CCACATACATTGCCTCAATTTATCTTTCAACAACTTGTGTGTTATAT
TTTGGAATACAGATACAAAGTTA
TTATGCTTTCAAAATATTCTTTTGCTAATTCTTAGAACAAAGAAAG
GCATAAATATATTAGTATTTGTGT
ACATCTGTTCCTTCCTGTGTGACCCTAAGTTTAGTAGAAGAAAGGA
GAGAAAATATAGCCTAGCTTATAA
ATTTAAAAAAAAATTTATTTGGTCCATTTTGTGAAAAACATAAAAA
AAGAACTGTCACATCTTAATTTAA
AAAATATATGCTTAGTGGTAAGGAGATATATGTCAACTTTTAAGAG
GTTGAAAAACAAACGCCTCCCATT
ATAAGTTTATACTTCACCTCCCACCACTATAACAACCCAGAATCCA
TGAGGGCATTATCAGGAGTGAGTG
GAAGAGTAAGTTTGCCAATGTGAAATGTGCCTTCTAGGTCCTAGAC
ATCTGTGGTATAACTGCTCATAAG
CAGTAGAAAGAATTTAGAGGGATCCAGGCTCTCATCACGTTGGCA
CAAAGTATATTACTTGGATCCATCT
ATGTCATTTTCCATGGTTAATGTTTAAAACCACAGGCTTTAAAGTA
AAAAACAAAGAGCTGGATTCAACT
CTACTGACTCTTATTAATCATGATTTTGGGCACATTACGTAGCTTTC
ATGAGCTTTAGTTTCTACATTTA
TAAACAGGAGATTATACCTATTATGCACGGTTATTATGAAGGAAAA
TGACAAAATAGATATAAATCAAAT
AGCCCACTTCGAGACATATTAAGCATGAATAAACATTAGATACTAT
TAAAATCCTATATATTAACAAAGC

CAAAAGTTTCAAACTTTACTTTTTCCCAACATTCTTGTGAAATATGA
CACATCCCAATCTTAACAGATGC
TCATTTGGGATACTGTACTTGTGAGTGGAAGTGTGTATATTTGTGTG
CAAGTGTGTACTCATATACTTCC
ACCTTACCACCCTAGAAAGGCATGATGAAAATTTAAGATAGAAGG
AAAATATAAATTGAAAAAAAAAAAC
CTTAACAAATGATTCTGACAAATATCTTCTCTTTCCAGGGAGAATC
ACTGAGCCAGAATAAAATTGAACA
CTAAATATTCTAAGAAAAAAGGAATCTAGTTTGTCAAAATGTGACT
TGAATTAATAGATAAGGAGAGTCA
GATGATAAGAGGGTCAAAATTATGTTTATCTTAGGAAAAGTAGAA
TAGAAAATTT**A**TAAGCAGATTAAAA

ACACATAATAAAAGTAGTAAATAATAATGACAGTATCTCAAATCA
GTGCAGGGGGGAAAGGCCTACTAAT
GTGATGGTGGGATAATTGGATAGCAATATGGGAAAAGATATATTT
AATTTATTTGCTACACCAAATGCCA
GGACAATCTCTAAGTGAATTCAAGACATAACTCTTTTTTCAAAAAA
ACTATGCAAATATTAAAAGAAAAC
AAGTTAATGTTTTTATAATCTATGAATATGGTAAAGATGGATAACA
TTGACTATCAAATTAATTTTTAAT
GCGTAATAAAACTATGAGAAAATTTAAAAGTGAGAAGAAACTACT
TGTAACTCACATAATAGACTAGTAC
TTCTAACACATAGGGAACTTCTAAAACAAAACCCAAAATATTAATA
GGAAAATGGGCAAAACAGTTAAAC
TTACAGTTCATACATAAGGAGAATCAGTCTTTTTTTTTTTTTTTACA
GTTGTAGGCAGAAAACTTTTATT
TTTCATTTATTTGTAAAATTTACCCCTAATTTATTCATAATTCATTTA
ACTGCTAAGGGCATTAATGTGT
ACAACGCCATGGGAGAAACCAGTATATTCAGAATTTCTCCTGAAAT
TTGACCAGAAGTTATGGACATCCC
TCCCCTGGGAAGGAGGCAGGCAGAAAAGTTTGGAATCTATGTAGT
AAAATATGTTACTCTTTTATATATA
TACATATATGTGTGTATATGTGTATATATATATACACACATATATA
CATACATACATACATACATACATA
CATATTATCTGAATTAGGCCTGGTCTTTTTTAATACTTTAAGTTCTG
GGATACATGTGCAGAATGTACAG

