

Wayward Effect of Polymorphism (TA)₈ in the Promoter Region of *UGT1A1* Gene in a Mexican Family

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ABSTRACT

Gilbert syndrome (GS) is a hereditary relatively common benign unconjugated hyperbilirubinaemia. The promoter region of uridine diphosphate glycosyltransferase 1 (UGT1A1) gene contains a normal A (TA)₆TAA element; variations in this motif (A(TA)_{7/8}TAA) are generally associated with this disorder. This is a report of the varied effects of GS in a Mexican Mestizo family with a non-common (TA)₈ repeat in this population. The proposita and her mother showed (TA)₇/(TA)₈ genotype, while her father and sister were (TA)₆/(TA)₇, but only the proposita showed clinical manifestations. This report supports that the (TA)₇ and (TA)₈ are necessary, but not enough to explain the features of GS. There are probably additional genetic variations ie, the presence of "modifier" genes or one can speculate that an oligogenetic trait can contribute to the expression of the final phenotype.

Keywords: Gilbert syndrome, hyperbilirubinaemia, UGT1A1, microsatellite

Efecto Caprichoso del Polimorfismo (TA)₈ en la Región del Promotor del Gene *UGT1A1* en una Familia Mexicana

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RESUMEN

El síndrome de Gilbert (SG) es un hiperbilirubinemia no conjugada, benigna, relativamente común y hereditaria. La región promotora del gen (UGT1A1) de la uridina difosfato glicosiltransferasa 1, contiene un elemento normal A (TA)₆ TAA. Las variaciones en este motivo (A (TA)_{7/8}TAA) se encuentran por lo general asociadas con este desorden. Éste es un reporte de los variados efectos del SG en una familia mestiza mexicana con una repetición (TA)₈ no común en esta población. La probando y su madre mostraron el genotipo (TA)₇/(TA)₈, mientras su padre y hermana eran (TA)₆/(TA)₇, pero sólo la probando mostró manifestaciones clínicas. Este informe sostiene que el (TA)₇ y (TA)₈ son necesarios, pero no suficientes para explicar los rasgos del SG. Probablemente hay variaciones genéticas adicionales, es decir, la presencia de genes "modificadores", o se puede especular que un rasgo oligogenético puede contribuir a la expresión del fenotipo final.

Palabras claves: Síndrome de Gilbert, hiperbilirubinemia, microsatélite, UGT1A1

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INTRODUCTION

Gilbert syndrome (GS) [OMIM #143500], a benign condition in which unconjugated hyperbilirubinaemia occurs without structural liver disease or overt haemolysis (1, 2), is characterized by episodes of mild intermittent jaundice that tends to fluctuate in severity, particularly after fasting (3). A bilirubin concentration range between 17 and 102 $\mu\text{mol/L}$ [1–6 mg/dL] is generally accepted in GS (4).

Gilbert syndrome has an unclear inheritance pattern, since many people do not have a clear family history, mainly because the syndrome often remains undiagnosed. Powell *et al* proposed an autosomal dominant inheritance with incomplete penetrance, and a recessive form has been suggested by Bosma *et al* (5, 6). The molecular mechanism in GS involves a polymorphism in the promoter of the *UGT1A1* gene (Uridine Diphosphate Glycosyltransferase 1 Family, Polypeptide A1; OMIM *191740); this region contains a normal run of six thymine-adenine repeats A(TA)₆TAA, localized at -53 to -38 upstream. Homozygosity of two extra TA nucleotides interferes with binding of the transcription factor IID and results in a decrease of the glucuronosyltransferase-1 activity up to about 30% of normal values and therefore higher mean serum bilirubin levels (6, 7).

CASE REPORT

A 19-year old girl was referred to our department with a probable diagnosis of GS. Neither scleral icterus, jaundice, fever or other evidence of gallbladder disease were observed on clinical evaluation, but ultrasound examination showed mild liver enlargement. The laboratory test revealed low-grade unconjugated hyperbilirubinemia (0.90 mg/dL), and increased levels of transaminases (TGO 70 U/ml, TGP 142 U/ml). Her apparently healthy father, mother and sister had normal indirect bilirubin levels (0.5, 0.40 and 0.30 mg/dL, respectively).

Molecular analysis of the TATA-box region of the *UGT1A1* gene was performed by polymerase chain reaction (PCR), according to Bosma *et al*. The amplified fragment was purified and directly sequenced with an ABI PRISM 310 PE Applied Biosystem analyser and the Big Dye Terminator v 3.0 cycle sequencing ready reaction kit (6).

