Study single nucleotide polymorphism in Promoter region of UGT1A1 Gene in Iraqi Patients with Gilbert’s syndrome

Marwa A. Kubba1,*, Abeer Ali Marhoon2 and Rafed Abbas Kadhum3

Abstract: This study aimed to detect genetic variants of the UGT1A1 gene in patients with Gilbert’s syndrome. To detect this, primers were designed; PCR and direct sequencing were done for the promoter area of the gene as a diagnostic tool for the detection of any polymorphism. Variation and polymorphism were detected within the promoter mutants of the UDP-glucuronosyltransferase (UGT1A1 gene) that causes hyperbilirubinemia in a group of Iraqi patients compared with a group of the normal healthy individual as controls. The patients with hyperbilirubinemia in this study were 30 in which the total bilirubin level was more than 12 mg/dl serum; they included 25 males and 5 females, while the control group consisted of 20 healthy individuals. This study was carried out from September 2019 till April 2021. The result displayed high occurrence of Gilbert syndrome within male patients than in females, and regarding the analyses of mutation of bilirubin UDP glucuronosyltransferase UGT1A1 gene, it is clear that the genotypic distribution of variation among the hyperbilirubinemia patients included all 30 patients, while SNP was detected in 18 patients out of 30 which indicate that the UGT1A1 gene mutation was a likely risk factor for the development of hyperbilirubinemia related Gilbert syndrome in Iraq. The homozygous and heterozygous polymorphisms A/G inside the promoter region of the UGT1A1 gene were effectively identified by sequencing. Our finding suggests that TA repeats and allele of UGT1A1 polymorphism A/G are associated with Gilbert’s syndrome and act as genetic markers of this disease in Iraqi patients. To analyze data and sequence variation in gene, generous software was used after amplifying the gene. All processes include DNA extraction, PCR amplification, sequencing, and assembly.

Key words: Gilbert’s syndrome, UGT1A1 gene, polymorphism, hyperbilirubinemia.

Introduction

Gilbert syndrome (GS) is a disorder conspicuous by intermittent unconjugated hyperbilirubinemia and jaundice. Protein formed from the UGT1A1 gene, named the bilirubin uridine diphosphate glucuronosyltransferase (bilirubin-UGT) enzyme, is a hepatic enzyme in which glucuronidated bilirubin; a material formed once red blood cells are broken down. The enzyme’s function changes the toxic form of bilirubin to its nontoxic form, making it capable of being thawed and detached from the body; this enzyme is required for the conversion (conjugation) and subsequent elimination of bilirubin from the body. Gilbert’s syndrome is supposed in patients that have unconjugated hyperbilirubinemia produced by reduced activity of the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene in the lack of irregular liver function and hemolysis, noticeable by unbalanced unconjugated hyperbilirubinemia, typically due to the polymorphism uridine diphosphate-glucuronosyltransferase inherited defects in uridine diphosphoglucuronate glucuronosyl-transferase (UGT) that encoded by the UGT1A1 gene at chromosome 2q37.1, the reduced enzyme action are lead to hyperbilirubinemia. The variation in the UGT1A1 gene can reason for Gilbert syndrome that characterized by phases of significant unconjugated hyperbilirubinemia. The main genetic variants in Gilbert’s syndrome are TATA-box repeats of the promoter region that are responsible for the manufacturing of the bilirubin-UGT enzyme and exon 1 G211A of the coding region, UGT1A1 gene mutations either decrease the affinity of UGT1A1 toward bilirubin or decrease enzyme activity. New studies presented serum bilirubin is related to a genetic variation of the UGT1A1 locus. In several people, the maximum shared change cause Gilbert syndrome happens in part near the UGT1A1 gene called the promoter region. This alteration must happen in both copies of the UGT1A1 gene to cause Gilbert syndrome.

Homozgyous polymorphism is the most public UGT1A1 genotype responsible for Gilbert’s syndrome when A(TA)7 TAA in the gene’s promoter region; this will make a 70-80% reduction in the gene’s promoter region glucuronidation activity. In contrast, the change at nucleotide 211 (G211A), which change arginine to replace with glycine at position T1 in the coding region of the UGT1A1 gene, is accountable for around 20% of Gilbert’s syndrome cases in Asian people. Therefore this study was aimed to detect the gene variation in UGT1A1 gene in blood samples as a diagnostic tool by PCR and sequencing in patients from Iraq and determine its susceptibility to with Gilbert’s syndrome.

