

1 **PREVALENCE OF UGT1A1 GENETIC VARIANTS IN ARGENTINEAN POPULATION,**
2 **POTENTIAL IMPLICATIONS FOR PHARMACOGENOMIC TESTING**

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15 **ABSTRACT**

16 The four groups of uridine diphosphate glucuronosyltransferase (UGT) enzymes form a
17 superfamily responsible for the glucuronidation of target substrates. These include hormones,
18 flavonoids, environmental mutagens and pharmaceutical drugs. Thus, UGT enzymes are
19 relevant for pharmacogenetic research.

20 Most of the members of the UGT family are expressed in the liver, but are also present in
21 intestinal, stomach or breast tissue.

22 The incidences and types of polymorphisms for different enzymes vary with geographical
23 regions and ethnic groups. This is the first study that examined the frequency of polymorphisms
24 for UGT1 isozymes for a population of 100 healthy argentinians.

25 The distribution of UGT1A1 in our population was: 70.5% (70.5) for the *1 allele, 21.5% for the
26 *28 allele and 1% for the *36 allele. 48% (48) presented the *1/*1 genotype, while 43 % (43) had

27 *1/*28, 2% (2) had *1/*36 and 7% (7) showed *28/*28. There was no preferential sex
28 distribution.

29 Since most Argentinians are of Caucasian descent, a European genotype frequency profile is to
30 be expected. That is evident in the wild type prevalence in our population. However, the
31 contribution of Native American ancestry to gene pool components may in part explain the
32 higher prevalence of the *28 genotype in UGT1A1 *1 in our population, in comparison with
33 European cohorts.

34 **INTRODUCTION**

35 The uridine diphosphate glucuronosyltransferase (UGT) enzymes conform a superfamily of
36 enzymes responsible for the glucuronidation of target substrates. The transfer of glucuronic acid
37 renders xenobiotics and other endogenous compounds water soluble, allowing for their biliary or
38 renal excretion. The UGT superfamily is responsible for the glucuronidation of hundreds of
39 compounds, including hormones, flavonoids and environmental mutagens. Most of the
40 members of the UGT family are expressed in the liver, as well as in other type of tissues, such
41 as intestinal, stomach or breast. A few members are expressed only outside the liver such as
42 UGT1A7, UGT1A8, UGT1A10 and UGT2A1. UGT superfamily is composed by four families:
43 UGT1A, UGT2, UGT3 and UGT8. UGT2 is further divided into two subfamilies, UGT2A and
44 UGT2B, both of which present on chromosome 4. Although limited studies are already available
45 on UGT2A enzymes, they appear to be involved in the glucuronidation of compounds such as
46 phenolic odorants and polycyclic aromatic hydrocarbon metabolites. UGT2B proteins are mainly
47 responsible for the metabolism of steroids. The roles of UGT3 and UGT8 family members have
48 not been well characterized yet.

49 The UGT1A family is located on chromosome 2q37(1), and members of this group
50 glucuronidate a large variety of compounds. Pharmaceutical drugs are also a common substrate
51 of the UGT family, turning the enzymes in this group relevant for pharmacogenetic research(2).

52 Bilirubin-UGT (*UGT1A1*) conjugates bilirubin with glucuronic acid, converting the bilirubin into a
53 water-soluble form that is readily excreted in bile. Mutations of bilirubin UDP-glucuronosyl
54 transferase causes hereditary unconjugated hyperbilirubinemias, including Crigler-Najjar and
55 Gilbert syndromes(3).

56 The genetic defect in patients with Gilbert syndrome involves the promoter region of
57 *UGT1A1*(4,5). Gilbert syndrome manifests only in people homozygous for the variant promoter.
58 As a result, its inheritance is most consistent with an autosomal recessive trait. However,
59 heterozygotes for the Gilbert genotype have higher average plasma bilirubin concentrations
60 compared with those with two wild-type alleles. It is estimated that 9 percent of individuals in the
61 Western general population world are homozygous for the variant promoter, and up to 42
62 percent are heterozygous(5).

