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IDENTIFICATION OF GILBERTS SYNDROME DUE TO PRESENCE OF TAA_7 ALLELE WITH THE HELP OF CAPILLARY ELECTROPHORESIS BASED DNA SEQUENCING

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Abstract: **Gilberts Syndrome is a mild a condition where the liver is unable to produce the enzyme bilirubin in the blood. The reason for instability might be stress, dehydration, fasting etc. It is caused due to mutation in the UGT1A1 gene, basically the insertion of another TATAA box which results in the formation of TA_7 gene in the sequence. In the present study we aim to focus on the allelic mutations in the UGT1A1 sequence that cause gilbert's syndrome. We have used capillary electrophoresis technology to obtain DNA sequences of the samples under study and compare it with UGTA1 sequence for detecting mutation in the UTGA1 gene. DNA was extracted and PCR amplified products of 363bp were provided to carry out the study. The PCR products were purified using magnetic beads. These purified products were used for setting up cycle sequencing reaction. Next, the extension products generated were purified with BDX Terminator kit. Purified extension products were subjected to CE on genetic analyzer 3500 xL. Data files generated were analyzed with Sequencing Analysis Software to view the electropherogram and generate FASTA files. The sequences obtained were blasted in the NCBI database.**

Keywords: **Capillary Electrophoresis, Cycle Sequencing, Gilberts Syndrome, PCR product purification, BLAST.**

1. INTRODUCTION

1.1 DNA Sequencing

DNA Sequencing is a process to produce a DNA Sequence, or nucleic acid sequence. It helps us understand, how the bases Adenine, Cytosine, Guanine and Thymine are aligned in a sequence.

There are various uses of DNA Sequencing such as identifying mutations and SNP's, comparing two sequences to diagnose different diseases and also find its treatment. Nowadays, DNA Sequencing has proven to be one of the most influential technology in the field of research in molecular biology and the medical industry. With the recent advances in DNA Sequencing, Personalized medicine has come to action which will revolutionize the health sector. [1]

Sanger Sequencing is basically a method to derive a nucleotide sequence of the DNA. Sanger Sequencing requires a DNA Template, a sequencing primer, DNA Polymerase, dNTP's, ddNTP's and reaction buffer. The dNTP's have 3'-OH group and hence chain elongation takes place. When ddNTP's are added, which do not have 3'-OH group, chain termination takes place, and then with the help of electrophoresis, sequence information can be retrieved. [2]

1.2 Capillary Electrophoresis

During CE, the extension products enter the capillary as a result of electro kinetic injection. A high voltage charge is applied and the negatively charged fragments are directed into the capillaries. The extension products are separated by size based on their total charge.

Before reaching the positive electrode, the fluorescently labeled DNA fragments, separated by size are moved across the path of laser beam. The laser beam causes the dyes on the fragments to fluoresce. An optical detection device detects the fluorescence. The data collection software converts signal to digital data. All 4 bases and colors can be detected and distinguished in one capillary injection.[3]

1.3 Gilberts Syndrome

Gilbert syndrome is an autosomal recessive bilirubin metabolism condition that affects the liver. Unconjugated hyperbilirubinemia and recurring episodes of jaundice result from reduced bilirubin glucuronidation. Approximately 95% of bilirubin is unconjugated in normal circumstances. Crigler-Najjar syndrome is another condition characterised by unconjugated hyperbilirubinemia. The majority of Gilbert syndrome patients are asymptomatic in terms of liver disease, but they may experience symptoms in response to triggers. Asthma, intercurrent sickness, menstruation, and dehydration are all triggers. [4]

2. MATERIALS AND METHODS

2.1 PCR Product Purification

Isolation of the DNA samples was done followed by PCR. PCR product purification was done by magnetic bead based elimination method to eliminate excess primers, dimers and impurities. At first, the ampure, Beckmann Coulter was taken out and kept at room temperature for 25 to 30 min. Ampure and BDT are usually kept at deep freezer at around 4°C. Label the tubes properly. Aliquot 18 µl of ampure in 5 separate tubes. Add 10 µl PCR product. Vortex and spin down (quick spin) these samples and then incubate for 5 min. After that, put the samples on magnetic stand. Discard 21 µl of supernatant. Perform ethanol wash by adding 200 µl 70% Ethanol and flip the tubes 4-5 times. Discard the Ethanol and repeat the previous step. Spin down the samples and discard 10 µl. Air dry the tubes for 7 min on the magnetic stand with the lids open. After that, add 11 µl of NFW (Nuclease Free Water). Vortex and spin down and then incubate for 5 min at room temperature. Put the samples in magnetic stand and incubate for 3 min. Take 10 µl of the final product and transfer it to new fresh tubes. This samples will be then proceeded to cycle sequencing.

