# The clinical application of *UGT1A1* pharmacogenetic testing: Gene–environment interactions

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## Abstract

Over the past decade, the number of pharmacogenetic tests has increased considerably, allowing for the development of our knowledge of their clinical application. The uridine diphosphate glucuronosyltransferase 1A1 gene (*UGT1A1*) assay is an example of a pharmacogenetic test. Numerous variants have been found in *UGT1A1*, the main conjugating enzyme of bilirubin and drugs such as the anticancer drug irinotecan. Recently, the US Food and Drug Administration (FDA) recommended testing for the presence of *UGT1A1*28*, an allele correlated with decreased transcriptional activity, to predict patients at risk of irinotecan toxicity. The administration of other drugs — such as inhibitors of the UGT1A1 enzyme — can clinically mimic the *28 phenotype, whereas inducers of UGT1A1 can increase the glucuronidation rate of the enzyme. The *28 polymorphism is not present in all ethnicities at a similar frequency, which suggests that it is important to study different populations to determine the clinical relevance of testing for *UGT1A1*28* and to identify other clinically relevant *UGT1A1* variants. Environmental factors such as lifestyle can also affect UGT1A1 activity. This review is a critical analysis of studies on drugs that can be affected by the presence of *UGT1A1*28*, the distribution of this polymorphism around the globe, distinct variants that may be clinically significant in African and Asian populations and how lifestyle can affect treatment outcomes that depend on *UGT1A1* activity.

***Keywords:*** *UDP-glucuronosyltransferase, UGT1A1, polymorphism, ethnicity, pharmacogenetics, drug therapy*

## Introduction

The uridine diphosphate glucuronosyltransferase (UGT) enzymes are a superfamily of conjugating enzymes that aid in the excretion of several molecules by transferring one glucuronic acid to their substrates. This makes them more hydrophilic molecules and enables their biliary or renal elimination.[1] This superfamily consists of two families (UGT1, UGT2) and three subfamilies (UGT1A, UGT2A, UGT2B). The UGT2 family comprises eight different proteins encoded by individual genes located on chromosome 4q13, while the first subfamily (UGT1A) comprises nine proteins and is coded by the *UGT1A* gene, located on chromosome 2q37. This locus contains each isoform's unique exon 1 and the common exons 2–5, present in all transcripts.[2] Some UGT isoforms are tissue specific.[3] There is evidence of substrate overlap, although some substrates are specific for one particular isoform, such as the conjugation of bilirubin, which is mainly catalysed by UGT1A1.[1–3] *UGT1A1* is the focus of this report.

To date, more than 150 functional polymorphisms have been identified on the *UGT1A* locus, and 113 functional variants have been identified specifically in UGT1A1.[1,4] These allelic variations

  

were found in both the exonic and promoter sequences. The most thoroughly studied of these polymorphisms is *UGT1A1*28*, representing seven thymine–adenine (TA) repeats in the promoter region of *UGT1A1*. Individuals with this variant have an extra TA repeat in this sequence, whereas the wild-type allele comprises six repeats and is denoted as *UGT1A1*1*.[1,2,5] The length of this TA repeat sequence is inversely correlated with the activity of the UGT1A1 enzyme; therefore, the *28 polymorphism results in reduced UGT1A1 activity, which affects the elimination of its drug substrates. When the *28 allele is present on only one chromosome, it results in a 25 per cent decrease in enzyme activity[6] and, when present in a homozygous fashion, *UGT1A1* transcription is reduced by 70 per cent.[1,2,4,5] In addition, the *UGT1A1*28* polymorphism has been associated with Gilbert's syndrome, a mild form of an inherited unconjugated hyperbilirubinaemia that does not indicate liver damage but can affect the metabolism of several substances.[3–5]

There is evidence that both endogenous and exogenous substances are metabolised by this UGT isoform. Zhang *et al*. showed that, *in vitro*, conjugation of bilirubin and 3-OH conjugates of oestradiol and ethinyl oestradiol was catalysed by UGT1A1.[5] It was also shown that liver microsomes from individuals homozygous for the *UGT1A1*28* allele had a decreased rate of glucuronidation when compared with heterozygous and wild-type samples, the latter being the ones with higher glucuronidation activity.[5] Given that UGT1A1 plays a role in oestradiol metabolism, studies were undertaken to identify a correlation between enzyme activity and the development of gynaecological cancers. Duguay *et al*. reported that the wild-type allele seemed to be related to a higher risk of endometrial cancer, which was more evident among premenopausal women.[7] It is not yet clear, however, how *UGT1A1*28* may influence risk for breast cancer. Guillemette *et al*. found a higher risk of breast cancer in premenopausal African-American women with longer promoter sequences, an association that was stronger for oestrogen receptor (ER)-negative ($ER^-$) breast cancers than for $ER^+$ ones.[8] Sparks *et al*. have reported a reduced risk of $ER^-$ breast cancer in Caucasian and Asian women with two *UGT1A1*28* alleles.[9]