**Finding out the restriction enzymes to be used to perform PCR-RFLP**

The restriction enzymes to be used to perform PCR-RFLP were selected using the insilico.ehu.es website.33 The selected regions were pasted in sequence info of the site, all default parameters were selected, then clicked on getting enzymes to find out the restriction enzymes cutting the region. Then the mutation was made at respective positions (i.e., at 1386) of the selected region and then pasted in sequence info of the site and again the map sites were found.

**Table 1:** The restriction sites for normal region of OCT1

| Restriction Enzyme | Cuts | Position |
|---|---|---|
| AatI, Eco147I, PceI, SseBI, StuI AGG^CCT | 1 | 201 |
| AcsI,ApoI.XapI R^AATT_Y | 2 | 120 |
| AseI, PshBI, VspI AT^TA_AT | 1 | 51 |
| BfaI, FspBI, MaeI, XspI C^TA_G | 1 | 26 |
| BshFI, BsnI, BspANI, BsuRI, HaeIII, PhoI GG^C | 1 | 201 |
| PsiI TTA^TAA | 1 | 126 |
| MaeIII ^GTNAC_ | 1 | 40 |

**Choosing and optimizing the primers**

The sequence of the regions to be studied was copied to the online software program Primer 3. All default parameters were selected and then primers were designed. The pair with the highest score was chosen. The selected primers were then put in BLAST[34] (Basic Local Alignment Search Tool) at the online Programme select nucleotide BLAST, feed the primer sequence in Enter query sequence, and in choose search set select Human genomic + transcript and click BLAST. This is done in order to check for any homology with other genomic regions. Considering the Max score and E value, the primers were optimized and selected.

**Table 2:** Choosing and Optimizing Primers

| | Sequence (5'->3') | Template strand | Length | Start | Stop | Tm | GC% | Self-complementarity | Self 3' complementarity |
|---|---|---|---|---|---|---|---|---|---|
| Forward primer | GGAGAGTCAGATGATAAGAGGGT | Plus | 23 | 62 | 84 | 58.52 | 47.83 | 4.00 | 0.00 |
| Reverse primer | CCCCCTGCACTGATTTGAGAT | Minus | 21 | 195 | 175 | 60.06 | 52.38 | 4.00 | 3.00 |
| Internal | | Plus | | | | | | | |

**Polymerase Chain Reaction- Restriction Fragment Length Polymorphism**

**Polymerase chain reaction (PCR)**

The polymerase chain reaction is one of the most revolutionary methods in genetics. The method gives amplification of specific DNA sequences by an enormous factor. For PCR, we need two oligonucleotide primers that flank the DNA sequence in question. The primers hybridize to opposite strands of denatured DNA and allow synthesis by DNA polymerase to begin. The principle of the PCR reaction is based on the fact that DNA molecules denature and anneal at different temperatures. These temperature changes occur in cycles to produce many copies of the DNA sequence. The first step is the denaturation of double-stranded DNA at 94 °C. Then, the primers anneal to their complementary sequences at about 55 °C (depending on the primer base composition and length). Extension by the DNA polymerase from the two primers has an optimal temperature of 72 °C. This cycle is repeated 25 to 40 times to give exponential amplification of the desired DNA sequence. After 30 cycles, theoretically, about 268 million copies are created. The DNA polymerase used in PCR is the thermo stable Tag polymerase isolated from the bacteria Thermusaquaticus. This enzyme can survive the high temperature and the temperature changes during the reaction.

