The UGT1A1*28 gene variant predicts long-term mortality in patients undergoing coronary angiography

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Abstract

Background: Uridine diphosphate glycosyltransferases 1A1 (UGT1A1) plays an essential role in detoxification and excretion of several endogenous and exogenous compounds. A functional polymorphism in the promoter of the UGT1A1 gene (TA repeat insertion, UGT1A1*28, rs3064744) has been associated with reduced UGT1A1 enzyme activity. The purpose of the present study was to investigate the role of UGT1A1 genotypes in mortality.

Methods: UGT1A1 genotypes as well as baseline plasma bilirubin levels were analyzed in participants of the Ludwigshafen Risk and Cardiovascular Health study (n = 3316). UGT1A1*28 genotypes were determined on an ABI PRISM 3730 genetic analyzer.

Results: As expected, UGT1A1 genotypes were associated with baseline bilirubin levels (*1/*1 genotype: 9.1±4.6 µmol/L; *1/*28 genotype: 10.8±5.3; *28/*28: 16.9±9.2; p<0.001). During a median follow-up of 10.4 years, 995 subjects (30.0%) died. In a multivariate regression analysis adjusting for age, sex, smoking, type 2 diabetes, dyslipidemia, alanine aminotransferase (ALT) levels and bilirubin levels, the UGT1A1*28 variant predicted lower overall mortality (hazard ratio [HR], 0.86; 95% confidence interval [CI], 0.78–0.95; p=0.003). Contrary to expected, higher baseline bilirubin levels predicted increased mortality (HR, 1.014; 95% CI, 1.002–1.025; p=0.019).

Conclusions: The UGT1A1*28 gene variant is associated with lower mortality rates. The protective effect of the UGT1A1*28 variant likely includes mechanism other than bilirubin metabolism.

Keywords: mortality; polymorphism; UGT1A1.

Introduction

UDP-glycosyltransferase 1A1 (UGT1A1) metabolizes unconjugated bilirubin to conjugated bilirubin by means of glucuronic acid and is the major enzyme influencing bilirubin levels. Additionally, UGT1A1 plays an important role in the detoxification and excretion of endogenous and exogenous lipophilic compounds [1].

A polymorphism in the promoter of the UGT1A1 gene, UGT1A1*28 (rs3064744) is characterized by six or seven TA repeats in the TATA-Box. The UGT1A1*28 variant (seven repeats) results in lower gene expression and subsequently lower enzymatic activity compared with the wild-type UGT1A1*1 variant (six repeats) [2, 3].

Although a meta-analysis provided clear evidence of an association between serum bilirubin and cardiovascular disease [4], the UGT1A1*28 variant seems to have no influence on cardiovascular risk [5–8]. In chronic hemodialysis patients, the UGT1A1*28 variant has been associated with reduced overall mortality [9].

The aim of the present study was to investigate the role of the UGT1A1*28 polymorphism for long-term outcome in a well-characterized cohort of patients referred for coronary angiography.

Materials and methods

Study subjects

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study includes consecutive Caucasian patients hospitalized for coronary angiography between June 1997 and January 2000. The Ethics Review
Committee at the “Landesärztekammer Rheinland-Pfalz” (Mainz, Germany) has approved the study and the participant obtained a written informed consent in accordance to the Helsinki Declaration.

A detailed description of the LURIC study design and baseline characteristics has been published [10]. Briefly, the study population comprised 3316 participants. According to the classification of the American Heart Association, coronary artery disease (CAD) was defined as the presence of a visible luminal narrowing (≥20% stenosis) in at least one of 15 coronary segments [11]. Individuals with stenosis <20% were considered as not having CAD. Cardiovascular risk factors such as type 2 diabetes, hypertension and smoking were assessed. Hypertension was defined as a systolic and/or diastolic blood pressure exceeding 140 and/or 90 mmHg or a history of hypertension documented in medical records. Individuals with either high-density lipoprotein cholesterol <0.91 mmol/L or total cholesterol more than 6.24 mmol/L or triglycerides more than 1.71 mmol/L were considered dyslipidemic [10]. Data on smoking habits were retrieved using questionnaires. Type 2 diabetes mellitus was diagnosed according to the criteria of the American Diabetes Association. Further, individuals with a history of type 2 diabetes or those receiving oral antidiabetics or insulin were considered diabetic [12]. To detect “hidden” smokers, plasma cotinine concentrations were determined using a commercial radioimmunoassay (cotinine RIA; DPC). Individuals suffering from acute illnesses other than acute coronary syndromes, chronic non-cardiac diseases and a history of malignancy within the past 5 years were not eligible.