2.2 Cycle Sequencing

We then proceeded to cycle sequencing. We used the kit BDT V3.1 Invitrogen biosciences, Thermo Fisher Scientific to perform cycle sequencing.

First, we created a master mix, which has Sequencing Buffer, RRM and Water.

Table 1: Master Mix

Contents	Amount	Amount * 3
Sequencing buffer	1.75µl	5.25µl
RRM	0.5µl	1.5µl
Water	2.75µl	8.25µl

5µl of master mix is then added to the plate in each well. After that, we add 4µl of DNA and 1µl of primer (5 pico mole/µl), making the whole reaction of 10µl per well. We put an adhesive film in order to maintain the mixture's stability. Vortex the plate and then spin down. Put the plate in PCR under the conditions – Denaturation at 96°C for 1 minute; Annealing at 96°C – 10 sec, 50°C – 0.05 sec, 60°C – 4 min; Hold at 4°C till infinity for 25 cycles.[5]

2.3 Extension Product Purification

We then proceeded to final step of wet lab. Extension product purification was done with the help BDX Terminator, Invitrogen biosciences, Thermo Fisher Scientific to remove all the salts, dNTP's and ddNTP's.

After removing the plates from PCR, we manually added 57 µl of BDX solution.[6]

Table 2: Constituents of BDX Terminator Solution

Constituents	Amount	Amount * 3
SAM Solution	45µl	135 µl
BDX Solution	10µl	30 µl

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2.4 Capillary Electrophoresis

We finally inserted the plates in Genetic Analyzer 3500xL and ran them. We then observed the electropherogram of the samples which helped us obtain the DNA sequence.

3. RESULT

After processing the samples on the genetic analyzer 3500 xL, we proceeded to the observation phase. The sequencing analysis software 6 generated electropherogram.