63 Characterization of the *UGT1A* gene locus has permitted an understanding of the molecular
64 defects responsible for the Gilbert syndrome. The mutation responsible for Gilbert syndrome is
65 in the promoter region, upstream to exon 1 of *UGT1A1*. The normal sequence of the TATAA
66 element within the promoter is A[TA]6TAA. Caucasian and black patients with Gilbert syndrome
67 are homozygous for a longer version of the TATAA sequence, A[TA]7TAA, which causes
68 reduced production of bilirubin-UGT. This variant is termed *UGT1A1*28*(6).

69 This longer TATAA element has been found in all individuals with Gilbert syndrome studied in
70 the United States, Europe, and countries of the Middle East and South Asia. However, other
71 factors are probably involved in the expression of Gilbert phenotype since not all patients who
72 are homozygous for the variant promoter develop hyperbilirubinemia. Furthermore, in the

73 Japanese population, other mutations within the coding regions of *UGT1A1* can be the
74 underlying cause of the Gilbert phenotype.

75 Since bilirubin-UGT is involved in the glucuronidation of several important drugs, individuals with
76 Gilbert syndrome may be more susceptible to the toxic effect of substances that require
77 bilirubin-UGT-mediated hepatic glucuronidation prior to excretion. Gilbert syndrome is known to
78 increase the risk of drug toxicity with irinotecan and the hyperbilirubinemia associated with
79 atazanavir(2,7).

80 The active metabolite of irinotecan, SN-38, is glucuronized in the liver mainly by bilirubin-UGT.
81 The major dose-limiting toxicity of this drug is diarrhoea. In patients who inherit certain *UGT1A1*
82 polymorphisms, reduced glucuronidation of SN-38 leads to an increased incidence of diarrhoea.
83 The symptoms can be severe enough to warrant switching to other drugs. Thus, it is currently
84 recommended to test the *UGT1A1* polymorphism prior to the administration of Irinotecan and to
85 adjust the dose according to the resulting genotype(8).

86 As previously stated, some drugs may induce hyperbilirubinemia in patients with Gilbert
87 syndrome. Atazanavir, an antiretroviral medication, is an inhibitor of bilirubin-UGT activity and is
88 associated with hyperbilirubinemia(9). Isolated hyperbilirubinemia has also been reported during
89 the treatment of hepatitis C with peginterferon and ribavirin and in patients receiving pazopanib.
90 In such cases, discontinuation of therapy is usually not necessary(10).

91 The incidences and types of the polymorphisms for these enzymes are quite different according
92 to geographical regions and ethnic groups. The aim of this study is to estimate the prevalence of
93 *UGT1A1* polymorphism in the Argentine population and to evaluate what clinical implications
94 this might have.

95 **MATERIAL AND METHODS**

96 [dx.doi.org/10.17504/protocols.io.6sjhecn](https://doi.org/10.17504/protocols.io.6sjhecn)

97 **Study population**

98 One hundred random and anonymized DNA samples from healthy donors were analysed. The
99 Hospital Italiano de Buenos Aires DNA Bank collection project has the approval of the local
100 ethic committee and all the volunteer subjects signed an informed consent.

101 **Sample collection and DNA extraction**

102 Following an informed consent process, 10 mL of peripheral blood were collected from each
103 subject in 5-mL EDTA tubes. Whole blood samples were stored at 4°C until the time of
104 processing. Genomic DNA was extracted and purified using the QI Amp DNA Blood Mini kit
105 (QIAGEN).

106 **Genotyping of UGT1A1**

107 Genomic DNA was extracted from 200 µL of whole blood using QIAamp DNA Mini kit (Qiagen,
108 GmbH, D-40724 Hilden, Germany). The polymerase chain reaction (PCR) was performed in a
109 final volume of 20 µL. The forward primer was 5'- CAGCCTCAAGACCCACACA – 3' and the
110 reverse primer was 5'- TGCTCCTGCCAGAGGTTC -3'. The PCR conditions were 5 minutes at
111 95°C, followed by 35 cycles of 30 seconds at 95°C, 60 seconds at 61°C, 60 seconds at 72°C,
112 and final extension for 10 minutes at 72°C. The PCR product was detected on 2% agarose gels
113 by means of ethidium bromide staining. The presence of variant UGT1A1 was confirmed by
114 direct sequencing of PCR products on an automated ABI 3100 capillary sequencer (Applied
115 Biosystems, Foster City, Calif) using the Big Dye Terminator Cycle Sequencing Kit (Applied
116 Biosystems).