The specificity of the PCR reaction depends on the

specificity of the primers. The primers should be 17-30 nucleotides long. A GC content of about 50% is ideal. A lower GC content would lower the melting temperature. Sequences of long runs of a single nucleotide should be avoided, and primers with significant secondary structures are undesirable. Complementarities primers will give primer dimers, which reduces the effectiveness of the amplification.

Equipment used were PCR Machine (Eppendorf); Gel Electrophoresis system (GENEI); Gel documentation system (Alpha Innotech corp.) Materials Required were Template DNA; Downstream oligonucleotide primer; Upstream oligonucleotide primer; Taq DNA polymerase;10×reaction buffer;25mM MgCl2 (optional); Nuclease free water; dNTP mix (10mM of each dNTP); Primers: The primers required for this study was designed using PRIMER 3 and were procured from Sakhala Enterprises, Bengaluru.

**Table 3:** Primers used

| | |
|---|---|
| Forward primer | TCCTGGCTGAGTATTTGGGC |
| Reverse primer | TGTATGCTCCAGGGAACACG |

**Procedure**

The 0.2ml PCR tubes were labelled for each genomic DNA and arranged in the rack. Added 2µl of template DNA to the already labelled tubes and kept in the PCR tube rack. Prepared the following reaction cocktail in a 1.5 ml Eppendorf tube for the required number of reactions, plus two reactions to compensate for the pipetting loss. (If more than one primers used, prepare a cocktail separately for each primer).

**Table 4**: Constituents required for makeup of 25µl

| Constituent | Quantity required to make up to 25µl |
|---|---|
| Sterile water | 18.70 |
| 10× PCR buffer | 2.50 |
| dNTPs 10mM | 0.15 |
| Forward primer | 1.0 |
| Reverse Primer | 1.0 |
| Taq polymerase | 0.30 |
| Magnesium chloride (25mM) | 1.5 |
| Total | 25µl |

(If many tubes are being processed, then the water, buffer, dNTPs, primer, Taq enzyme can be made up as a single solution. Just prior to use. While preparing the PCR reactions, it is important to keep the reaction mixture in ice; add the components in the order as indicated).

Added 23µl of the reaction mixture to the 0.2ml PCR tube, which is already loaded with 2µl of template DNA, and make the final volume to 25 µl. Mixed by flicking the tube repeatedly with finger and then centrifuge for few seconds to bring the contents to the bottom of the tube. (Do not use the vortex mixer to mix the enzymes mixtures, as this will inactivate the enzymes).

**Restriction enzyme's digestion of the PCR product**

The restriction enzymes to be used were selected using insilico.ehu.es. The restriction enzymes recognize specific sequences of DNA and cleave the phosphodiesterase bond on each strand at that sequence. After digestion with the restriction enzyme, the resulting DNA fragments can be separated by agarose gel electrophoresis, and their corresponding sizes can be estimated. The restriction enzymes were Streptomyces photochromogens (SphI) procured from Sakhala Enterprises, Bengaluru.

**Table 5**: Performing the PCR reaction in a thermocycler (Eppendorf) as follows below

| | Temperature ($^0$C) | Time (minutes) | Cycles (No.) |
|---|---|---|---|
| Initial Denaturing | 94 | 10 | One |
| Denaturing | 94 | 1 | |
| Annealing* | Tm-2 $^0$C | 10 | 30-35 |
| Extension | 72 | 2 | |
| Final extension | 72 | 10 | One |
| Storage | 4 | Forever | |

Materials required were DNA sample in water or in TE buffer (PCR amplified product); 10×digestion buffer; Restriction enzyme; Agarose gel 1 % (or different depending on expected band sizes).

