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# IDENTIFICATION OF GILBERTS SYNDROME DUE TO PRESENCE OF TAA<sub>7</sub> 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<sub>7</sub> 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. <sup>[1]</sup>

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.<sup>[2]</sup>

#### **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.

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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.<sup>[3]</sup>

## **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.<sup>[4]</sup>

# 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  $\mu$ l of ampure in 5 separate tubes. Add 10  $\mu$ l 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  $\mu$ l of supernatant. Perform ethanol wash by adding 200  $\mu$ l 70% Ethanol and flip the tubes 4-5 times. Discard the Ethanol and repeat the previous step. Spin down the samples and discard 10  $\mu$ l. Air dry the tubes for 7 min on the magnetic stand with the lids open. After that, add 11  $\mu$ l 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  $\mu$ l 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.

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

Table 1	1: Master	Mix
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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.<sup>[5]</sup>

#### **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.<sup>[6]</sup>

# **Table 2: Constituents of BDX Terminator Solution**

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

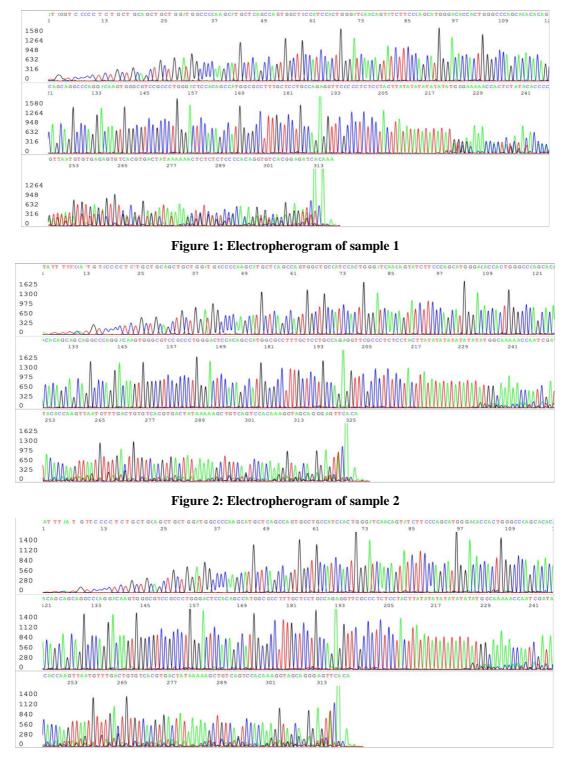
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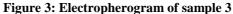
## 2.4 Capillary Electrophoresis

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.





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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.

			onosyltransferase		r A1 (UGT1A1), R	efSeqGene (LRG_733) on chromoso
Range 1	1: 4871	to 5176 GenBan	Graphics		Vext Match	Previous Match
Score 466 bit	s(252)	Expect 7e-127	Identities 288/306(94%)	Gaps 0/306(0%)	Strand Plus/Minus	
Query			AGCTGCTGGATGGCCCC			67
Sbjct Query	5176 68		ÁGCTGCTGGÁTGGCCCC ATCTTCCCAGCATGGGA			5117 127
Sbjct	5116	GGGATCAACAGT	ATCTTCCCAGCATGGGA	CACCACTGGGCCCAG	CACACACAGCAGCAGG	5057
Query Sbjct	128 5056		GGGCGTCCGCCCTGGGA			187 4997
Query	188	GAGGTTCGCCCT	CTCCTACT <mark>tatatatat</mark>	atatat <mark>GGGAAAAAC</mark>	CACTCTATACACCCCG	247
Sbjct Query	4996 248	ĠĂĠĠŦŦĊĠĊĊĊŦ	ĊŦĊĊŦĂĊŦŦĂŦĂŦĂŦĂŦĂ AGTGTCACGTGACTATA		ĊĂAŦĊĠĂŦĂĊĂĊĊAAĠ	4937 307
Sbjct	4936	HAATGTTTGAC	TGTGTCACGTGACTATA		ACAAAGGTAGCAGGGA	4877
Query	308 4876	GATCAC 313        GTTCAC 4871				
Sbjct	40/0	GTTCAC 4871				

