Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 42 (2009) 1635–1641
CLINICAL BIOCHEMISTRY

Haplotypes in the *UGT1A1* gene and their role as genetic determinants of bilirubin concentration in healthy German volunteers

Katrin Borucki [a], Cornelia Weikert [b,d], Eva Fisher [b], Sibylle Jakubiczka [c], Claus Luley [a], Sabine Westphal [a], Jutta Dierkes [a,*]

[a] *Institute of Clinical Chemistry, Medical Faculty University Magdeburg, Germany*
[b] *Department of Epidemiology, German Institute of Human Nutrition, Nuthetal, Germany*
[c] *Institute of Medical Genetics, Medical Faculty University Magdeburg, Germany*
[d] *Institute for Social Medicine, Epidemiology, and Health Economics, Charité University Medicine Berlin, Germany*

Received 11 May 2009; received in revised form 20 August 2009; accepted 22 August 2009
Available online 2 September 2009

Abstract

Background: Genetic variations of UDP-glucuronyltransferase 1A1 (UGT1A1) influence the concentration of serum bilirubin. We investigated the association of four common polymorphisms including UGT1A1-53$(TA)_n$, and common haplotypes of the *UGT1A1* gene with bilirubin levels in 218 Caucasian volunteers.

Methods: Total bilirubin was measured in serum of 218 healthy Caucasian volunteers. Genotyping of four genetic variants was performed: UGT1A1-53$(TA)_{6/7}$, UGT1A1c.-3279T>G, UGT1A1c.-3156G>A, and UGT1A1c.211G>A. The association between polymorphisms/haplotypes and bilirubin levels were determined.

Results: Minor allele frequencies were 0.36 for UGT1A1-53$(TA)_7$, 0.47 for c.-3279G, 0.33 for c.-3156A and 0.006 for c.211A. The three promoter polymorphisms were in close linkage disequilibrium. Common haplotypes were: -53$(TA)_6$/c.-3279T/c.211G (frequency 0.530), -53$(TA)_7$/c.-3279G/c.211G (frequency 0.365), and -53$(TA)_6$/c.-3279G/c.211G (frequency 0.099). Male sex, UGT1A1-53$(TA)_{6/7}$ and the c.-3279GG variant were significantly associated with higher bilirubin concentrations.

Conclusions: Two UGT1A1 promoter polymorphisms (-53$(TA)_{6/7}$ and c.-3279T>G) and a common haplotype of the *UGT1A1* gene are associated with serum bilirubin concentrations in Caucasians.

Keywords: UDP-glucuronosyltransferases; Haplotypes; Bilirubin; Caucasians

Introduction

UDP-glucuronosyltransferases (UGT) catalyze the conjugation of the glucuronyl group from uridine 5-diphosphoglucuronic acid with endogenous and exogenous substrates. The resulting glucuronide products are more polar, less toxic, and more easily excreted. One prominent endogenous substrate is bilirubin, derived from the catabolism of hemoglobin. The UGTs are a superfamily of phase II biotransformation enzymes, which in humans are divided into two families: the gene encoding UGT1A protein family is located on chromosome 2, while the gene encoding UGT2B is located on chromosome 4 [1,2]. The proteins of the UGT1A family share four common exons (exons 2–5), but differ in exon 1 and the promoter region of the gene. Several polymorphisms have been reported, especially in the promoter region and in exon 1, which occur with highly variable frequencies in different populations and regions of the world. Some of them are highly relevant as they exert a significant effect on enzyme function [1,3].

Bilirubin is a non-polar metabolite formed in the catabolism of hemoglobin, which is bound to glucuronic acid in the liver by the UGT1A1 enzyme to form so-called conjugated bilirubin.

* Corresponding author. Institute of Clinical Chemistry, Leipziger Str. 44, D-39120 Magdeburg, Germany. Fax: +49 391 6713902.
E-mail address: jutta.dierkes@med.ovgu.de (J. Dierkes).

0009-9120/$ - see front matter © 2009 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2009.08.011

Unconjugated hyperbilirubinemia occurs if the activity of the hepatic UGT1A1 enzyme is limited or absent. If the enzyme activity is severely affected, this results in the Crigler–Najjar syndrome type I (OMIM 218800) and type II (OMIM 606785). Gilbert's syndrome (OMIM 143500) is a benign hyperbilirubinemia caused by reduced enzyme activity. A tandem repeat in the 5′ promoter region (UGT1A1-53 $(TA)_n$) has been identified as a main cause of Gilbert's syndrome [4]. Usually, six copies of this tandem repeat are present and are associated with normal enzyme activity, thus representing the wild type enzyme. Fewer than six copies are rare in Caucasian populations and are associated with increased enzyme activity, while more than six copies are associated with reduced enzyme activity. The frequency of an allele with seven copies is about 0.35 in Caucasians, leading to a homozygous genotype in about 10% of the population. More than seven copies of the TA repeat occur only occasionally in Caucasians. Heterozygous subjects usually have normal or slightly elevated bilirubin levels [4–6].

