Clinical Variability among Patients with Gilbert Syndrome

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Abstract

Introduction: Gilbert syndrome (GS) is due to a defect in uridine diphosphate glucuronosyl transferase (UGT1A1) gene and belongs to the group of the most common human metabolic disorders and is characterized by an elevated level of bilirubin in blood serum. Genotyping functional polymorphisms in UGT1A1 gene is an important step in the determination of the etiology of free hyperbilirubinemia of unknown origin. Herein, we aimed to explain the genetic profile and the clinical variability of this disease in Tunisian patients.

Material and methods: We explored a total of 30 subjects including 7 unrelated isolated cases and 23 subjects distributed to 7 families. The exploration of these subjects included the genotyping of two Functional polymorphisms of UGT1A1 gene associated with GS namely: A(TA)nTAA and G71R, the bilirubin level, the hematological parameters, the determination of glucose-6-phosphate dehydrogenase activity (G6PD) and echo dense images within the gall bladder with acoustic shadowing or gravitational change in position.

Results: The exploration of the hematological parameters showed that all subjects enrolled in this study are out of hemolytic disease with the exception of two individuals, it is a father and his son who have a profile of a β thalassemia minor. The exploration of G6PD activity showed that all patients presented normal values. All patients presented hyperbilirubinemia among whom some patients presented benign asymptomatic jaundice, two patients presented kernicterus and 4 patients presented cholelithiasis. The genetic profile studied of UGT1A1 revealed that the mutant allele A of the G71R polymorphism was absent for all subjects and that the genotype (TA)7/(TA)7 was associated with hyperbilirubinemia and with GS.

Keywords: Gilbert syndrome; UGT1A1 polymorphism; Clinical variability

Abbreviations:

GS: Gilbert Syndrome; G6PD: Glucose-6-phosphate Dehydrogenase; SCA: Sickle Cell Anemia; UGT1A1: Uridine Diphosphate Glucuronosyl Transferase

Introduction

The protein produced from the UGT1A1 gene, called the bilirubin uridine diphosphate glucuronosyl transferase, is the only enzyme that glucuronidates bilirubin (bilirubin-UGT). This enzyme converts the toxic form of bilirubin (unconjugated bilirubin) to its nontoxic form (conjugated bilirubin), making it able to be dissolved and removed from the body [1]. The bilirubin-UGT enzyme is primarily found in cells of the liver, where bilirubin glucuronidation takes place. Conjugated bilirubin is dissolved in bile, a fluid produced in the liver, and excreted with solid waste [2]. Changes in the UGT1A1 gene can cause GS. This condition is characterized by periods of mild unconjugated hyperbilirubinemia, which rarely leads to episodes of jaundice [3-6]. GS occurs worldwide, but some mutations are seen more often in particular populations. In many populations, the most common genetic change that causes Gilbert syndrome occurs in an area near the UGT1A1 gene called the promoter region, which controls the production of the bilirubin-UGT enzyme. This change must occur in both copies of the UGT1A1 gene to cause GS. The common genetic change involved in GS, called UGT1A1*28, results from the addition of two DNA building blocks (nucleotides) to an important sequence in the promoter region known as the TATA box [7,8]. The normal UGT1A1 TATA box sequence is written as A(TA)6TAA [6]. Moreover, three mutant allelic forms exist depending on the number of (TA): UGT1A1*36 (n=5), UGT1A1*28 (n=7) and UGT1A1*37 (n=8). The UGT1A1*28 change, however, is uncommon in Asian populations. Asians with GS often have a mutation in one copy of the UGT1A1 gene that results in the change of a single protein building block (amino acid) in the bilirubin-UGT enzyme. The most common mutation in this population is a restriction polymorphism at exon 1 G211A replaces the amino acid glycine with the amino acid arginine at position 71 of the enzyme (written as Gly71Arg or G71R) [5]. This type of mutation, known as a missense mutation, results in reduced enzyme function. People with Gilbert syndrome have approximately 30 percent of normal bilirubin-UGT enzyme function [9,10]. As a result, unconjugated bilirubin is not glucuronidated quickly enough, and it builds up in the body, causing mild hyperbilirubinemia. Symptoms can range from clinical jaundice to nausea, malaise and discomfort in the right hypochondrium or even abdominal pain.

In a previous studies we have reported the implication of the gilbert mutation in cholelithiasis among patients without hemolytic disease and among patients with sickle cell anemia [11,12]. Our findings have been similar to further studies which have been reported the role of gilbert mutation in cholelithiasis.
Herein, we aimed to determine the genetic profile of GS among Tunisian patients and to discuss the clinical variability going of the asymptomatic in kernicterus.