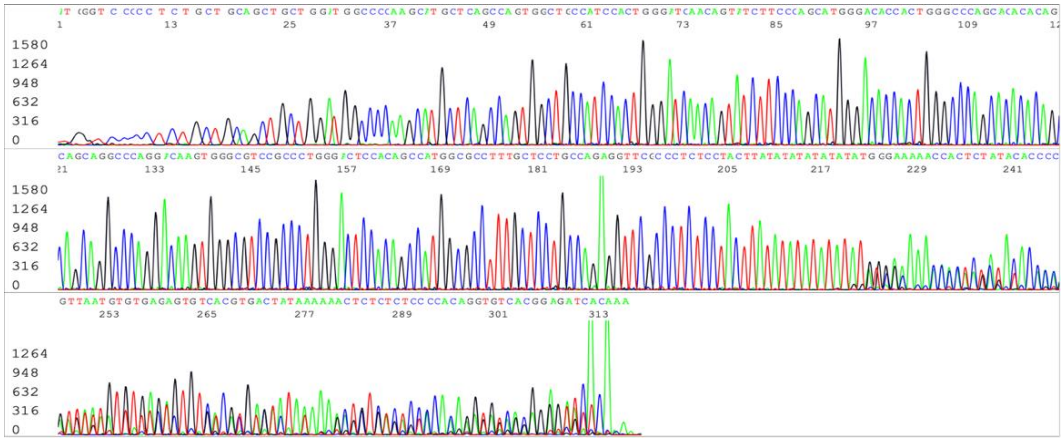

Figure 1: Electropherogram of sample 1

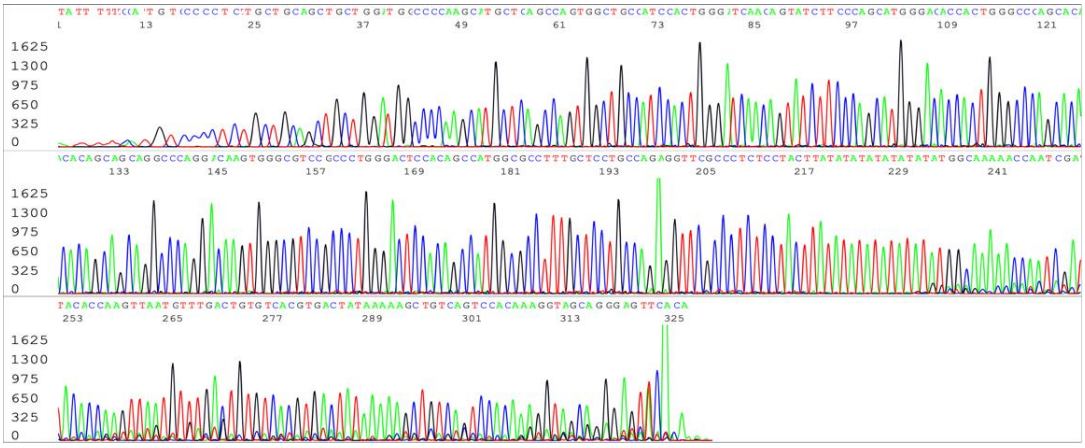

Figure 2: Electropherogram of sample 2

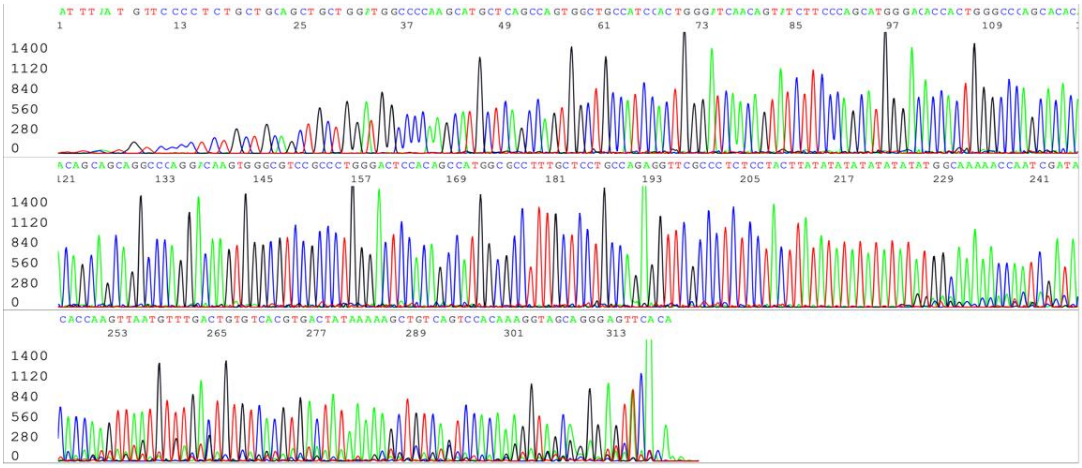

Figure 3: Electropherogram of sample 3

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The DNA Sequence was then determined. FASTA sequence was generated and then inserted in BLAST to compare the sequences and find the possible mutations.