117 **STATISTICAL ANALYSIS**

118 Allelic and genotypic frequencies were expressed in absolute and percentage values in the
119 study population. Sample size calculation based on the estimation of a 4% prevalence of TT
120 genotype as published for european population was of 64 samples, while for a prevalence of 7
121 % as in global population was of 110 samples, with an alpha error of 5 %. The results obtained
122 were further evaluated for Hardy-Weinberg equilibrium.

123 **RESULTS**

124 The genotyping of all samples was according to the method previously described. Among
125 the 100 subjects analysed, 49% (49) were male and 51% (51) were female with a median age
126 of 43 and a range of 32-80 years.

127 In our population, the distribution of UGT1A1 was -Figure 1 attached separately-: 70.5%
128 (70.5) for the *1 allele, 21.5% for the *28 allele and 1% for the *36 allele. 48% (48) presented
129 the *1/*1 genotype, while 43 % (43) had *1/*28, 2% (2) had *1/*36 and 7% (7) showed *28/*28.
130 No preferential sex distribution was observed between genotypes.

131 **DISCUSSION**

132 UGT 1A1 is the major UGT isoform responsible for glucuronidation of bilirubin in human liver,
133 and it is capable of conjugating various phenols, anthraquinones and flavones. Mutations of
134 bilirubin UDP-glucuronosyl transferase causes hereditary unconjugated hyperbilirubinemia and
135 can also alter the metabolism of certain drugs.

136 In our population of 100 healthy argentinian volunteers, the distribution of the UGT1A1 showed
137 a predominance of the *1 allele whereas the *28 allele showed a relatively high prevalence of
138 21,5%.

139 Compared to this, in a cohort of 245 healthy men and women, aged 20-40 years of Caucasians
140 and Asians the frequencies of the UGT1A1 genotypes were 53,7% for 6/6 *1, 34,8% for 6/7 *1-
141 28, 9,8% for 7/7 *28, 0.8% for 5/6 and 0.8% for 6/8 *1-38 promoter TA repeats. Both allele and
142 genotype frequencies varied by race ($P < 0.02$), with 11% of the Caucasians and none of the
143 Asians having the 7/7 *28 genotype. Overall, 8% were homozygous variant for both UGT1
144 polymorphisms and 43% had at least one variant allele for both UGT1A1*28 and
145 UGT1A6*2(11).

146 The incidences and types of the polymorphisms for these enzymes vary according to
147 geographical regions and ethnic groups. This is the first study that has examined the frequency
148 of polymorphisms for UGT1 isozymes for a population of healthy argentinians.

149 Since most Argentinian inhabitants are of Caucasian descent, a European genotype frequency
150 profile is to be expected. That is evident in the wild type prevalence in our population and the
151 one from Lampe et. al. However, the contribution of Native American ancestry to gene pool
152 components may in part explain the higher prevalence of the *28 genotype in UGT1A1 *1 in our
153 population, in comparison with the 11% observed in the european study. This finding provides
154 additional information to the ongoing controversy regarding which population -if any- can be
155 used as a general reference for Argentinian genotypic profiles.

156 A limitation of our results is that all the volunteers evaluated are from the Capital City of Buenos
157 Aires, Argentina. This does leave aside a considerable amount of variant genomics particularly
158 in a vast country that has a longstanding history of racial mixture.

159 Understanding these polymorphisms and the characterization of these in different populations is
160 essential for the prevention of adverse effects of a considerable number of drugs and also for
161 the reduction of cancer-associated risks.

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Figure 1: UGT1A1 Genotypes distribution in Argentinian population