Material and Methods

Materials

We explored a total of 30 subjects including 7 unrelated isolated cases and 23 subjects distributed to 7 families with persistent indirect hyperbilirubinemia serum bilirubin levels and diagnosed as affected by Gilbert syndrome. Table 1 summarizes the main characteristics of the studied population.

| Number | Patients jaundice | Patients without jaundice | p |
|--------|------------------|---------------------------|---|
| Age (range) | 2-45 | 3-45 | NS |
| Sex ratio (M/F) | 12/5 | 7/6 | NS |
| Hb (g/dL) | 12.3 ± 0.9 | 12.9 ± 1.3 | NS |
| RBC (10^12/L) | 4.89 ± 1.02 | 4.29 ± 0.9 | NS |
| MCV (fl) | 74.2 ± 1.3 | 79.7 ± 0.9 | NS |
| MCH (pg) | 35.7 ± 1.02 | 34.9 ± 2.1 | NS |
| RDW (%) | 5.29 ± 1.02 | 4.83 ± 0.5 | NS |
| HbA | 97 | 97 | NS |
| HbA2 | 3 ± 0.1 | 3 ± 0.2 | NS |
| G6PD values (U/gHb) | 6.86 ± 1.01 | 6.54 ± 0.9 | NS |
| Total bilirubin level (µmol/L) | 90 ± 45 | 20 ± 2 | 0.01 |
| Unconjugated bilirubin level (µmol/L) | 70 ± | 10 ± 2.2 | 0.006 |
| Conjugated bilirubin level (µmol/L) | 20 ± | 10 ± 1.8 | 0.04 |
| Cholelithiasis | 2 | 0 | 0.05 |
| Kernicterus | 3 | 0 | 0.045 |

Table 1: Repartition of hematological and demographic and clinical data according to the presence or the absence of jaundice

Methods

For each patient we explored different parameters including:

Definition of clinical events, hematological data, hemoglobin profile, biochemical parameters, G6PD dosage and genotyping of UGT1A1 polymorphism.

Definition of clinical events

Cholelithiasis was diagnosed on the basis of echodense images within the gall bladder with acoustic shadowing or gravitational change in position. Kernicterus was diagnosed on the basis of MRI and physical examinations.

Laboratory methods

After consent of each patient venous blood samples of 2.5 ml volume were drawn from the study subjects and were collected in K2-EDTA anticoagulant containers. The complete blood counts including counts of red blood cells (RBC), white blood cells (WBC), and the measurement of hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW) were performed using a an automated cell counter (ABX pentra 60c+). Diagnosis of hemoglobinopathy was performed using cation-exchange high performance liquid chromatography (HPLC) (D10 Biodar) Total unconjugated and conjugated bilirubin concentrations were determined in serum by a standardized colorimetric procedure using automate Konelab 20. The determination of G6PD activity was performed using spectrophotometric method as described by Bendaoa et al. [13].

A(TA)nTAA and G71R genotyping

After consent, 10 ml of EDTA blood were collected from each volunteer. The DNA was extracted by the standard phenol / chloroform method from peripheral blood leukocytes. We used PCR / sequencing for the study of microsatellite polymorphism A(TA)nTAA and G71R. The PCR reaction was performed in a reaction volume of 25 µl containing 150 ng of genomic DNA, 0.2 mM dNTP, 5 pmol of each primer ([TA] F: TAACTTGTTATCGATTG and (TA) R: CTTTGCTCCTGCCAGGTT), 1 unit of Taq DNA polymerase (Invitrogen, 5 U/µl) and 2.5 µl of 10X PCR buffer in the presence of 1 µl of 50 mM of MgCl2. PCR products were purified by the enzymatic method with exonuclease I and Shrimp Alkaline Phosphatase (SAP) and subsequently sequenced using an automated sequencer (310 DNA sequencer ABI PRISM PE Applied Biosystems, Foster City, United States).

Statistical analysis

The demographic and hematologic data are normally distributed, so we used means and standard deviations. The bilirubin data are not normally distributed, so we used medians. The two polymorphisms studied were tested for deviation from the Hardy-Weinberg equilibrium using the software package Arlequin (version 3.01). Chi Square test or fisher test was used to determine genetic differences between patients using compare 2(version 1.02)

Results

As for the hemoglobin exploration the results show that all individuals implicated in this study have a normal hemoglobin profile (HbA with a percentage equal to 97%, HbA2 with a percentage equal to 3%). With the exception of two subjects, it is a father and his son who have a profile of a β thalassemia minor: HbA with a percentage equal to 94%, HbA2 with a percentage equal to 6%. This phenotype was confirmed by molecular study of β globin gene (data not shown). These two individuals have the mutation C/T codon39 in the
heterozygous state. The G6PD assay revealed normal values for all individuals studied. The hematological analysis based on complete blood counts (CBC) revealed that all individuals have normal hematological parameters except for two individuals who have thalassemia microcytosis. Biochemical analysis showed that the values of total bilirubin ranging from 10 μM to 246 μM. Knowing that the maximum limit of the normal values of total bilirubin corresponds to 17 μM, our sample was classified into two groups according to two ranges of values of total bilirubin: the first includes the following values A: (10 μM - 17 μM), the second corresponds to the following values B: (17 μM - 246 μM). The 13 non-icteric individuals are classified in the interval A. 17 jaundiced individuals are classified in the interval B.