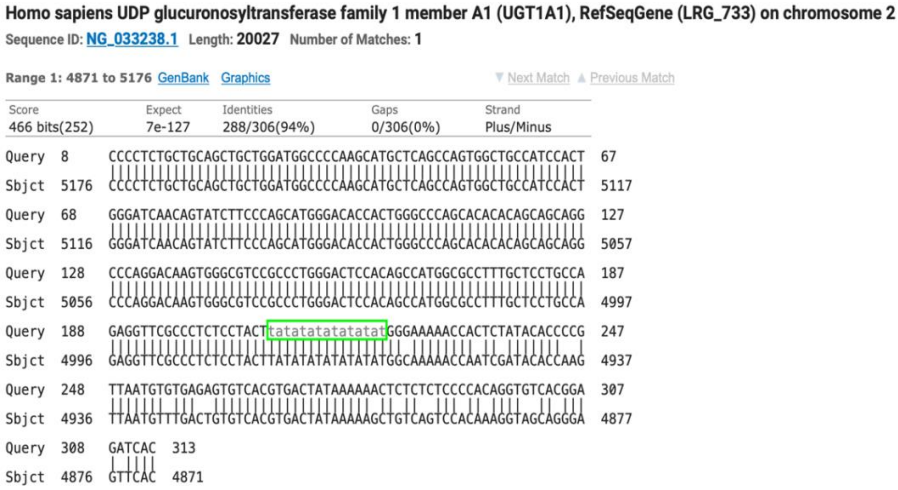

Figure 4: After BLAST we found 7 repetitive mutation in TA box in sample 1

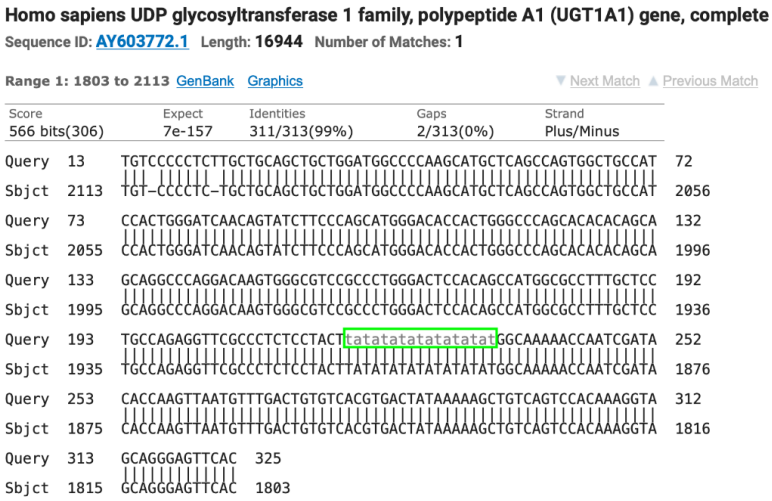

Figure 5: After BLAST we found 8 repetitive mutation in TA box in sample 2

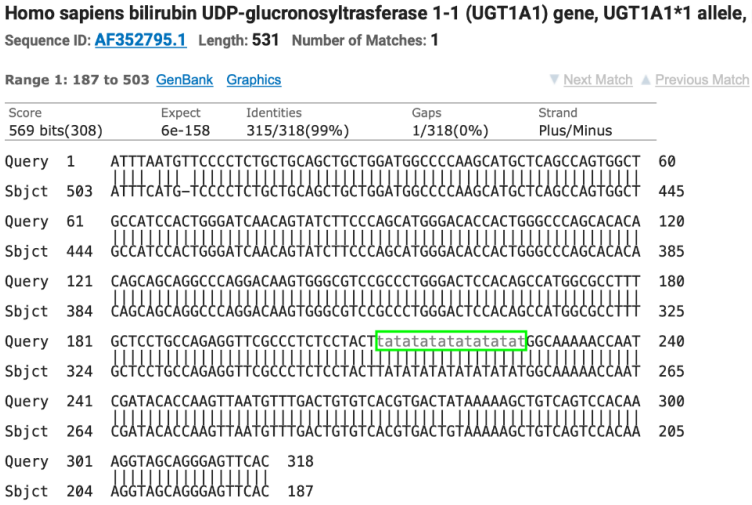

Figure 6: After BLAST we found 8 repetitive mutation in TA box in sample 3

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4. DISCUSSION

The samples showed the presence of gilberts syndrome. To cross check, we pasted the samples accession numbers in the gene database of NCBI.