**Table 6:** Constituents for Restriction enzyme's digestion of the PCR product

| Constituents | Volume Required |
|---|---|
| PCR amplified product | 10 µl |
| Restriction enzyme | 1 µl |
| Assay Buffer 10X | 5 µl |
| Distilled water (Deionized) | 9 µl |
| Total | 25 µl |

Mixed the final solution (Do not vortex as this will rip the DNA). Spinning down the tube in the microcentrifuge to ensure all liquid is at the bottom. Placed the tube in the 37 °C bath for about 2 hours to facilitate digestion. After two hours, placed in a 65 °C bath for about 5 mins. (This destroys the enzyme and stops digestion). Allowed it to cool for some time. Then it was run on an agarose gel to check the bands.

**Checking the amplified product**

Cast the 1% agarose gel with a suitable gel electrophoresis system. 2. Added 2µl of 6× loading dye to 10µl of the PCR product and mixed well. 3. Loaded the gel with PCR product along with the molecular weight marker (DNA ladder of 1000bp was used) 4. The gel was run in 1×TAE at 120V till the bromophenol blue reached the end of the gel.5. Stained for 5 minutes in the 0.001% ethidium bromide solution. 6. De stained in water for 15minutes.7. The gel was viewed using a UV transilluminator and documents gel using a photo documentation system. 8. The banding patterns were analyzed and discussed

## RESULTS AND DISCUSSION

We sequenced all the SLC22A1 from 12 South Indian healthy adult male volunteers whose ages ranged between 12-35 years. The mean age was found to be 18±0.5. The mean bodyweight of the volunteers was found as 63.5±9.0 kg that ranging from 52 to 78 kg. The height of the volunteers ranged from 158 to 180 cm, with a mean

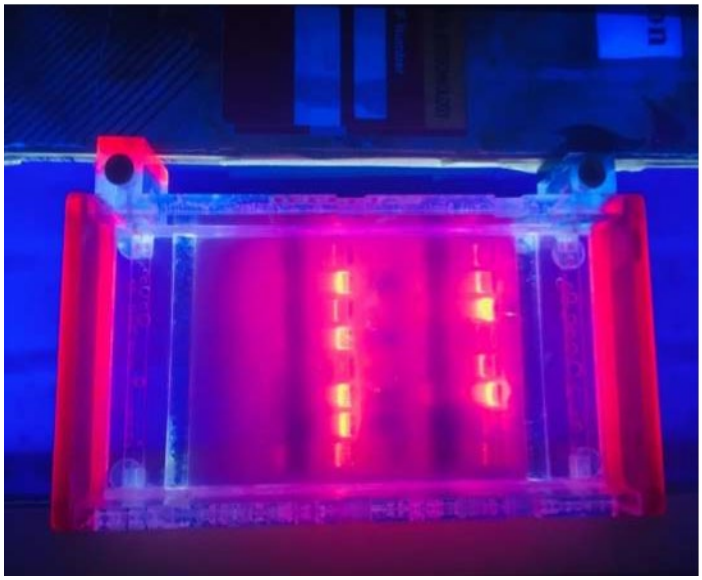
value of 171.5±6.39. All the general physiological parameters of the volunteers were found within the normal reference values, and based on these values, the volunteers were certified as healthy individuals by a physician. The mean value of the body temperature was 98.4±0.16 (°C), heart rate was (per min) 74.0±2.94, and systolic and diastolic blood pressure were found 109±8.75 mmHg and 72.0±6.32mmHg respectively. The mean hemoglobin and random blood sugar values were found as 16.0±0.73 (g/dL) and 77.0±6.41 (mg/dL) respectively. The serum bilirubin total was 1.19±0.60 and direct 0.58±0.20and indirect bilirubin was 0.61±0.43. The SGOT and SGPT were measured and found as 11.5±5.79 &7.3±5.43 (U/dL) respectively.