Many exogenous substances, mutagenic xenobiotics and therapeutic drugs are UGT1A1 substrates. Examples of therapeutic drug substrates of UGT1A1 are: irinotecan (SN-38), acetaminophen (paracetamol), carvedilol, etoposide, lamotrigine and simvastatin.[1–3] To date, however, anticancer drugs seem to be the predominant drug substrates clinically affected by the *UGT1A1*28* polymorphism. There are also many drugs that alter the activity of UGT1A1 by acting as inducers or inhibitors.[1–3] Rifampicin and phenobarbital are examples of UGT enzyme inducers.[10–13] Patients being treated concomitantly with a UGT1A1 drug substrate and an inducer may require higher doses of the drug substrate to ensure successful treatment. UGT1A1 inhibitors can have a greater impact on the treatment outcome of individuals with the *UGT1A1*28* polymorphism, however, since these individuals already have a reduced UGT1A1 basal activity and further inhibition of enzyme activity while being administered a UGT1A1 substrate can lead to drug accumulation and toxicity. Therefore, testing for the presence of the *UGT1A1* reduced activity polymorphism can provide invaluable information on the potential for either drug toxicity or efficacy when prescribing certain drugs (Table 1). The scoring system presented in Table 1 illustrates the strength of currently available studies that assess the relevance of testing for the presence of *UGT1A1*28*.

## Irinotecan

Irinotecan, a topoisomerase I inhibitor, is used in the treatment of metastatic colorectal cancer, often in combination with other drugs.[6] Irinotecan is a prodrug, activated to SN-38, which is then conjugated by UGT1A1 to glucuronides (SN-38G), which are excreted.[2] It is the most exhaustively studied drug concerning the *UGT1A1*28* polymorphism. Patients with this variant may be at higher risk for adverse reactions to irinotecan treatment, since they express lower rates of glucuronidation.[6] This correlation was investigated in several studies and it was

**Table 1.** Clinical relevance of testing for the presence of UGT1A1*28

| Drug | UGT1A1 | Ref. | Summary | Indication for pharmacogenetic testing | Rationale |
|---|---|---|---|---|---|
| Irinotecan (SN-38) | Substrate | 14–16 | The presence of the *UGT1A1*28* allele is a risk factor for the development of adverse reactions to irinotecan treatment | **** | Testing prevents drug toxicity at high dose |
| Raloxifene | Substrate | 17 | Patients under raloxifene treatment that are homozygous for *UGT1A1*28* are more exposed to its active metabolite, exhibiting a superior increase in hip bone mineral density | ** | Testing could identify patients that need a higher dose of raloxifene |
| Raltegravir | Substrate | 18,19 | Homozygosity for *UGT1A1*28* is correlated with an increase in raltegravir plasma concentrations, but does not seem to affect the safety profile of this drug | * | Testing does not seem to be useful |
| Indinavir | Inhibitor | 4,20, 23,26 | Indinavir raises unconjugated and total bilirubin concentrations by inhibiting UGT1A1. This effect can lead to clinical jaundice in patients with the *UGT1A1*28* genotype | ** | Testing could help avoid hyperbilirubinaemia |
| Atazanavir | Inhibitor | 20–22 | Atazanavir inhibits the UGT1A1 enzyme, which leads to hyperbilirubinaemia. Patients with the *UGT1A1*28* genotype are at greater risk of developing clinical jaundice when taking this drug | *** | Strong evidence that testing may prevent clinical jaundice |
| Sorafenib | Inhibitor | 24,25 | Sorafenib at high doses inhibits UGT1A1 activity, which can cause hyperbilirubinaemia | ** | Further studies are required to understand sorafenib's influence on UGT1A1 activity |

*Testing does not seem necessary to guide treatment.
**Testing may be relevant to guide treatment, but further studies are required.
***There is some evidence that testing may be an important indicator to guide treatment.
****There is a strong evidence that testing provides essential information to guide treatment.

repeatedly shown that *UGT1A1*28* is predictive of irinotecan toxicity, especially grade 4 neutropenia.[14] In 2005 these results led the US Food and Drug Administration (FDA) to issue a recommendation for *UGT1A1*28* testing on the irinotecan drug label.[2] Later, Hoskins *et al.* showed that at low doses of irinotecan ($<150$ mg/m$^2$), the risk for neutropenia was similar between patients with this polymorphism and patients with the wild-type allele; however, when administering higher doses of irinotecan ($>150$ mg/m$^2$), neutropenia was more likely to occur in patients with the *UGT1A1*28* polymorphism.[15] If this is *bona fide*, then the irinotecan labelling information should be altered to reflect the real association between irinotecan dose and *UGT1A1*28*.[15] Some other reports suggest that testing for a haplotype within the *UGT* gene, instead of only testing for *UGT1A1*28*, would be more accurate when predicting treatment outcome, since additional isoforms, such as UGT1A7 and UGT1A9, also seem to influence irinotecan haematological toxicity.[16] Besides neutropenia, severe diarrhoea is also a side effect of irinotecan treatment;[6] however, diarrhoea is not clearly associated with the