The exploration of the polymorphism A(TA)nTAA revealed the presence of five genotypes. In our sample we counted four individuals (TA)5/(TA)7, 1 (TA)6/(TA)5, 1 (TA)5/(TA)5, 14 (TA)5/(TA)7 and 10 (TA)7/(TA)7 (genotype associated with Gilbert’s syndrome). Genotypic comparison between patients in the two intervals of total bilirubin showed a statistically significant association between genotype (TA)7/(TA)7 and hyperbilirubinemia. In addition, patients who have the genotype (TA)7/(TA)7 have variable depending on the extent of jaundice hyperbilirubinemia.

The genotypic and allelic comparison between patients according to the presence or absence of jaundice showed a statistically significant association between genotype (TA)7/(TA)7 and jaundice. Among patients with jaundice 2 have nuclear jaundice and 3 have cholelithiasis. The exploration of genetic profile of UGT1A1 polymorphisms studied, our results show the association of (TA)7/(TA)7 with hyperbilirubinemia, jaundice, cholelithiasis and kernicterus. The exploration of G71R polymorphism showed the absence of mutant allele in the studied sample. The homozygous (TA)7/(TA)7 has a meaning because of its relationship to the onset of Gilbert. Indeed, 17% of Gilbert’s disease appears to be associated with a homozygous (TA)7 in various populations: Caucasian, African American, and Japanese [19-23]. In asian population the GS is associated with G71R [9].

### Table 2: Repartition of different genotypes depending two intervals representing bilirubin level

| A(TA)nTAA genotypes | A | B | p  |
|----------------------|---|---|----|
| (TA)5/(TA)5          | 1 | 0 |   |
| (TA)6/(TA)5          | 1 | 0 |   |
| (TA)5/(TA)7          | 4 | 0 | 1*|
| (TA)7/(TA)7          | 7 | 5 | 0.082|
| (TA)n/(TA)7          | 0 | 12| 10^-3|
| Total                | 13| 17|    |

A: Total bilirubin level comprising between 10 μM and 17 μM. B: Total bilirubin level comprising between 17 μM and 246 μM. Usual value of bilirubin level: Total bilirubin <17 μmol/l. 1*: reference group. P: index of significance, each p<0.05 is considered as significant.

### Discussion

Polymorphism A(TA)nTAA has been explored in different populations indicating its association with hyperbilirubinemia [14,15] and the appearance of pigment stones [16-18]. In the Tunisian population we have been reported previously that subjects carrying (TA)7 or (TA)5 variant in their genotypes are associated with high bilirubin level and with cholelithiasis [11,12]. Furthermore, the comparison between patients and controls according to A(TA)nTAA variation demonstrated that (TA)5/(TA)7 and (TA)7/(TA)7 genotype and (TA)7/(TA)7 and (TA)5 alleles were significantly associated with an increased risk of gallstone diseases p=0.0017, p= 6.1 10^-6, p=1.5 10^-6 and p=0.025 respectively [5] (Tables 2 and 3).

We also performed all clinical laboratory tests ordered that we considered relevant to evaluating whether hemolysis contributed to the hyperbilirubinemia. Our results showed that all patients included in this study are out of hemolytic disease. The G6PD assay revealed normal values for all individuals studied. Of the 30 subjects studied 17 had hyperbilirubinemia and jaundice, 13 presented normal values of bilirubin. Among patients with jaundice 2 have nuclear jaundice and 3 have cholelithiasis. As for the genetic profile of UGT1A1 polymorphisms studied, our results show the association of (TA)7/(TA)7 with hyperbilirubinemia, jaundice, cholelithiasis and kernicterus. The exploration of G71R polymorphism showed the absence of mutant allele in the studied sample. The homozygous (TA)7/(TA)7 has a meaning because of its relationship to the onset of Gilbert. Indeed, 17% of Gilbert’s disease appears to be associated with a homozygous (TA)7 in various populations: Caucasian, African American, and Japanese [19-23]. In asian population the GS is associated with G71R [9].
strategies in all of healthcare, resulting in a healthy baby rather than a child with a life-time of debilitating neurodevelopment handicaps.

|            | Absence | Presence | p     |
|------------|---------|----------|-------|
| (TA)₆/(TA)₈ | 4       | 0        | 1*    |
| (TA)₆/(TA)₉ | 1       | 0        | 1     |
| (TA)₇/(TA)₇ | 1       | 0        |       |
| (TA)₉/(TA)₇ | 9       | 5        | 0.119 |
| (TA)₁₀/(TA)₇| 0       | 12       | 10⁻³  |

1*: reference group.
P: index of significance, each p<0.05 is considered as significant.

Table 3: Repartition of different genotypes depending the presence or the absence of jaundice.

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