**Development and validation of Reverse Transcriptase- PCR method for identifying OCT1 (rs622342) group were achieved.**

DNA Extraction and identifying rs622342 polymorphic group:

The plasma removed from the volunteer's blood was subjected to DNA isolation using the phenol-chloroform method. The obtained DNA samples were then run-on Agarose gel electrophoresis to identify the same. **The DNA bands are shown in Figure–1**

**Figure 1**: Figure showing DNA bands after the isolation using the Phenol Chloroform method



The restriction enzyme (PsiI) was then added to the obtained DNA samples in order to identify the polymorphic group (rs622342) as shown in Figure -2

After the addition of restriction enzyme (PsiI) in the ten samples, none of the samples showed fragmentation, which shows that there is a lack of rs622342 genetic polymorphism in the above ten samples.

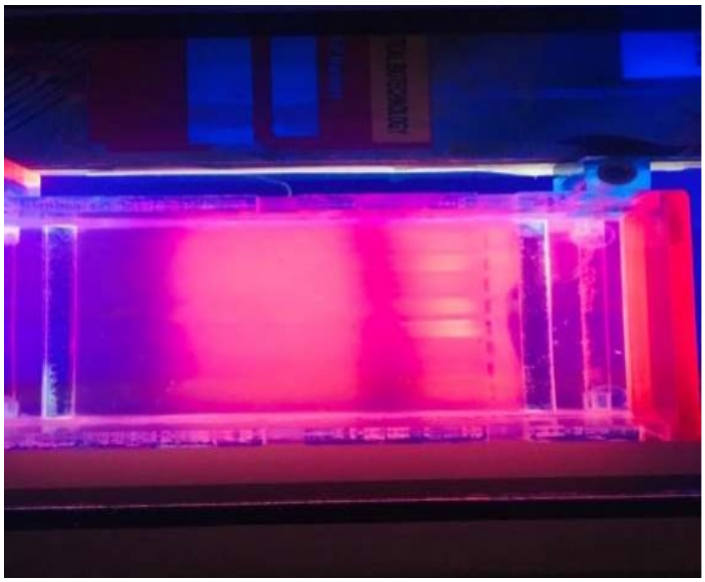
**Limitations**

As the sample size was very less, the genetic polymorphism in patients was not established.

**Future Directions**

The methodology established in this pilot study shall be used further to repeat the study with the same objectives with a large sample size, which could help in finding the influence of genetic polymorphism in a healthy population.

**Figure 2:** DNA bands after adding restriction enzymes



## CONCLUSIONS

Individual variations in response to a drug are a result of differences in the pharmacodynamics and pharmacokinetics, including receptor and transporter polymorphisms. The genetic makeup of an individual patient can influence the metabolic pathways of drug action and elimination. These variations range from failure to respond to drugs to adverse drug reactions. Organic Cation Transporter (OCT) plays a major role in the detoxification of many exogenous and endogenous compounds. OCT isozymes were found to be highly polymorphic, and some polymorphisms can lead to both transcriptional and functional changes of the enzymes. Literature studies have stated that genetic polymorphisms in OCT1 drug transporters are significant and contribute to a greater incidence of drug ineffectiveness. Genetic variants of the organic cation transporter (OCT1) gene could influence interindividual variation in clinical response to drug therapy.

This study showed a lack of OCT1 genetic polymorphism in ten healthy volunteers, which shows the need for more sample size to confirm. Once the study is further carried out with larger sample size, we could expect the presence of genetic polymorphism as stated in the works of literature and assist in relating the effect of genetic polymorphism on the pharmacokinetics of drugs. This standardized methodology could be useful for many other studies to carry out the genetic polymorphism of OCT1 with many other substrates. The major limitation which we came across was that as the sample size was very less, the genetic polymorphism in volunteers was not established. The future direction of this study is the methodology established in this pilot study shall be used further to repeat the study with the same objectives with a large sample size, which could help in finding the

influence of genetic polymorphism in the pharmacokinetic parameters of drugs in South Indian healthy population.

## DECLARATION

Ethical statement informed consent form has been obtained from all the volunteers and the study has been approved by the Institutional Ethics committee, JSS College of Pharmacy, Rocklands, Ooty.

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**CONFLICT OF INTERESTS** The authors declare none

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