presence of *UGT1A1*28* polymorphism. There are other enzyme families that contribute to the metabolism of irinotecan. Irinotecan is activated to its active form, SN-38, by carboxylesterases and is then converted into SN-38G by the UGT1A1 enzyme. Irinotecan is oxidised to two inactive metabolites, 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC), by the cytochrome P450 3A4 (CYP3A4) enzyme.[6] ATP-binding cassette (ABC) transporters such as the multi-drug resistance 1 P-glycoprotein gene (ABCB1) and the multi-drug resistance-associated protein 2 gene (ABCC2) are also thought to contribute to the elimination of irinotecan and its metabolites.[2,17] This complex metabolic pathway, involving different highly polymorphic enzymes, suggests that testing for *UGT1A1*28* alone may not be enough in some cases to predict irinotecan toxicity, especially severe diarrhoea (Figure 1).

## Raloxifene

Raloxifene is a selective ER modulator indicated for the treatment and prevention of osteoporosis in postmenopausal women and, since 2007, for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast cancer.[18] UGT1A1 is thought to be the major UGT isoform conjugating raloxifene, so it would be expected that individuals with the *UGT1A1*28* genotype would have reduced levels of its metabolites. Trontelj *et al*. showed otherwise, ie that postmenopausal women homozygous for seven (TA) repeats on the promoter sequence of *UGT1A1* had higher concentrations of raloxifene's glucuronides, particularly the raloxifene-4′-glucuronide (M2).[18] These women also had a visibly higher exposure to raloxifene compared with women with at least one wild-type allele. This was not statistically significant, however, which the authors suggested was due to insufficient sample size and the prominent data variability. The hypothesis formulated to explain the paradoxical increase of the concentration of glucuronides in women homozygous for *UGT1A1*28* (*28/*28) was that the metabolic enzyme–transporter interaction could be responsible for the high M2 levels, meaning that the decreased conjugating activity would lower the formation and excretion rates of raloxifene conjugates, which could instigate the accumulation of raloxifene.[18] Given that the raloxifene glucuronides can form raloxifene via enterohepatic cycling, an additional explanation for the unexpected findings was that this enterohepatic cycle was stimulated by the presence of higher concentrations of glucuronides. Consequent to the higher exposure to raloxifene, there was a greater increase in hip bone mineral density in *28/*28 women. Further studies are required fully to understand raloxifene pharmacokinetics and the effect of the *28 polymorphism on raloxifene treatment outcomes. If these results can be replicated, testing for *UGT1A1*28* could be useful for defining appropriate raloxifene dose requirements.

## Raltegravir

Raltegravir, a human immunodeficiency virus-1 (HIV-1) integrase inhibitor, is another UGT1A1 substrate. The pharmacokinetics of raltegravir were evaluated in individuals homozygous for the *UGT1A1*28* and for the wild-type allele

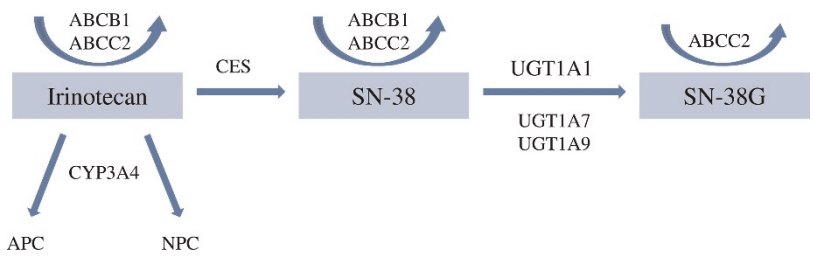


**Figure 1.** Metabolism of irinotecan.

(*UGT1A1*1*). Individuals homozyous for *28 exhibited a higher area under the concentration–time curve ($AUC_{0-\infty}$) of raltegravir but the higher plasma levels were well tolerated, since there was not an altered safety profile.[19,20] This is probably due to the drug's relatively large therapeutic window. Therefore, testing for the presence of *UGT1A1*28* does not seem to provide additional guidance on the safe use of raltegravir.