Three different alleles were identified (TA)₆, (TA)₇, and (TA)₈. The probanda and her mother were compound heterozygotes (TA)₇/(TA)₈, the genotypes of both father and sister were (TA)₆/(TA)₇.

Bilirubin is usually measured as part of a panel of liver function tests, such as liver alkaline phosphatase and liver transaminases. In patients with liver disease, abnormal levels of these enzymes are often combined with increased values of serum bilirubin (8).

The TATA box at the promoter region of the *UGT1A1* gene has been widely studied; variation of these motifs [(TA)₇ and (TA)₈] has been associated with low UDP-glucuronosyltransferase-1 enzymatic activity (9). Some individuals with the (TA)₇/(TA)₈ genotype have been described and only few of them display the clinical features of GS (10, 11), suggesting

that environmental and additional genetic factors play a role in the marked inter-individual phenotypic variability (12). Previous reports, based on population studies, have shown increased bilirubin levels after a low caloric diet (13) with significant gender variations higher in men (0.72 $\pm$ 0.004 mg/dL) than in women (0.52 $\pm$ 0.003 mg/dL), and smoking status with reduced bilirubin levels in active smokers (14). In a Japanese population, a substitution at -3279 T $\rightarrow$ G, in the phenobarbital-responsive enhancer module of *UGT1A1*, reduces its transcriptional activity and is associated with increased levels of bilirubin in plasma (15).

Besides the familiar A(TA)_nTAA polymorphism, the insertion in the CAAT box of *UGT1A1* promoter (nucleotide positions -85 to -83) reduces the gene transcription. Normally, there is one copy of the CAT trinucleotide in the promoter of the *UGT1A1* gene, the addition of an extra CAT element has shown a significantly elevated unconjugated bilirubin level (6.13 $\pm$ 1.61 mg/dL), when compared to those without the insertion (2.93 $\pm$ 0.28 mg/dL). This insertion produces structural changes in the DNA-folding of the promoter region, which leads to a 20-fold decrease in transcription efficiency, compared to a two-fold decrease for the (TA)₇TAA variant (16).

The allele frequencies for (TA)₆ and (TA)₇ repeats in Mexican Mestizos is 0.654 and 0.334, respectively (17), such frequencies are similar to those reported in several populations of European origin (6, 9, 18). However, the (TA)₇ allele is more common among people of African origin than in Caucasians (9). On the other hand, the (TA)₈ allele in Caucasian populations is extremely rare, but in the African population the (TA)₈ allele reaches a frequency of 0.069 (9).

Since the Mexican Mestizo population has a complex process of racial admixture, mainly between Amerindians (69%) and Caucasians (26%) and to a lesser extent Africans [5%] (19), the presence of (TA)₈ allele could be explained by this genetic admixture. Nevertheless, the hypothesis of a new mutation resulting from a recent genetic event, as suggested by Tsezou *et al*, cannot be ruled out. Repeated sequences are extremely unstable and may be modified as a result of unequal crossing-over in meiosis or a polymerase template slippage. This latter explanation is the primary mutational mechanism leading to changes in microsatellite length, which has been shown to be higher in dinucleotide than in trinucleotide repeats, by a factor of 2.2, and much higher than the tetranucleotide repeats rate by a factor of 9.2 (20, 21). It seems reasonable therefore that the (TA)₈ repeat pattern can be found.

The mild hyperbilirubinaemia in the probanda, as well as the lack of clinical features in her mother, support the idea that (TA)₇/(TA)₈ insertion would only give a proclivity to develop jaundice, but it is not enough to display the clinical features described in GS. Although we do not have enough elements to explain this finding, we speculate that known additional genetic variations within the *UGT1A1* gene (15, 16, 22, 23) and/or in other genes involved in bilirubin metabolism, like the organic anion transporter polypeptide 1B1 [OATP1B1] (24), in addition to age, gender, co-morbidities and/or envi-

ronmental factors are necessary to develop the clinical and biochemical features. But another possibility to consider is that GS is behaving as an oligogenic trait. The unclearness of its inheritance pattern may be explained by different reasons, such as undiagnosed cases and absence of clearer family histories.

Further studies are required to assess the molecular mechanism(s) leading to GS, as well as the functional role of each one of the above mentioned elements that contribute to the syndrome, contributing to a better understanding of bilirubin metabolism.

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