A Combinatorial Haplotype of the UDP-Glucuronosyltransferase 1A1 Gene (#60-#IB) Increases Total Bilirubin Concentrations in Japanese Volunteers

To the Editor:

UDP-glucuronosyltransferases (UGTs) are a family of enzymes that glucuronidate many endogenous and exogenous substrates *(1)*. Of the *UGT1A* gene isoforms, *UGT1A1* is primarily responsible for glucuronidation of bilirubin *(1)*. In east Asians, 2 well-known genetic variants, $A(TA)_6TAA>A(TA)_7TAA$ (allele **28*, reduced transcription) and G71R (211G>A, allele **6*, reduced activity), are causative factors for increased plasma bilirubin concentrations in Gilbert syndrome *(1)*. The **28* allele is almost always linked to the **60* allele (-3279T>G), with reduced in vitro transcription *(2)*.

In a previous study *(2)* in which we divided *UGT1A1* into 2 haplotype blocks (the 5′-flanking region and exon 1 in block 1 and common exons 2 to 5 in block 2), **60* and **IB* (perfectly linked 1813C>T, 1941C>G, and 2042C>G in the 3′-untranslated region in Japanese persons) showed increased total bilirubin concentrations in non-**28* patients. Because of the small number of patients, however, it was not clear whether bilirubin concentrations were affected by **60* and **IB* acting independently or cooperatively when they were on the same chromosome. To clarify this point, we reinvestigated the associations between the *UGT1A1* haplotypes and total bilirubin concentrations in 554 healthy Japanese volunteers. The ethical review boards of the participating institutions approved this study, and informed consent was obtained from all participants.

For genotyping of **60*, **28*, **6*, and **IB* marker variations, DNA was extracted from Epstein-Barr-virus–transformed lymphoblastoid cells. The genotyping methods for the **60*, **6*, and **IB* alleles were described previously *(3, 4)*. For **IB*, 1941C>G was genotyped *(3)*. For **28*, −364C>T, which is perfectly linked with the **28* allele in Japanese persons *(2)*, was used as a surrogate polymorphism, as described in Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol53/issue2, which also shows the allele frequencies of the variations. The diplotype configuration (combination of haplotypes) for each volunteer was inferred by an expectation-maximization–based program, LDSUPPORT, as

described previously (2, 5). Diplotype configurations of the 521 volunteers without heterozygous *60 and *IB were obtained at 1.00 probability. Previously, we reported that *UGT1A3* 17A>G is linked with both *60 and *IB (5), and *IB is not linked with *28 and *6 (2). When *UGT1A3* 17A>G was included, all the diplotype configurations were inferred with >0.95 probability.

To differentiate between allele and haplotype names, haplotypes are indicated by the # symbol plus the representative allele name. The haplotypes without marker variations were designated #1 for Block 1 and #IA for Block 2 (2). Note that the *28 allele was perfectly linked with the *60 allele, but only half of the *60 allele, approximately, was linked with the *28 allele. Thus, the #28 haplotype harbors both *28 and *60 alleles, whereas the #60 haplotype harbors only the *60 allele, as reported previously (2). The most frequent haplotype was #1-#IA (frequency, 0.545), followed by, in order, #6-#IA (0.171), #28-#IA (0.107), #60-#IA (0.079), #1-#IB (0.060), and #60-#IB (0.038).

We investigated the association of *UGT1A1* haplotypes with total bilirubin concentrations (Fig. 1). *P* values <0.05 were considered significant. We used the Kruskal–Wallis test ($P < 0.0001$) for statistical analysis of the differences in bilirubin concentrations among all diplotypes, followed by the nonparametric Dunnett multiple comparison test. Significant increases in bilirubin concentrations were observed in the #6-#IA/#28-#IA, #6-#IA/#6-#IA, #6-#IA/#60-#IB, #60-#IA/#28-#IA, and #28-#IA/#28-#IA volunteers compared with the #1-#IA/#1-#IA volunteers. An increasing trend (statistically not significant) in bilirubin concentrations (2.4-fold increase) was seen in the two #60-#IB/#28-#IA volunteers compared with the #1-#1A/#1-#1A volunteers. Significant increases in bilirubin concentrations have already been reported for #6 (#6-#IA in this study) and #28 (#28-#IA) (1). Note that the median of total bilirubin values was not increased in the heterozygotes of #6-#IA (#1-#IA/#6-#IA) and #28-#IA (#1-#IA/#28-#IA; Fig. 1).