## Protease inhibitors

### Indinavir and atazanavir

Indinavir and atazanavir are protease inhibitors — drugs used in the treatment of HIV infection and chronic viral hepatitis. They are competitive inhibitors of the UGT1A1 enzyme,[21] so they can induce hyperbilirubinaemia by competing with the binding site on UGT.[4,21,22] When used in combination with a UGT1A1 substrate, they can induce toxicity, since they decrease the elimination of the substrate. A number of studies were carried out to evaluate the pharmacokinetics of protease inhibitors in the presence of *UGT1A1* reduced function alleles such as the *UGT1A1*28* polymorphism[23,24] and haplotypes.[4,21,22] They all reported that higher levels of bilirubin were observed in study subjects with at least one variant allele of *28 and who were receiving one of these protease inhibitors. The development of clinical jaundice was considerably higher in individuals homozygous for the *28 allele, which suggests that they are more susceptible to adverse reactions when treated with indinavir or atazanavir.[4,21,22] By comparison with indinavir, atazanavir exhibited a stronger association with high concentrations of bilirubin.[21] Therefore, testing for the *28 polymorphism may be more useful when atazanavir, as opposed to indinavir, is implemented in a treatment regimen (Table 1).

### Lopinavir and ritonavir

The concomitant administration of lopinavir and ritonavir, a formulation used as first-line treatment in naïve HIV patients, with irinotecan at 150 mg/m$^2$, was evaluated in seven Caucasian male patients infected with HIV and diagnosed with Kaposi's sarcoma.[17] Patients were administered 400mg of lopinavir and 100mg of ritonavir on a single formulation (kaletra) twice a day. A lower concentration of the oxidised metabolite of irinotecan, APC, was found and was attributed to the inhibition of CYP3A4 by the protease inhibitors and also possibly due to the inhibition of ABCB1 transporters; although this could not be confirmed. The serum concentrations of irinotecan, SN-38 and SN-38G were higher when patients were also treated with lopinavir and ritonavir. The authors hypothesised that the increase in irinotecan and SN-38 levels was due to simultaneous inhibition of CYP3A4 and UGT1A1 by the protease inhibitors.[17] The concentration of SN-38G was increased only due to the higher availability of SN-38, because the glucuronidation rate of SN-38 was, indeed, decreased by 36 per cent, which was suggested to be due to the inhibition of UGT1A1 by lopinavir and ritonavir. The occurrence of neutropenia was prevented by prophylaxis with granulocyte colony-stimulating factor (G-CSF) but a higher rate of severe diarrhoea was observed, suggesting that clinical consequences may arise from the simultaneous use of protease inhibitors and substrates for UGT1A1, an interaction that seems to affect the UGT1A1, CYP3A4 and ABCB1 gene products.

## Sorafenib

Sorafenib is an anticancer drug that inhibits tumour cell proliferation through the Raf/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK signalling pathway, and tumour angiogenesis by targeting the receptor tyrosine kinases of vascular endothelial growth factors 2 and 3, and platelet-derived growth factor.[25] It is metabolised by CYP3A4 and UGT1A9. The combined treatment of sorafenib with irinotecan was evaluated in patients with advanced, refractory solid tumours.[26] It was found that at high doses (400 mg twice daily), sorafenib increases the exposure to irinotecan and SN-38. This higher exposure did not increase toxicity, which could be explained by the fact that a low dose of irinotecan (125 mg/m$^2$) was being administered. This result suggests that

sorafenib may inhibit the glucuronidation of irinotecan, thereby making it a plausible UGT1A1 inhibitor. Recently, a single-case report showed that one patient diagnosed with hepatocellular carcinoma developed unconjugated hyperbilirubinaemia after one infusion of cyclophosphamide and doxorubicin and seven days of sorafenib 400 mg twice daily.[25] The patient was genotyped for *UGT1A1* and was found to have one *UGT1A1*28* allele, which, in combination with his concomitant diagnosis of liver cirrhosis, was thought to induce the high levels of bilirubin. The occurrence of unconjugated hyperbilirubinaemia is an indication of impaired UGT1A1. Conversely, conjugated hyperbilirubinaemia indicates progression of liver disease. Further studies of potential interactions between sorafenib and the *UGT1A1*28* polymorphism are required in order to evaluate if testing patients starting on this anticancer agent is useful, or whether performing a bilirubin fractionation is sufficient.

## Etoposide

Another drug that may show relevance for testing for the *UGT1A1*28* polymorphism is the anticancer drug and topoisomerase II inhibitor, etoposide. UGT1A1 was shown to be the major glucuronidating isoform of etoposide, *in vitro*.[27] If this metabolic pathway also takes place *in vivo*, the presence of seven (TA) repeats in the promoter region of *UGT1A1* can possibly lead to a decreased rate of excretion of this anticancer drug, hence an indication for testing for the presence of the *UGT1A1*28* allele may become clinically relevant.

## The significance of ethnicity

In most studies of *UGT1A1* genotype and phenotype correlation, Caucasians make up the majority of the study subjects. This is a limitation of these investigations. While similar polymorphisms of *UGT1A1* exist in both Caucasian and non-Caucasian people, and they can result in the same physiological effect, the frequency of *UGT1A1* variants is not the same across all populations (Table 2). For instance, among different countries in Europe, the frequency of homozygosity for *UGT1A1*28* is in the range 9–16 per cent.