Fasting blood samples were obtained in the morning before coronary angiography. Selected variables were measured after samples were frozen and stored at −80 °C. Information on mortality rates was obtained from local registries. Death certificates were used to classify the deceased into those who died from cardiovascular versus non-cardiovascular causes. This classification was done independently by two experienced clinicians who were blinded to any data on the study participants except the information that was required to classify the causes of death.

Genotyping

Genomic DNA was extracted from the white blood cells in 9 mL of EDTA blood using a salting out method. UGT1A1 genotypes were determined on an ABI 3730 sequencing system (Applied Biosystems, Vienna, Austria). Briefly, polymerase chain reaction was performed using a fluorescence labeled forward primer (FAM-GTCACGTGACACAGTCAAACATTAAC) and an unlabeled reverse primer (5’-ACAAGTGGGCGTCGCC). Two microliters of the polymerase chain reaction product was mixed with 10 μL HI-Di™ Formamide (Applied Biosystems) and 0.5 mL Genscan 400HD ROX size standard (Applied Biosystems). After denaturation (95 °C for 2 min) and cooling (4 °C for 5 min), fragments were analyzed on the ABI 3730 sequencing system. Genotyping was done using the GeneMapper version 3.7 software (Applied Biosystems).

Statistics

Statistical analysis was done using SPSS 23.0 software (IBM). Continuous variables were compared between groups by univariate analysis of variance. A linear regression model was performed to identify predictors of bilirubin levels. Cox regression was used to estimate effects on mortality. For regression analyses, an allelic model based on additive gene-dose effects was used, and genotypes were coded as 0 (“wildtype”), homozygous *1/*1 genotype, 1 (heterozygous *1/*28 genotype) or 2 (homozygous *28/*28 genotype). The criterion for statistical significance was p < 0.05.

Results

Baseline data of the LURIC cohort are presented in Table 1. Genotypes for the UGT1A1*28 gene polymorphism were determined in 3245 (97.9%) members of the LURIC study. UGT1A1 genotype frequencies were 42.2% (*1/*1), 45.4% (*1/*28) and 12.4% (*28/*28) and did not deviate from those predicted by the Hardy-Weinberg equilibrium.

As expected, UGT1A1 genotypes were strongly associated with bilirubin levels, with levels in carriers of the *28/*28 genotype being almost twice as high as in carriers of the *1/*1 genotype (Table 1). In a multivariate regression analysis, baseline bilirubin levels were predicted by UGT1A1 genotype, sex, age, CAD, smoking status and alanine aminotransferase (ALT) level (p < 0.001 for all predictors).

During a median follow-up of 10.4 years, 995 (30.0%) subjects died. In a multivariate regression analysis adjusting for age, sex, CAD, smoking, type 2 diabetes, dyslipidemia,