We next analyzed the additive effects of #60-#IB and #60-#IA on #6-#IA and #28-#IA, respectively. A significant increasing effect of #60-#IB on #6-#IA was observed for #6-#IA/#60-#IB compared with #1-#IA/#6-#IA ($P = 0.0093$; Mann–Whitney *U*-test). However, when #60-#IA/#28-#IA was compared with #1-#IA/#28-#IA, the effect of #60-#IA was not statistically significant ($P = 0.0513$).

This study shows that either #60 or #IB alone has a slight effect on total bilirubin concentrations. The presence of both #60 and #IB on the same DNA strand (#60-#IB), however, significantly increased bilirubin concentrations when present with #6-#IA on the other chromosome. Thus, at least in the Japanese population, #60 and #IB marker variations should also be incorporated into the *UGT1A1* genotyping in addition to #6 and #28 markers.

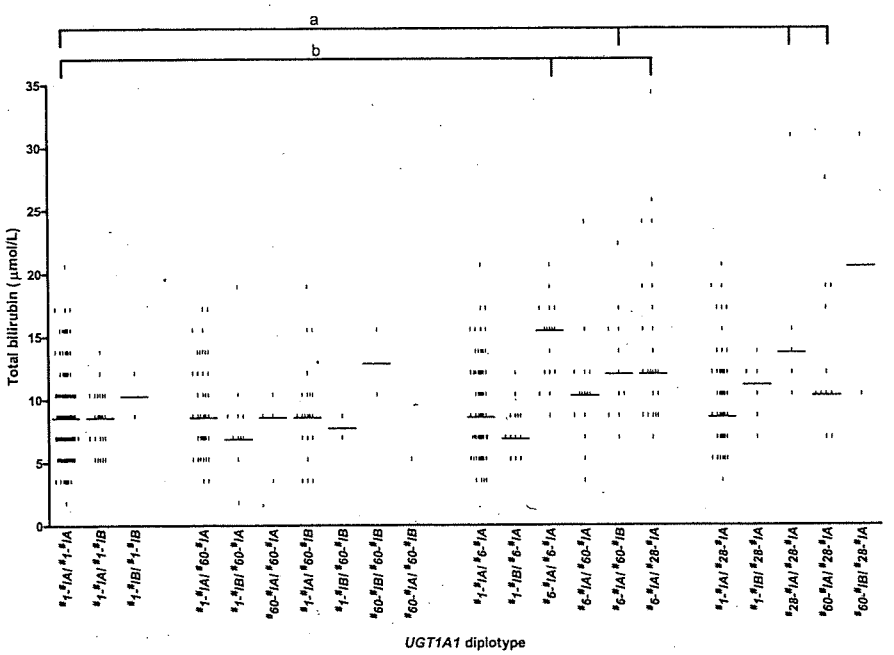

Fig. 1. Association of the *UGT1A1* diplotypes with increased total bilirubin concentrations in 554 Japanese healthy volunteers.

Haplotypes are shown with # plus representative allele name. Note that haplotype #28 harbors both *28 and *60 alleles (see Fig. 1 in the online Data Supplement). Each *point* represents 1 volunteer, and the median is indicated by a *horizontal bar*. The Kruskal–Wallis test for the 21 diplotypes yielded a *P* value of <0.0001. Significant increases in bilirubin concentrations were detected in #6-#IA/#28-#IA ($P < 0.0001$), #6-#IA/#6-#IA ($P < 0.0001$), #6-#IA/#60-#IB ($P = 0.0133$), #60-#IA/#28-#IA ($P = 0.0186$), #28-#IA/#28-#IA ($P = 0.0213$), compared with #1-#IA/#1-#IA (nonparametric Dunnett multiple comparison test). *a*, $P < 0.05$; *b*, $P < 0.0001$.

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