**Table 2.** Global population frequencies of UGT1A1*28

| Continent | Ethnicity | Frequency of UGT1A1*28 homozygotes (%) | Ref. |
|---|---|---|---|
| Europe | Scottish | 12 | 1, 28 |
| | Italian | 9; 16 | 16; 28 |
| | Dutch | 12 | 29 |
| | Spanish | 10 | 30 |
| North America | Canadian Eskimos | 18 | 31 |
| | Caucasian American | 9 | 14 |
| | African-American | 23 | 1 |
| South America—Brazil | Caucasian-descendant | 13 | 32 |
| | African-descendant | 17 | 32 |
| | Parakanã Indian | 3 | 32 |
| Africa | Egyptian | 8 | 1 |
| | Côte d'Ivoire | 8 | 33 |
| | Kenyan (Luo) | 18 | 33 |
| | Madagascan | 6 | 33 |
| Asia | *Chinese* | 2 | 34 |
| | Dong origin | 1 | 35 |
| | Han origin | 1 | 35 |
| | She origin | <1 | 35 |
| | Indian | 13 | 1, 34 |
| | Thai | 1 | 36 |
| | Japanese | <3 | 31, 37 |

There is evidence of intra-ethnic allele frequency differences concerning the presence of *UGT1A1* variants. One example of this is the reported frequency of the homozygous *UGT1A1*28* allele in two Italian studies, possibly distinct due to the differences in the study populations observed. Cecchin *et al*. specifically studied patients from north-east Italy, while Biondi *et al*. assessed allele

frequency from Italians sampled throughout the country.[16,28] In three subpopulations in China, where more than 50 ethnic groups co-exist, the frequency of *UGT1A1*28* homozygotes was also described to be different.[35] Subjects of Dong and Han origin exhibited a very similar frequency, approximately 1 per cent, whereas individuals of the She group had a frequency of less than 1 per cent (Table 2). The presence of haplotypes spanning the $(TA)_7$ polymorphism was also shown to be different between these groups. One particular haplotype, including three polymorphisms reducing the activity of *UGT1A1* (*UGT1A1*60*, −3156G>A, *UGT1A1*28*) was found to be of significant frequency only in Dong and Han individuals.[30] These findings indicate that people from the same country may have different genotypes, which can denote differential responses to drugs. Therefore, labelling individuals according to their geographical location as an approximation of genotype may not provide useful information.

There is also evidence of inter-ethnic variability concerning the presence of the *28 polymorphism. In North America, Caucasian Americans are reported to have a frequency of *28 homozygotes, comparable with Caucasian Europeans;[14] Canadian Eskimos have a higher frequency (18 per cent)[31] and African-Americans reportedly have the highest frequency of *UGT1A1*28* (23 per cent).[1] The *UGT1A1* promoter sequence was studied in three distinct Brazilian populations.[32] The frequencies of homozygosity for the *28 allele in Caucasian descendants, African-derived individuals and Parakanã Indians were shown to be 13 per cent, 17 per cent and 3 per cent, respectively. In Africa, sample populations from Egypt and Côte d'Ivoire reportedly have an allele frequency of 8 per cent;[1,33] this is in contrast to Kenya's Luo tribe, which has an allele frequency of 18 per cent.[33] In Asia, frequencies of the *28 allele are lower, occurring in less than 5 per cent of Chinese,[34] Japanese[31,37] and Taiwanese[36] populations; however, this polymorphism can be identified in 13 per cent of individuals from Indian populations.[1,34] All these examples of intra- and inter-ethnic diversity show how geography and ethnicity may not be accurate predictors of genotype.

If most studies concerning *UGT1A1*28* are performed in Caucasian populations, and it is clear that the allele frequency is different in diverse populations, the question that has to be answered is: how relevant are these results for people belonging to different ethnic and racial groups? For instance, the presence of *UGT1A1*28* as a homozygous trait is uncommon in Asian people compared with the *6 variant. If dose adjustments of the drugs mentioned in the previous section, such as irinotecan (UGT1A1 substrate), need to be performed based on the standard *UGT1A1* pharmacogenetic assay that only tests for the *28 allele, the interpretation of the *UGT1A1* genotype and phenotype may not be accurate for the Asian individual compared with the Caucasian individual. Compared with the *28 allele, the *UGT1A1*6* variant is more common among Asian populations and produces the same phenotype of hyperbilirubinaema as does the presence of the *28 variant.[2] The *UGT1A1*6* allele frequency ranges from 13–23 per cent in Asians,[35] with homozygosity found in 7 per cent of one Korean population[38] and in 4 per cent of one Japanese population.[37] This polymorphism is found in exon 1[2] and results in the substitution of an arginine for a glycine (G71R) at position 71.[36] It reduces UGT1A1 activity by 40 per cent in heterozygotes and by 70 per cent in homozygotes, similar to *UGT1A1*28*, and is also correlated with Gilbert's syndrome in Asian populations.[31] Boyd *et al.* showed that, in a Thai population, the coding region polymorphism was of greater significance for the risk of indinavir-induced hyperbilirubinaemia, than the TA polymorphism on the promoter sequence. In addition, the presence of both *UGT1A1*6* and *UGT1A1*28* polymorphisms in the same individual further increased bilirubin levels.[36] These findings suggest that testing for *UGT1A1*6* in Asians may be more informative than testing for **28* alone for predicting treatment outcomes.