Table 1: Baseline data of the LURIC cohort.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>UGT1A1 *1/*1</th>
<th>UGT1A1 *1/*28</th>
<th>UGT1A1 *28/*28</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3316</td>
<td>1370</td>
<td>1473</td>
<td>402</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>62.7 ± 10.7</td>
<td>62.6 ± 10.9</td>
<td>62.8 ± 10.5</td>
<td>62.3 ± 10.5</td>
<td>0.67</td>
</tr>
<tr>
<td>Male</td>
<td>2310 (69.7%)</td>
<td>961 (70.1%)</td>
<td>1018 (68.9%)</td>
<td>279 (69.4%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>2583 (77.9%)</td>
<td>1071 (78.2%)</td>
<td>1135 (77.1%)</td>
<td>327 (81.3%)</td>
<td>0.18</td>
</tr>
<tr>
<td>Current smoker</td>
<td>654 (19.7%)</td>
<td>257 (18.8%)</td>
<td>296 (20.1%)</td>
<td>81 (20.1%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Bilirubin, μmol/L</td>
<td>10.8 ± 6.2</td>
<td>9.1 ± 4.6</td>
<td>10.8 ± 5.3</td>
<td>16.9 ± 9.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are number of subjects (%) or mean ± standard deviation. UGT1A1 genotypes were determined in 3245 (97.9%) subjects. p-Values indicate significance of differences between genotype groups.
baseline ALT and baseline bilirubin levels, the UGT1A1*28 variant predicted lower overall mortality (hazard ratio [HR], 0.86; 95% confidence interval [CI], 0.78–0.95; p = 0.003) in a dose-dependent manner (Figure 1). Contrary to expected, in the same model higher baseline bilirubin levels predicted increased mortality (HR, 1.014; 95% CI, 1.002–1.025; p = 0.019). Analysis of various subgroups showed a stable association of UGT1A1*28 variant with lower overall, cardiovascular and non-cardiovascular mortality (Figures 1 and 2).

Discussion

In the present study, the number of UGT1A1*28 alleles was associated with lower cardiovascular or overall mortality.

This association remained significant when adjusted for baseline bilirubin levels and conventional risk factors, indicating a genotype effect independent of bilirubin levels.

Some studies reported a protective effect of bilirubin on cardiovascular disease and/or mortality, but results have been inconsistent and conflicting [4–8]. In the present study, higher baseline bilirubin levels were associated with higher mortality, which was seemingly contradictory to the protective effects of the UGT1A1*28 allele. Current data suggest a complex network of UGT1A1 genotypes, traditional cardiovascular risk factors and bilirubin influencing mortality. Some conventional risk factors, such as age, are associated with elevated bilirubin levels, while others, such as smoking or male sex, are associated with decreased bilirubin levels [13]. Furthermore, liver disease leads to strongly elevated bilirubin levels, and bilirubin levels are directly related to liver-related mortality [14, 15]. Beyond being a marker for other risk factors, bilirubin itself could additionally show causal effects on mortality by acting as inhibitor of low-density lipoprotein oxidation and platelet activation [16, 17]. The net effects of bilirubin levels on mortality are therefore hard to predict, strongly dependent on other risk factors, and may change during life time [18].

Nevertheless, the main aim of the present study was to investigate the role of UGT1A1 genotypes in mortality. UGT1A1 genotype was, independent of baseline bilirubin levels, a stable and causal predictor of cardiovascular and overall mortality. UGT1A1 conjugates a variety of endogenous and exogenous substances other than bilirubin [1]. The UGT1A1*28 variant, leading to reduced UGT1A1 activity, leads therefore pleiotropic effects, including reduced conjugation of hormones or drugs. It is
likely that these effects, rather than bilirubin levels, are responsible for the decreased mortality conferred by the UGT1A1*28 allele.

Our results are in line with several other studies, showing an association of the UGT1A1*28 allele with reduced mortality in the general population as well as cohorts with different underlying diseases [9, 19, 20]. The strengths of the present study include the large number of participants from an ethnic homogenous population, as well as its prospective design. Nevertheless, some limitations of the present study should be kept in mind: LURIC is a hospital-based cohort, and the majority of participants had a prevalent CAD, resulting in a higher mortality compared with the general population. Effect sizes of the UGT1A1*28 variant may depend on the risk profile of the study participants and cannot be extrapolated to other populations.

In the present study, UGT1A1*28 was not associated with a lower prevalence of CAD or MI, which is in contrast to data from the Framingham study [21]. Furthermore, no association of UGT1A1 genotypes with severity of CAD was seen in the present study (data not shown). The present study consisted of patients admitted for coronary angiography, resulting in a large proportion of subjects with CAD or previous MI. This may have obscured the potential associations of UGT1A1 genotypes with CAD risk.

In conclusion, we show that the UGT1A1*28 variant is independently associated with reduced mortality. The mechanisms for this association remain to be established, but are likely other than bilirubin metabolism.

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References


