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Gilbert's syndrome and hyperbilirubinemia in protease inhibitor therapy – An extended haplotype of genetic variants increases risk in indinavir treatment☆

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***Background/Aims*: Gilbert's syndrome is a frequent genetic conjugation abnormality associated with adverse drug effects. Genetic *UDP glucuronosyltransferase (UGT)1A* gene variants can influence gene transcription, inducibility and glucuronidation activity. Protease inhibitors used in human immunodeficiency virus (HIV) infection and chronic viral hepatitis can inhibit UGTs. Indinavir (IDV) can lead to hyperbilirubinemia in Gilbert's syndrome (UGT1A1∗28), which does not explain interindividual severity differences and may thus involve additional *UGT1A* variants.**

***Methods*: One hundred and twenty-five HIV patients receiving IDV and 427 healthy blood donors were genotyped for the presence of UGT1A1∗28, UGT1A3 -66T/C, UGT1A7 -57T/G, UGT1A7$^{N129K/R131K}$ using Taqman 5′ nuclease assays.**

***Results*: Hyperbilirubinemia was observed in 42%. UGT1A1∗28 frequencies did not differ between HIV patients and controls but were significantly higher in hyperbilirubinemic patients. The frequency of homozygous carriers of the 4 *UGT1A* marker haplotype increased with hyperbilirubinemia affecting all patients with bilirubin levels >85 μmol/l.**

***Conclusions*: In IDV treatment the risk of severe hyperbilirubinemia is associated with genetic variants of the *UGT1A3* and *UGT1A7* genes in addition to Gilbert's syndrome (UGT1A1∗28). This haplotype is a useful predictor of protease inhibitor-induced side effects.**

***Keywords*: Protease inhibitor; Gilbert's syndrome; Pharmacogenetics; UGT1A1; Hyperbilirubinemia; Irinotecan; Indinavir**

1. Introduction

Gilbert's syndrome represents one of the most common reasons for hyperbilirubinemia in Caucasians. It is the result of a 70% reduction of *UDP-glucuronosyltransferase* (*UGT*) *1A1* gene transcription found in carriers of the UGT1A1∗28 variant, which is characterized by a $A(TA)_7TAA$ promoter sequence differing from the more prevalent $A(TA)_6TAA$ sequence that exhibits full transcriptional activity [1]. Although Gilbert's syndrome does not lead to progressive liver damage it

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has attracted considerable attention regarding the pharmacogenetics of drug metabolism [2–6], and as a risk factor for cancer [7–10]. Apart from being the only physiological UGT capable of bilirubin glucuronidation, UGT1A1 also catalyzes the glucuronidation of 2-hydroxy-estrone and estradiol, and a number of therapeutic drugs such as ethinylestradiol, gemfibrozil, metabolites of irinotecan, simvastatin and buprenorphine [11]. Furthermore, mutagenic xenobiotics such as *N*-hydroxy-2-amino-1-methyl-6-phenylimidazol[4,5-β]pyridine (PhIP) and benzo(α)pyrenes undergo conjugation and detoxification by UGT1A1 [12]. The glucuronidation of the irinotecan metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) has led to the establishment of a link between reduced UGT1A1 activity in Gilbert's syndrome (UGT1A1∗28) patients and SN-38 toxicity. Since not all patients with UGT1A1∗28 variants experienced toxicity reactions it has recently been suggested that a haplotype combining *UGT1A1* (Gilbert's syndrome: UGT1A1∗28) and UGT1A7 variants encoding UGT proteins that are both capable of SN-38 glucuronidation may more accurately reflect the toxicity risk [13]. The association of a haplotype of different UGT1A gene variants has also been reported as a risk factor for antiretroviral protease inhibitor-associated adverse drug reactions [4]. Protease inhibitors such as atazanavir (ATV) and indinavir (IDV) have been reported to lead to hyperbilirubinemia in a significant number of treated patients [6,14,15]. Based on these and other recent reports combinations of variants of *UGT1A* and other genes appear to represent pharmocogenetic risk factors for clinically observed adverse drug effects. Among these drugs are protease inhibitors, which are currently being studied as a novel therapeutic strategy in chronic viral hepatitis and thus are of considerable interest in hepatology [16].