African populations seem to have greater microsatellite diversity, which is also evident at the *UGT1A1* locus. The *UGT1A1*36* and the *UGT1A1*37* variants, coding for a $(TA)_5$ and a $(TA)_8$ promoter sequence, respectively, are common in many African populations,[2,33] but those variants rarely, if ever, occur

in populations outside of Africa.[33] Given that UGT1A1 activity increases with shorter (TA) repeats in its promoter, the glucuronidation rate of this isoform is increased in *UGT1A1*36* and decreased in *UGT1A1*37*.[1] In addition to these rare variants, African populations also tend to have a reasonable frequency of combinations of reduced activity alleles. For example, the presence of one *UGT1A1*28* allele and one *UGT1A1*37* allele was found in 1 per cent of one population of Madagascar, in 3 per cent of one Malawian population, in 14 per cent of one Ivorian population, and in 6 per cent of a North and Central America population with varying degrees of African ancestry.[33,39]

UGT1A1 is important in the metabolism of protease inhibitors used for the treatment of HIV. HIV/AIDS is one of the top three health burdens in most sub-Saharan African countries. Therefore, it is important to address the influence of polymorphisms in *UGT1A1* in African populations in order to improve HIV treatment outcomes. It is clear that the pharmacokinetics of protease inhibitors can be affected by the presence of the reduced activity *UGT1A1* variant, *UGT1A1*28*. If the frequency of an increased activity allele, *UGT1A1*36*, is common in African populations, there is a possibility that some HIV patients on protease inhibitors may have sub-therapeutic responses because they metabolise some of these drugs very quickly. If, instead, they have the reduced activity allele, *UGT1A1*37*, or even its combination with *UGT1A1*28*, they can develop toxicity to protease inhibitors, ranging from the relatively benign yellowing of the skin and sclera to more severe neurological dysfunctions such as seizures and schizophrenia.[40] For all the stated reasons, *UGT1A1* variation should be thoroughly studied in African populations in order to understand which variants are better predictors of treatment outcome in such a diverse population.

## Lifestyle interactions

It has become clear that lifestyle and environment can affect the phenotypic expression of our genes. The effect of cigarette smoking on the pharmacokinetics of irinotecan was recently studied.[41] The results showed lower SN-38 exposure in smokers, which suggested that UGT1A1 was induced. Less haematological toxicity (neutropenia) was observed in these individuals and, although it could not be investigated, it was hypothesised that they could be at risk for treatment failure. If this hypothesis is tested and proven correct, smokers that are *UGT1A1*1* homozygotes and being treated with irinotecan may be resistant to the drug and, therefore, at higher risk for a negative therapeutic outcome.

Several reports have shown that alcohol induces UGT1A1 activity.[31,42] A recent study established a relationship between alcohol consumption and increased UGT1A1 glucuronidation rates on samples from human liver banks.[43] The long-term consequence of alcohol on this protein's activity is still controversial, however, and is likely to be due to the paucity of studies on this subject. Drinking habits can affect a patient's response to drugs that are substrates, inhibitors or inducers of the UGT1A1 enzyme.

Some studies have analysed how diet can influence UGT1A1 expression, particularly in people with *UGT1A1*28* (Table 3). Peterson *et al*. showed that, when determining the influence of four botanical groups, *Cruciferae*, *Rutaceae*, *Liliaceae* and *Leguminosae*, on UGT1A1 activity as measured by serum bilirubin, there was a significant association between *Cruciferae* intake (eg cabbage and broccoli) and the **28* polymorphism.[42] Specifically, participants homozygous for the **28* genotype experienced a decrease in bilirubin levels with *Cruciferae* intake compared with the group that had no intake of vegetables from this botanical family. It was suggested that *UGT1A1*28* heterozygous and *UGT1A1* wild-type homozygous individuals — since they have intrinsically lower levels of bilirubin compared with **28* homozygotes — may require a higher intake of cruciferous vegetables in order to detect the same correlation.