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

Homo sapiens UDP glycosyltransferase 1 family, polypeptide A1 (UGT1A1) gene, complete cds Sequence ID: <u>AY603772.1</u> Length: 16944 Number of Matches: 1

ange 1	: 1803	to 2113 GenBank	Graphics		Vext Match
<sup>core</sup> 66 bits	(306)	Expect 7e-157	Identities 311/313(99%)	Gaps 2/313(0%)	Strand Plus/Minus
lery	13				GCCAGTGGCTGCCAT
ojct	2113	+6+-6666+6-+66	ttgcygctgctgctggytggc	ccaagcatgctca	ŚĊĊĂĠŦĠĠĊŦĠĊĊĂŦ
Jery	73	CCACTGGGATCAA	CAGTATCTTCCCAGCATGG	GACACCACTGGGC	CAGCACACACAGCA
ojct	2055	ccactgggatcaa	CAGTATCTTCCCAGCATGG	GACACCACTGGGC	
uery	133	GCAGGCCCAGGAC	AAGTGGGCGTCCGCCCTGG	GACTCCACAGCCA	rggcgcctttgctcc
ojct	1995	GCAGGCCCAGGAC	AAGTGGGCGTCCGCCCTGG	GACTCCACAGCCA	reececcttttectcc
iery	193	TGCCAGAGGTTCG	CCCTCTCCTACT tatatat	atatatatat <mark>GGC/</mark>	AAAAACCAATCGATA
ojct	1935	tgccagaggttcg	╘сстстсстасттатата	atatatatatagda	AAAACCAATCGATA
Jery	253	CACCAAGTTAATG	ГТТGACTGTGTCACGTGAC	татааааадстдт	CAGTCCACAAAGGTA
ojct	1875	CACCAAGTTAATG	+++GAC+G+G+CACG+GAC	tataaaaagctgto	
Jery	313	GCAGGGAGTTCAC	325		
ojct	1815	GCAGGGAGTTCAC	1803		

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

Homo sapiens bilirubin UDP-glucronosyltrasferase 1-1 (UGT1A1) gene, UGT1A1\*1 allele, partial cds Sequence ID: <u>AF352795.1</u> Length: 531 Number of Matches: 1

Range 1	l: 187	to 503 GenBank G	raphics		Vext Match	Previous Match
Score 569 bits	s(308)	Expect 6e-158	Identities 315/318(99%)	Gaps 1/318(0%)	Strand Plus/Minus	_
Query	1	ATTTAATGTTCCCC	rctgctgcagctgctggat	GGCCCCAAGCATGC	TCAGCCAGTGGCT	60
Sbjct	503	AtttcAtg_tcccc	ŀċŦĠċŦĠċĂĠċŦĠċŦĠĠĂŦ	GGCCCCAAGCA+GC-	tcagccagtggct	445
Query	61	GCCATCCACTGGGA	rcaacagtatcttcccagc	ATGGGACACCACTG	GCCCAGCACACA	120
Sbjct	444	GCCATCCACTGGGA	rcaacagtatcttcccagc	ATGGGACACCACTG	GCCCAGCACACA	385
Query	121	CAGCAGCAGGCCCA	GGACAAGTGGGCGTCCGCC	CTGGGACTCCACAG	CATGGCGCCTTT	180
Sbjct	384	CAGCAGCAGGCCCA	GACAAGTGGGCGTCCGCC	ctgggyctccycyg	ccateecect+++	325
Query	181	GCTCCTGCCAGAGG	TTCGCCCTCTCCTACT tat	atatatatatatat	GCAAAAACCAAT	240
Sbjct	324	GCTCCTGCCAGAGG	HCGCCC+C+CC+AC++A+	************	GCAAAAACCAA†	265
Query	241	CGATACACCAAGTT	ATGTTTGACTGTGTCACG	TGACTATAAAAAGC	IGTCAGTCCACAA	300
Sbjct	264	CGATACACCAAGTT,	latettteactetetekee	tgactetaaaaagc	fgtcagtccacaa	205
Query	301	AGGTAGCAGGGAGT	TCAC 318			
Sbjct	204	AGGTAGCAGGGAGT	TCAC 187			

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

Gene	Gene B NG_033238.1				
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<ol> <li>Showing Current items.</li> </ol>					
UGT1A1 UDP gluc	uronosyltransferase family 1 member A1 [ Homo sapiens (human) ]				
Gene ID: 54658, updated on	27-Feb-2022				
Summary	8 ?				
Official Symbol	UGT1A1 provided by HGNC				
Official Full Name					
Primary source	HGNC:HGNC:12530				
See related	Ensembl:ENSG00000241635 MIM:191740; AllianceGenome:HGNC:12530				
Gene type					
RefSeq status					
Organism	Homo sapiens				
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae: Homo				
Also known as	nominidae, nomo GNT1: UDPGT: UGT1A: HUG-BR1: BILIQTL1: UDPGT 1-1				
Summary					
· · · · · · · · · · · · · · · · · · ·	steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. This gene is part of a complex locus that encodes several UDP-				
	glucuronosyltransferases. The locus includes thirteen unique alternate first exons followed by four common exons. Four of the alternate first exons				
	are considered pseudogenes. Each of the remaining nine 5' exons may be spliced to the four common exons, resulting in nine proteins with different				
	N-termini and identical C-termini. Each first exon encodes the substrate binding site, and is regulated by its own promoter. The preferred substrate of				
	this enzyme is bilirubin, although it also has moderate activity with simple phenols, flavones, and C18 steroids. Mutations in this gene result in				
127 14 102	Crigler-Najjar syndromes types I and II and in Gilbert syndrome. [provided by RefSeq, Jul 2008]				