The frequency distribution of this polymorphism is highly variable in different ethnicities with an allele frequency of the $(TA)_7$ allele equal to about 0.10 in Asians, and one of about 0.45 in African Americans [7,8].

The association between the (UGT1A1-53 $(TA)_n$ variant and Gilbert's syndrome was identified in 1995 by Bosma and colleagues [4]. Since then, numerous studies investigated the association between this variant and hyperbilirubinemia [9,10]. It has thus been found that this variant is not the only common genetic variant causing hyperbilirubinemia and that not all cases of hyperbilirubinemia are caused by this variant [11–13].

As a result of many investigations carried out during the last few years, it has appeared that this gene variation occurs in linkage disequilibrium with other variations in the promoter region (c.-3156G>A or c.-3279T>G). It has been suggested that the c.-3156G>A polymorphism is also involved in toxicity of irinotecan [14]. The c.-3279T>G polymorphism is located in a phenobarbital-responsive enhancer module of the promoter and it has also been suggested that it is associated with reduced enzyme activity [11] and involved into irinotecan toxicity [15]. A polymorphism in exon 1, (c.211G>A) causing an amino acid change in the protein (G71R), is a genetic cause of hyperbilirubinemia in Asian populations [16,17], but the frequency of this variant in Caucasian populations appears to be low [18].

The combined effects of these polymorphisms on bilirubin concentrations in healthy subjects have not been studied systematically. There is also a lack of information on UGT1A1 haplotypes. We therefore studied the common polymorphisms in the *UGT1A1* gene in 343 healthy Caucasian Germans and their effects on bilirubin levels.

Patients and methods

Recruitment of patients

Participants were recruited by advertisements on the campus of the School of Medicine of Otto-von-Guericke University in Magdeburg and during lectures. Participation was voluntary and was not associated with attendance in courses, lectures or examinations. The advertisements invited students to take part in a screening procedure for a clinical trial which would be limited to subjects with a -53 $(TA)_{7/7}$ genotype. Thus, it cannot be ruled out that subjects who felt anxious about their bilirubin levels (e.g. those with "yellow eyes") are over-represented in the sample. However, it was not the aim to arrive at a representative sample but to collect as many healthy subjects as possible.

The criteria for inclusion in the screening procedure were as follows: (1) written informed consent to testing of the variants of the *UGT1A1* gene, (2) good health and (3) not taking any drugs or medications and reporting a low or moderate alcohol consumption. Volunteers were only included after signing informed consent for genetic testing and if they did not use any medication (except for contraceptives) and if there was no doubt on their reported alcohol consumption. Furthermore, we included only volunteers of Caucasian ancestry.

For one part of the study, the screening procedure involved the collection of a venous blood sample during an arranged visit to the Lipid Clinic of the Institute in order to obtain blood cells for DNA analysis and serum for bilirubin measurements. This visit was usually in the morning between 7 and 10 a.m. but did not require that the person was fasting.

To increase the effectiveness of the screening procedure a number of subjects were also screened without an appointment. These subjects gave only their written consent, provided a sample of buccal cells, and filled in a health questionnaire. This non-invasive procedure was also carried out in the Lipid Clinic but without appointments.

In total, 343 healthy adult volunteers of Caucasian descent were included (mean age 25.3 ± 6.2 years, 193 women and 150 men), recruited between May 2004 and February 2008. Bilirubin was determined in a subcohort ($n=218$) due to the availability of serum (see above).

The study design and all procedures involving patients were first approved by the Ethics Committee of the School of Medicine of the University of Magdeburg. Each participant gave his or her written informed consent. All procedures were in accordance with the data protection laws and the declaration of Helsinki in its revised form. All data were analyzed after blinding and steps taken to ensure the subjects' anonymity.

Genetic and biochemical methods

DNA was isolated from white blood cells using the QIAamp DNA blood mini kit or from buccal cells using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

The following gene variations were analyzed: within the promoter sequence: UGT1A1-53$(TA)_{6/7}$, UGT1A1c.-3279T>G, UGT1A1c.-3156G>A, and within exon 1: UGT1A1c.211G>A. Our nomenclature follows the recommendations of Mackenzie [1,19].

For studying the polymorphisms DNA was amplified by polymerase chain reaction (Master Mix including Taq DNA Polymerase from Promega, Madison, USA) followed by genotype-determination by using in-house dHPLC methods