Table 3: Samples and their Accession Numbers

Sample	Gilberts Syndrome	Accession Number
Sample 1	Present	NG_033238.1
Sample 2	Present	AY603772.1
Sample 3	Present	AF352795.1

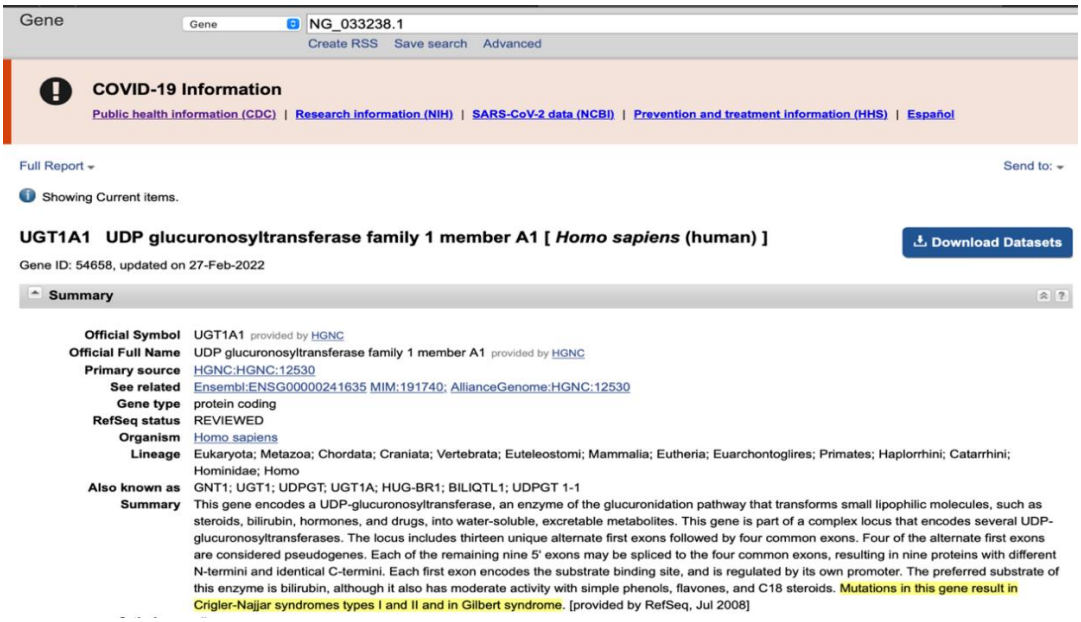

Figure 7: Sample 1 gene database search using accession number

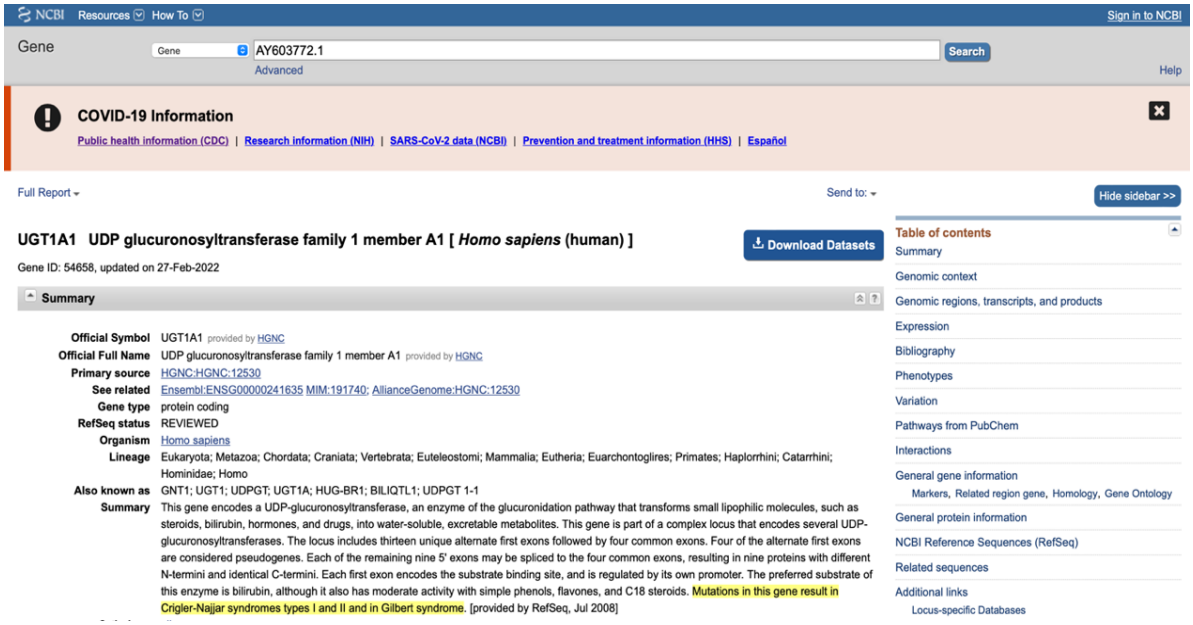

Figure 8: Sample 2 gene database search using accession number

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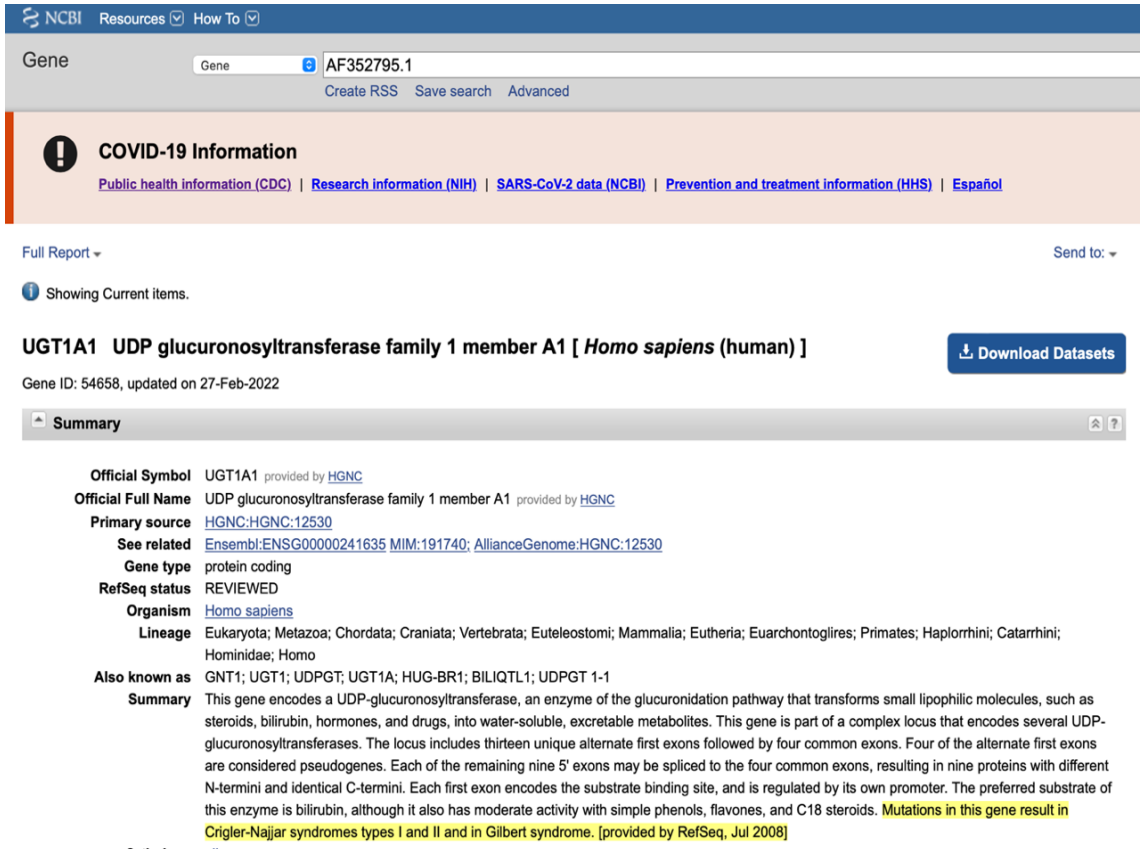

Figure 9: Sample 3 gene database search using accession number

5. CONCLUSION

All the samples showed the presence of gilberts syndrome.

Table 4: Deciphering Gilbert's Syndrome mutations from Samples

Sample	Mutation	Gilberts Syndrome
Sample 1	$A(TA)_7TAA$	Present
Sample 2	$A(TA)_8TAA$	Present
Sample 3	$A(TA)_8TAA$	Present

$A(TA)_7TAA$ mutation is the main cause of gilberts syndrome worldwide, but $A(TA)_8TAA$ is another variant which is specific to African continent.[7]

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