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•	uronosyltransferase family 1 member A1 [ Homo sapiens (human) ]	tasets	Table of contents Summary	
ne ID: 54658, updated on	27-Feb-2022		Genomic context	
Summary		* ?	Genomic regions, transcripts, and products	
Official Symbol	UGT1A1 provided by HGNC		Expression	
	UDP glucuronosyltransferase family 1 member A1 provided by HGNC		Bibliography	
	HGNC:HGNC:12530		Phenotypes	
See related	Ensembl:ENSG00000241635 MIM:191740; AllianceGenome:HGNC:12530		Variation	
	protein coding			
RefSeq status	Homo sapiens		Pathways from PubChem	
	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini;		Interactions	
	Hominidae; Homo		General gene information	
	GNT1; UGT1; UDPGT; UGT1A; HUG-BR1; BILIQTL1; UDPGT 1-1		Markers, Related region gene, Homology, Gene On	itol
Summary This gene encodes a UDP-glucuronosyltransferase, an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as			General protein information	
	steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. This gene is part of a complex locus that encodes several UDF glucuronosyltransferases. The locus includes thirteen unique alternate first exons followed by four common exons. Four of the alternate first exons		NCBI Reference Sequences (RefSeq)	
	glucuronosyltransierases. The locus includes thineen unique alternate linst exons followed by four common exons. Four of the alternate linst exc	are considered pseudogenes. Each of the remaining nine 5' exons may be spliced to the four common exons, resulting in nine proteins with different		
	are considered pseudogenes. Each of the remaining nine 5' exons may be spliced to the four common exons, resulting in nine proteins with diff	ferent		
		ferent	Related sequences Additional links	

# Figure 8: Sample 2 gene database search using accession number

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Summary	2
Summary	8
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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) <sub>7</sub> TAA	Present
Sample 2	A(TA) <sub>8</sub> TAA	Present
Sample 3	A(TA) <sub>8</sub> TAA	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.<sup>[7]</sup>

## REFERENCES

- Green, E. D., Rubin, E. M., & Olson, M. V. (2017). The future of DNA sequencing. *Nature*, 550(7675), 179–181. https://doi.org/10.1038/550179a
- Griffin, H. G., & Griffin, A. M. (1993). DNA sequencing. Applied Biochemistry and Biotechnology, 38(1–2), 147– 159. https://doi.org/10.1007/bf02916418
- [3] Wenz, H. M., Robertson, J. M., Menchen, S., Oaks, F., Demorest, D. M., Scheibler, D., Rosenblum, B. B., Wike, C., Gilbert, D. A., & Efcavitch, J. W. (1998). High-Precision Genotyping by Denaturing Capillary Electrophoresis. *Genome Research*, 8(1), 69–80. https://doi.org/10.1101/gr.8.1.69
- [4] Bosma, P. J. (2003). Inherited disorders of bilirubin metabolism. *Journal of Hepatology*, *38*(1), 107–117. https://doi.org/10.1016/s0168-8278(02)00359-8

Vol. 9, Issue 2, pp: (64-70), Month: April - June 2022, Available at: www.paperpublications.org

- [5] Sasaki, N., Izawa, M., Watahiki, M., Ozawa, K., Tanaka, T., Yoneda, Y., Matsuura, S., Carninci, P., Muramatsu, M., Okazaki, Y., & Hayashizaki, Y. (1998). Transcriptional sequencing: A method for DNA sequencing using RNA polymerase. *Proceedings of the National Academy of Sciences*, 95(7), 3455–3460. https://doi.org/10.1073/ pnas.95.7.3455
- [6] Fujikura, K. (2016). Data on single-step purification method for dye-labeled DNA sequencing. *Data in Brief*, 7, 873–876. https://doi.org/10.1016/j.dib.2016.02.050
- [7] Shiu, T. Y., Huang, H. H., Lin, H. H., Shih, Y. L., Chu, H. C., Chang, W. K., & Hsieh, TY. (2015). Restriction fragment length polymorphism effectively identifies exon 1 mutation of UGT1A1 gene in patients with Gilbert's Syndrome. *Liver International*, 35(8), 2050–2056. https://doi.org/10.1111/liv.12785