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Comparison of three methods for genotyping the *UGT1A1* $(TA)_n$ repeat polymorphism

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Abstract

Objectives: The *UGT1A1* promoter contains a $(TA)_n$ repeat polymorphism. The 7 repeat allele is associated with decreased enzyme activity and patients homozygous for this allele treated with irinotecan may experience life-threatening toxicity. Here, we have compared three methods [DNA sequencing, fragment analysis, and the Invader® assay (Third Wave Technologies)] for genotyping this polymorphism.

Results: All of the DNA samples ($n=119$) had concordant genotype calls between the sequencing and size-based methods. The Invader® method was also concordant if the genotypes were 6/6, 6/7, or 7/7. Both the size-based method and the Invader® method had straightforward data analysis, while interpretation of the sequencing results was occasionally more challenging. The Invader® method required more concentrated DNA for analysis, was more expensive, and had a limited genotyping spectrum.

Conclusion: All three methods were valuable for genotyping the *UGT1A1* $(TA)_n$ repeat, with the sequencing and size-based assays having the fewest drawbacks.

Keywords: Capillary electrophoresis; Fragment analysis; Genotyping; Irinotecan; Sequencing; TATA box; *UGT1A1*

Introduction

Glucuronidation is an important phase II reaction catalyzed by the UDP-glucuronosyltransferases (UGTs) and is involved in metabolism of xenobiotic and lipophilic endobiotic substances, including the chemotherapeutic drug, irinotecan, and bilirubin. *UGT1A1* is one of thirteen different gene isoforms in the UGT1A subfamily on chromosome 2q37 [1]. Multiple polymorphisms in the *UGT1A1* gene have been shown to be associated with altered activity of the UGT1A1 enzyme, including the extensively studied promoter region $(TA)_n$ repeat region. The wild-type sequence of this motif contains six TA repeats (*UGT1A1*1*) but can range from five to eight repeats with variable effects on transcriptional activity. The $(TA)_5$ repeat (*UGT1A1*36*) is associated with normal to possibly increased transcriptional activity, while the $(TA)_7$ and $(TA)_8$ repeats (*UGT1A1*28* and *UGT1A1*37*, respectively) are associated with a 20–30% reduction in *UGT1A1* expression and approximately 50% reduction in UGT1A1 activity [2–6]. Individuals homozygous or compound heterozygous for the $(TA)_7$ and $(TA)_8$ alleles may develop Gilbert syndrome, which is characterized by mild unconjugated hyperbilirubinemia.

The most common $(TA)_n$ variant is the $(TA)_7$ repeat, which has an allele frequency of 0.33–0.4 in Caucasians, 0.48 in Africans, and 0.14 and 0.17 in the Chinese and Japanese populations, respectively [3,7–11]. The $(TA)_5$ and $(TA)_8$ variants are found more frequently in the African population compared to other ethnic groups. Numerous other polymorphisms in *UGT1A1* have been described and characterized, including G71R in exon 1, which is associated with decreased enzyme activity and chemotherapy-induced hyperbilirubinemia [12–15].

Irinotecan is a camptothecin analogue that inhibits topoisomerase I and is widely used for the treatment of certain

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cancers, especially colorectal and lung carcinomas. A significant percentage of patients taking irinotecan may experience life-threatening leukopenia, neutropenia, and/or diarrhea, depending on the dosing regimen. This adverse drug reaction has been linked to certain *UGT1A1* polymorphisms, including $(TA)_7$ and $(TA)_8$, which are associated with reduced elimination of the active metabolite of irinotecan, SN-38 [16–18]. The incidence of irinotecan-associated toxicity can be decreased if such polymorphisms are identified in patients prior to undergoing irinotecan-based therapy and appropriate measures to modify treatment are taken [19]. In fact, the Federal Drug Administration (FDA) recently approved revisions to the safety labeling on irinotecan to recommend reduced dosing in patients who are homozygous for the $(TA)_7$ allele.

In order to aid clinical decisions pertaining to individualized irinotecan therapy, it is important to perform rapid and accurate genotyping of the $(TA)_n$ repeat region (and other *UGT1A1* polymorphisms that may affect irinotecan metabolism). The FDA recently cleared the Invader® UGT1A1 Molecular Assay for genotyping the *UGT1A1* promoter $(TA)_n$ repeat region [20]. Other methods for genotyping the UGT1A1 $(TA)_n$ polymorphism have been previously reported, including real-time fluorescent PCR, pyrosequencing, denaturing high performance liquid chromatography (dHPLC), single strand conformation polymorphism (SSCP), and melt-curve analysis [21–28]. Here, we have evaluated and compared the Invader® assay along with two other methods, an automated fluorescent sequencing assay and a capillary electrophoresis allelic size-based method (fragment analysis), for genotyping the *UGT1A1* TATA box.