The effect of a fruit and vegetable diet on UGT1A1 activity was also studied, by means of bilirubin concentrations measured on days 8 and 15 after the start of a prescribed two-week feeding period consisting of a basal diet either

**Table 3.** Lifestyle factors that influence UGT1A1 activity

| Environmental factor | Summary | Ref. |
|---|---|---|
| *Cruciferae* intake | Homozygotes for the UGT1A1*28 showed decreased bilirubin concentrations after consuming cruciferous vegetables | 42 |
| Citrus fruit intake | Women with two UGT1A1*28 alleles that consume citrus fruit may exhibit a higher activity of this gene than those who do not include it on their diet | 44 |
| Fruit and vegetable diet | The intake of cruciferous vegetables, soy foods and citrus fruit seems to influence the decrease in bilirubin levels in women that are UGT1A1*28 homozygous | 45 |
| Cigarette smoking | Smoking cigarettes can be related to a lower exposure to the metabolite of irinotecan, which can lead to less haematological toxicity | 41 |

supplemented with fruits and vegetables or devoid of fruits and vegetables.[45] A decrease in bilirubin levels was found in women homozygous for *UGT1A1*28* on the supplemented diet;[44] however, the significance of the decrease in bilirubin was not readily gleaned from the paper. A limitation of this study was the composition of the study group. Among the **28* homozygous males there were no Asians, despite the fact that Asians were well represented in the *UGT1A1* wild-type group. As is known, in Asians the *UGT1A1*6* polymorphism is more relevant than the *UGT1A1*28* polymorphism for predicting hyperbilirubinaemia, which indicates that it could have been important also to analyse the presence of this variant in the study population. In the female group of *28 homozygotes, only Caucasian women were represented and studied. This indicates that the correlation identified may be relevant only in Caucasian women.

Citrus fruit was also found to be associated with a decrease in bilirubin concentrations, an association that may be relevant only among *28 homozygous Caucasian women.[44] This result, in combination with the previous one, suggests that gender may affect the way that individuals respond to diet, as well as the intrinsic activity of UGT1A1, since the basal bilirubin concentration was shown to be higher in men than in women.[42,44,45] Despite studying the effects of several botanical families on UGT1A1 activity, Saracino *et al.* found that only the citrus fruit interaction proved significant.[44] The correlation identified between *Cruciferae* intake and UGT1A1 activity referred to above was not replicated in the Saracino *et al.* study, which, according to the authors, could be due to the much lower intake of such vegetables.[44]

These three studies were performed in the Seattle area, which raises the matter of extrapolation. It is possible that environmental factors, to some extent, account for the observed results. The interaction of diet with UGT1A1 activity should be analysed in subjects of diverse ethnic backgrounds living in different geographical locations, as they would be exposed to a different environment, lifestyle and diet.

## Future direction

The findings presented here point to a slightly different direction for future investigations. It is of great importance to assess the clinical relevance of *UGT1A1* variants in individuals of ethnicities other than Caucasian, since the results that are found in Caucasian populations may not stand extrapolation. There is a need to identify ethnically specific *UGT1A1* variants because they can initially be better predictors of treatment outcome in different populations, which can aid in identifying the best therapeutic option available for every patient. In order to do so, it is important to include non-Caucasian individuals in studies and to sequence their entire *UGT1A1* gene. In so doing, novel functionally significant variants in this gene may be identified. Furthermore, the relevance of these functional variants should be studied in clinical settings. For instance, rather than looking only for the presence of *UGT1A1*28*, African people routinely should be tested for *UGT1A1*36* and *UGT1A1*37*, since these variants occur more frequently in those populations and may have some predictive value in terms of treatment outcome or drug toxicity. The same

scenario can be inferred for Asian individuals concerning *UGT1A1*6*, which should be clinically examined along with *UGT1A1*28*.

The impact of lifestyle on the basal enzymatic activity of UGT1A1 also needs further clarification, because altering quotidian and dietary habits may be useful in improving the response to drug treatment. To analyse these possible interactions, randomised controlled clinical trials should be performed where, for instance, subjects in one arm would be fed a reasonable intake of *Cruciferae* vegetables and citrus fruit, and in the other arm subjects would not have any intake of these vegetables and fruits. A clear baseline measure of bilirubin should be obtained. In terms of length of study period, the results would probably be more reliable if the trial was designed for more than two weeks and bilirubin measurements were performed periodically, at least once a week during the study period. It is not unusual for diet to affect the function of enzymes and the activity of drugs, consequently affecting treatment outcome. A clear example of diet–drug interaction is the well documented interaction between green leaves and the anticoagulant agent, warfarin.[46,47] A high intake of green tea or green leafy vegetables — particularly turnip greens and broccoli — antagonises the anticoagulant effect of the drug, due to their high vitamin K content, which can lower the international normalized ratio (INR) and disturb the anticoagulant properties of warfarin. In terms of *UGT1A1*, it is essential to ascertain which individuals would benefit from genotype-directed dietary alterations, their usefulness in different ethnicities and what exactly the dietary alterations may entail.