The variable activity of human glucuronidation is the result of several features [17]. The human *UGT1A* gene locus is expressed in a tissue specific fashion [18–21] leading to differing overall glucuronidation activities in different organs and tissues [18,22]. UGT activity is inducible by xenobiotics and drugs [23–28], but inhibitory action by different drugs has also been shown [6,14,29]. Furthermore, genetic variation within the coding regions of a *UGT* gene can lead to lower or altered activity protein species [30–32], single nucleotide polymorphisms (SNPs) within promoter regions can affect transcriptional levels [1,25,33] as well as inducibility of *UGT* genes [25]. Genotyping studies demonstrate that many genetic variants of the *UGT1A* genes are found in linkage dysequilibrium with each other [30,31,34] leading to haplotypes which can include the variant present in the majority of Caucasian Gilbert's syndrome patients, UGT1A1∗28 [4,13].

In this study we analyzed hyperbilirubinemia in 125 human immunodeficiency virus (HIV) infected patients treated with IDV. We hypothesized that the risk of this adverse protease inhibitor effect may be associated with a more complex genotype of *UGT1A* SNPs than Gilbert's syndrome alone, which would confirm a genetic risk factor for the treatment with this class of drugs suggested in a previous report with the protease inhibitor atazanavir. We provide evidence for a risk haplotype including Gilbert's syndrome (UGT1A1∗28) combined with promoter and coding region variants of the *UGT1A3* and *UGT1A7* genes, suggesting that pharmacogenetic predisposition should be considered in drugs with a potential to inhibit detoxification by glucuronidation.

2. Materials and methods

2.1. Study population

2.1.1. Indinavir-treated patients with HIV infection

A total of 125 patients were recruited from the outpatient clinic of the Department of Clinical Immunology, Hannover Medical School, Germany and from the outpatient clinic of the Department of Internal Medicine, Bonn, Germany from March 1997 to May 2004. Blood samples were collected from patients receiving 3×800 mg of the protease-inhibitor indinavir or 2×800 mg IDV and 200 mg Ritonavir once daily for at least one month. Total bilirubin levels were measured before and after six weeks to six months after the beginning of treatment. Hyperbilirubinemia was graded according to the Division of AIDS (WHO) table for grading the severity of adverse events (total bilirubin levels): (WHO) grade 0 (normal) <19 μmol/l; grade 1 (mild), 19–26 μmol/l (1.1–1.5 × upper limit of normal (ULN)); grade 2 (moderate), 26–43 μmol/l (1.6–2.5 × ULN); grade 3 (severe), 43–85 μmol/l (2.6–5.0 × ULN); grade 4 (serious), ⩾85 μmol/l (⩾5.0 × ULN). Jaundice was defined as a total bilirubin level >43 μmol/l.

2.1.2. Healthy blood donors

Blood samples were obtained from a total of 427 anonymous healthy blood donors from the Department of Transfusion Medicine/Blood Bank of Hannover Medical School, Germany.

Informed consent was obtained from all patients and the study was approved by the Ethics Committee of Hannover Medical School.

2.2. Methods

2.2.1. Genomic DNA

Genomic DNA was isolated from full blood samples by the NucleoSpin Blood XL Kit according to the recommendations of the manufacturer (Machery & Nagel, Dueren, Germany).

2.2.2. Allelic discrimination genotyping of UGT1A7 −57T/G, UGT1A3 −66T/C, UGT1A7$^{N129K/R131K}$

Approximately 10 ng of genomic DNA were used as a template in Taqman 5′-nuclease assays. Primers and Probes specific for each SNP were designed with Primer Express software (Applied Biosystems) and labelled with either 6-FAM or VIC as reporter dyes and MGB-NFQ (Applied Biosystems) as a quencher as described previously [4]. The Taqman assays were performed using 600 nM primer concentrations and 200 nM probe concentrations (Applied Biosystems) and qPCR Mastermix Plus (Eurogentec, Seraing, Belgium). Each run consisted of a hot start at 95 °C for 10 min and 35 cycles of 94 °C for 15 s and 61 °C for 1 min. All assays were performed in 25 μl reactions in 96-well trays using an ABI 7000