Citation: Kubba, M. A.; Marhoon, A. A.; Kadhum, R. A. Study single nucleotide polymorphism in Promoter region of UGT1A1 Gene in Iraqi Patients with Gilbert’s syndrome. Revis Bionatura 2022;7(1). DOI: 10.21931/RB/2022.07.01.31
Received: 16 October 2021 / Accepted: 20 November 2021 / Published: 15 February 2022

Publisher’s Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).
Materials and methods

This study was held from September 2019 to April 2021 at the University of Al-Rasheed / Biology Department, and the molecular study was held in the molecular laboratory of ASCo. They were a learning center in Iraq. Blood samples were collected from a population consisting of 30 subjects with Gilbert syndrome disease who complained of high bilirubin level in blood and have familial history of Gilbert syndrome with no clinical features refer to a disorder of liver functions; they were selected from those attending private Hospital in Baghdad. The age of these patients ranged from 17 - 55 years, in which 25 patients were males, and only 5 were female. The control group involved 20 healthy individuals were their ages and sex thoroughly similar to the patients and had no history of any disease.

An aliquot of 5 ml blood sample was gained from the vein of patient and control groups and divided into two parts, the first part (2 ml) was collected into an EDTA tube and kept at -20 °C for molecular analyses while serum was used for biochemical tests for liver enzyme ALT, AST, alkaline phosphatase, was recovered from the second blood part (3 ml).

Biochemical analyses of liver enzymes (ALT, AST, alkaline phosphatase) were estimated. The levels of all enzymes were examined for both the experimental and control groups.

DNA extraction

First DNA was isolated according to the procedure ReliaPrep™ Blood gDNA Miniprep System, Promega as the steps in manufacturer producer (table 1).

Polymerase Chain Reaction (PCR)

PCR was performed for 50 samples (30 patients and 20 control groups) using specific primers designed to amplify and sequence the promoter region of the UGT1A1 gene as described in table (2). PCR amplifications were performed as presented in (table 3).

DNA Standard Sequencing

To analyze the nucleotides sequences for all samples to determine the presence of single nucleotide polymorphism (SNP) and determine the genetic variation of this gene within the population, sequence analysis of the promoter area of the UGT1A1 gene was done accomplished on Gilbert syndrome patients. PCR product was directed for Sanger sequencing by ABI3730XL, automated DNA sequences, Macrogen Corporation – Korea. The obtained results were established through email and investigated by generous software.
Results

Liver function test results of Gilbert syndrome patients

The patients with hyperbilirubinemia suffering from Gilbert syndrome in this study were 30 by a whole bilirubin level of more than 12 mg/dl, which comprise 25 patients were males and 5 patients were females, samples for patients and control groups have normal liver enzyme ALT, AST, alkaline phosphatase values.

Distribution of Cases According to Gender

The distribution of GS according to gender showed higher rates in males than females when the male cases were 83.33% and 16.66% for females (figure 1).

Purity and concentration of DNA extracted from

The DNA was effectively extracted from blood samples. The purity of DNA extracted from blood samples was extended from 1.8 to 2, and the concentration of DNA was extended from 70 -120 µg/ml.

Amplification of UGT1A1 gene

The molecular genetic markers related to patients with hyperbilirubinemia were examined, the segment of the UGT1A1 gene can be amplified by using the PCR, including the promoter region with a size of about 892bp shown in figure (2). The primers in this study were designed using the NCBI Primer-Design online tool to detect single nucleotide polymorphism (SNPs) that may be found in the target gene leading to hyperexpression bilirubin that leads to Gilbert syndrome.

Sequencing and Analysis of UGT1A1 gene

An investigation of any genetic variation and mutations in the UGT1A1 gene was done. The sequence analysis result for the promoter region in the UGT1A1 gene showed there were two genetic variants within the gene’s promoter area. The first variant was, 8 repeats of TA copies was observed in the sequence of the gene of all patients samples; the results were directly compared with the Iraqi healthy control group in which it's clear that 7 TA repeats were detected in this region as shown in table 4; also it is compared databases at www.ncbi.nlm.nih.gov by the BLAST check-up tool and besides using Genus software program figure (3-a,b). Also, there is the second variant in the promoter region of UGT1A1 region of the gene was detected in which among the thirty Gilbert Syndrome patients, there are one SNP detected in the promoter region, which is A substitution with G in site -208, as shown in table 4, and the number of patients who had this polymorphism (A instead of G) was 18 out of 30 patients (60 %) in comparison with that of healthy individuals included in this study, while the other 12 patients (40%) no variation appeared in this studied part of the gene, might have in another coding region of UGT1A1 gene, as shown in figure (4-a,b).

The outcomes of PCR analysis of UGT1A1 (-208 A/G) polymorphism subdivided GS patients into three genotypes groups: the first group is homozygous wild type (AA) in which no variation appeared when they have two identical alleles for a gene, the second group is homozygous mutant (GG), and the third group is heterozygous (AG) having two different alleles for a gene one dominant and one recessive, this is a heterozygous mutation, As a result of this observation, AG genotype may be considered as a risk factor, while GG genotype appeared only in 8 patients out of 30 patients (26.6%) when they harbored two AA/GG variations and cannot be considered as a protective factor for Gilbert syndrome in the Iraqi population examined. This observation indicated that these genotypes are associated with Gilbert’s genetic predisposition. The percentage of genetic variation of the promoter region of UGT1A1 (-208 A/G) was illustrated in Table 5.
In this study, the distribution of patients with GS according to gender showed higher rates of infection in males (25 out of 30) (83.33%) than females (5 out of 30) (16.6%), when this results supported by the results of Sieg A. et al. (1987). who found that males are more frequently affected than females, this might be clarified by the existence of a high bilirubin load per kilogram body weight in males, also may the androgen steroid inhibition of bilirubin enzymatic glucuronidation, Bosma PJ, et al. (1995).20,21.