Methods

Sample population

The collection of clinical data and peripheral blood samples was approved by the institutional review board of the Mayo Clinic. Anonymized genomic DNA (gDNA) samples were obtained from 119 unrelated African American individuals. We chose to study an African American population due to the higher frequency of the $(TA)_5$ and $(TA)_8$ variants in this ethnic group. A subset of the samples ($n=24$) were part of the African American Human Variation Panel and were purchased from the Coriell Cell Repositories (Camden, NJ). The remainder of the gDNA samples ($n=95$) was isolated using the QIAamp® 96-well DNA Blood Kit (Qiagen, Inc., Valencia, CA) from the peripheral blood leukocytes of subjects who submitted blood samples for genetic studies in our laboratory.

Genotyping by sequencing

Amplification of a region (1190 bp) of *UGT1A1* (GenBank accession number NG_002601) containing the TATA box was achieved on a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Hayward, CA). PCR primer sequences were as follows: forward-5′-TCT CTG AAA GTG AAC TCC CTG CTA CCT T-3 and reverse-5′-CCA GAA GAT GAT GCC AAA GAC AGA CTC AA-3′, corresponding to NG_002601 at nucleotides 175391–175418 and 176581–176553, respectively. Polymerase chain reaction (PCR) components included 25 ng gDNA, 0.5 μL each of 25 μM forward and reverse primers, 12.5 μL of 2× AmpliTaq Gold Master Mix (Roche, Alameda, CA), and 10.5 μL PCR-grade water (Fisher, Pittsburgh, PA), for a 25 μL total reaction volume. PCR cycling conditions were as follows: 94 °C for 10 min, 72 °C for 30 s (then decreased by 1 °C over the first 10 cycles with the final 25 cycles performed at an annealing temperature of 61 °C), 72 °C for 1 min, and a final extension at 72 °C for 10 min, followed by a 4 °C hold. PCR product was treated with shrimp alkaline phosphatase and exonuclease, followed by desalting and resuspension in 10 μL of PCR-grade water. Separate (forward and reverse) sequencing reactions were prepared by addition of 0.5 μL of 25 μM forward or reverse sequencing primer, 0.5 μL DMSO (Sigma, St. Louis, MO), and 4 μL PCR-grade water to 1 μL of the purified PCR product. Sequencing primer sequences were as follows: forward-5′-TCT CTG AAA GTG AAC TCC CTG CTA CCT T-3′ and reverse-5′-AAA CAT TAT GCC CGA GAC TAA CAA-3′, corresponding to NG_002601 at nucleotides 175391–175418 and 175819–175842, respectively. Sequencing was performed according to the ABI Dye Terminator Sequencing Protocol (Applied BioSystems). Sequence chromatograms for each sample were analyzed visually, and in some cases via Mutation Surveyor (SoftGenetics, LLC, State College, PA), by at least two individuals and compared in forward and reverse direction and against control samples.

Genotyping by fragment (size-based) analysis

A PCR protocol was performed combining 150 ng of gDNA, 5 μL of a 5× reaction buffer that included dNTPs (Perkin Elmer, Boston, MA) and $MgCl_2$ (Perkin Elmer), an additional 1.0 μL of 25 mM $MgCl_2$ (Perkin Elmer), and 0.50 μL each of a 20 μM forward (6-FAM fluorescent dye labeled; Applied Biosystems, Foster City, CA) and reverse (5′-GTTTCTT-3′ Tailed; Idaho Technology, Inc., Salt Lake City, UT) primer mix. The reverse primer was tailed to promote adenylation and prevent double peaks from interfering with proper data analysis [29]. The forward primer sequence, 5′-6FAM-GAGGTTCTGGAAG-TACTTTG-3′, was previously described by Akaba et al. [30] and the reverse primer sequence was 5′-GTTTCTTGGTTCGC-CCTCTCCTACT-3′ (the underlined portion represents the tail). The reaction also included 0.25 μL of Taq Gold Polymerase (5U/μL; Perkin Elmer) and 12.75 μL of DNAse/RNAse-free water (Sigma, St. Louis, MO) for a total volume of 25 μL. The reactions were amplified using a Hybaid™ Thermal Cycler (Thermo Electron Corporation, Basingstoke, Hampshire) programmed with the following parameters: 95 °C for 10 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. Products underwent a final extension for 10 min at 72 °C. The amplicons were then diluted 1:10 with DNAse/RNAse-free water before combining the diluted DNA (1 μL) with formamide (12.5 μL; Applied Biosystems) and a 500-bp ROX size standard (0.5 μL, Applied Biosystems). The samples were separated via capillary electrophoresis on the ABI