The incorporation of *UGT1A1* genotyping into clinical care depends on the drug or drugs that are going to be administered to the patient. In the case of irinotecan, testing is not required but it is recommended. Hoskins *et al.* demonstrated that low to medium doses in patients homozygous for *UGT1A1*28* do not give rise to toxicity; however, when a high dose is administered, neutropenia is more likely to occur.[15] Therefore, the pharmacogenetic test should be performed at the outset of treatment if the dose is going to be higher than 150 mg/m$^2$. If the dose is lower than this, the test should be performed if the patient experiences severe adverse reactions after the first treatment cycle. Lowering the dose of irinotecan following *UGT1A1* genotyping is a complex decision that has to be thoroughly studied because it has been reported that a reduction in tumour responsiveness may occur.[48]

Non-genetic factors also have to be considered when creating therapeutic algorithms. As an example, if a correlation between the intake of certain vegetables or fruits and a significantly higher UGT activity in people with the *28 allele is established, the knowledge of their dietary habits may partially indicate how they will react to irinotecan, and those habits can be modelled to help to improve treatment outcome. For example, a *28/*28 patient on a 250 mg/m$^2$ dose of irinotecan could be advised to eat a high number of servings of broccoli and cabbage per day to increase their elimination of SN-38 (Figure 2). Algorithms like this, which take genetic and non-genetic factors into account, could be modelled to help to improve treatment outcome.

To perform genotyping assays routinely may be cost-effective for some drugs and not for others. A decision-analytical model evaluating the cost-effectiveness of *UGT1A1*28* genotyping before administering 5-fluorouracil, leucovorin and irinotecan suggested that, although genotyping may have the ability to improve treatment outcome and quality-adjusted life years, it would only be cost-saving if the clinical efficacy was maintained after a dose reduction in *28 homozygotes.[49] Obradovic *et al.* also studied the cost-effectiveness of *UGT1A1*28* genotyping in second-line, high-dose, three-weekly irinotecan monotherapy treatment of colorectal cancer.[50] Effectiveness was evaluated in terms of prevention of severe neutropenia and number of life-years gained. This base-case model showed that reducing the dose of irinotecan in patients identified as homozygous for the *28 variant was cost-saving in Caucasian and African-descendant subjects, but not cost-effective in Asian-descendant individuals. The use of C-GSF as prophylaxis was not cost-effective in any population under study. These observations support the

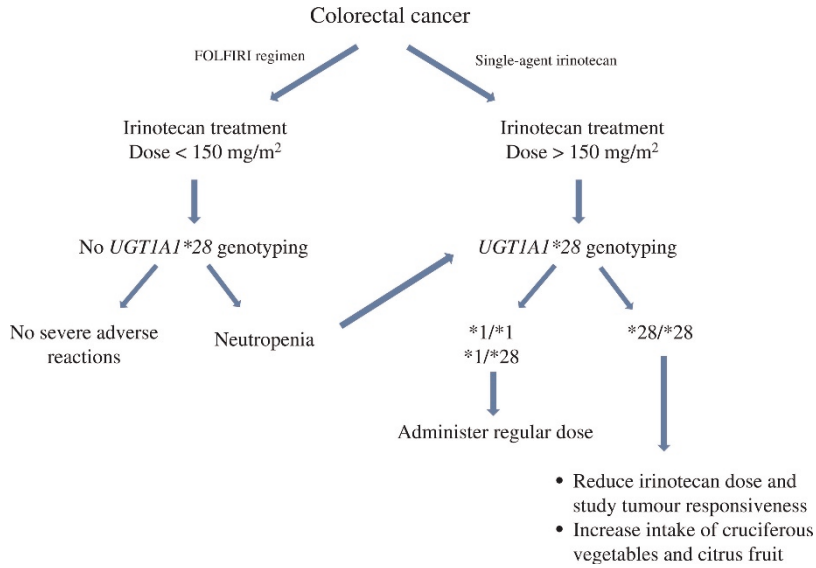


**Figure 2.** Irinotecan and UGT1A1 genotyping clinical decision algorithm.

need for identification of *UGT1A1* variants relevant in individuals of different ethnicities. That is crucial information for the design of cost-effectiveness studies like the one referred to above, in which a cost-saving relationship may have been found between Asian subjects and *UGT1A1*, if the **6* allele had also been genotyped.

Finally, when planning the incorporation of pharmacogenetic testing into clinical practice, physician education and that of other healthcare professionals cannot be disregarded. It is very important that physicians recognise the benefits of pharmacogenetics and learn how to interpret the results of such tests. Future generations of physicians, pharmacists and nurses should be educated on the matter during their professional training, not only during their careers. The cooperation between physicians, pharmacists and nurses should also be strengthened so that there is an interdisciplinary approach to treatment decisions based on pharmacogenetic tests. With the incorporation of all the steps outlined above, the ultimate goal of personalised medicine can become a reality for all populations.

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