The promoter area of the UGT1A1 gene in Gilbert syndrome patients compared to control samples (healthy individuals) showed two types of variation; this is agreed with Canu. et al. 2013, showed that more than 130 variations in both regulatory and coding regions of UGT1A1 had been recognized in hereditary hyperbilirubinemia patients22. the first one was the repeats of (TA) copies in promoter region appeared in all patients in which it repeated 8 times in sequences of patients while 7 times repeated in the sequence of the control group.

Bosma et al., 1995; Monaghan et al., 1996; Beutler et al., 1998; Biondi et al., 1999; Kadakol et al., 2000; Farheen et al., 2006)14-19, who found that the determined mutual UGT1A1 genotype accountable intended for Gilbert’s syndrome, in which the homozygous polymorphism A(TA)7 TAA found within promoter area of the gene, make a 70-80% decrease in glucuronidation activity, when they repeated 7 times in a sequence of patients while 6 times in control healthy group, while in our Iraqi patients it repeats 8 times in compared with the sequence of healthy patients, it repeats only 7 times, (rs 3064744).

The second variation in the promoter area of the UGT1A1 gene show one SNP in 18 patients, A substitution with G in -308 site in the promoter region of the gene indicate that these mutations show a part in this disease in which these polymorphisms modify the expression that altered transcription factor gene binding. Genetic factors are considered as an essential factor for the disease, and it’s clear that the variation in the promoter area of the gene contributes to GS disease, in which the promoter area controls the manufacture of the bilirubin-UGT enzyme.

Results were the same in the study Xiao-xiao Mi ., 2019, which detected the association between Gilbert syndrome and UGT1A1 G/C-46 SNP (rs873478) in the proximal promoter region gene and Gilbert syndrome.

Besides, homozygous genotypes (AA/GG) in the promoter region were more frequent in the patients than in the controls. These results disagree with another study in which they found this variation in exon 1 while in our study, one heterozygous SNPs were detected (AA/AG) in eight Gilbert syndrome samples.

Our finding agreed with Xiao-xiao Mi et al., 201913, who screened many coding areas of the UGT1A1 gene in 60 Gilbert syndrome patients, who noted proximal promoter area of UGT1A1 variation in 85% of patients, but no mutations were identified (Glasow et al., 2001). New genome association studies exposed that serum bilirubin is related to a genetic variation of the UGT1A1 locus9.

Changes in the UGT1A1 gene either decrease the affinity of UGT1A1 toward bilirubin or reduce enzyme activity10. The patients may have changes in another coding region of the UGT1A1 gene.

Single nucleotide polymorphisms (SNPs) were the most common variation in a specific gene that influences how a person responds to the environmental factor, which may alter the disease risk23.
Figure 3. A-Alignment demonstrates promoter area of UGT1A1 gene a- control, B- Gilbert syndrome using a sequencer analyzed by BLAST tool. Query number represented the present data, whereas the subject represented the reference gene sequence.
position to higher levels of bilirubin in the Iraqi population, thereby leading to increased susceptibility to Gilbert syndrome. In conclusion, the UGT1A1 gene mutation was a possible risk factor for Gilbert syndrome in Iraq.

Author Contributions
Conceptualization, software, investigation, methodology, data duration, funding acquisition Marwa A. Kubba; validation, formal analysis, resources, visualization, Abeer Ali Marhoon; Writing—original draft preparation, writing—review and editing, Supervision, project administration, Rafed Abbas Kadhum. All authors have read and agreed to the published version of the manuscript.

Acknowledgment
I am sincerely thankful to Al-Nahrain University, College of Biotechnology for providing me with the opportunity to make paper; I am also thankful to Dr. Sahar M. Hussein for guiding us in many stages during our work.

Funding
This research received no external funding.

Ethical Clearance
The ethical research committee at scientific research by ethical approval of both environment and health and higher education and scientific research ministries in Iraq

Conflicts of Interest
The authors declare that they have no conflict of interest.

Informed Consent Statement
Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Bibliographic references
13. Xiao-xiao Mi, Jian Yan, Xiao-jie Ma, Ge-li Zhu, Yi-dan Gao,2


Study single nucleotide polymorphism in Promoter region of UGT1A1 Gene in Iraqi Patients with Gilbert’s syndrome