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Original Article

EFFECT OF THE GLOBAL VARIATION OF THEGENETIC BIOMARKER URIDINE DIPHOSPHATE GLUCURONOSYL TRANSFERASE

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ABSTRACT

Objective: Uridine diphosphate glucuronosyltransferase (UGT1A1) has been used as a pharmacogenetic marker in irinotecan treatment, and in assessing neonatal jaundice and acetaminophen hepatotoxicity. The study aims at genotyping new population samples and investigating for the first time the global genetic variation of UGT1A1. In addition, the study examined the cost effectiveness of using UGT1A1.

Methods: UGT1A1*28 polymorphismsgenotyping was performed by PCR followed by Pyrosequencing.Extracteddata includedTA_7allele frequencies (AF)in different ethnic human populations across the world. The cost effectiveness of UGT1A1 pharmacogenetic tests was determined based on the irinotecan dose reduction in patients with TA7 allele.

Results: The results showed a new finding; i.e., UGT1A1*28 diversity reflects not only interethnic differences, but also a geographic distribution. In particular, decreased-function variants UGT1A1*28 were common in African and Southern Asian populations (AF= 0.41 and 0.43, respectively). The highest frequency of the active gene was found in Eastern and South Eastern Asian populations (AF= 0.88 and 0.87), respectively. Additionally, the implication for a pharmacogenetic biomarker use in irinotecan dosing would reduce the cost by 11%.

Conclusion: The study shows thatstratifying patients for drug response shall depart from the heterogeneity among different ethnic populations that must be considered in developing pharmacogenetic biomarkers.

Keywords: Cost Effectiveness, Genotype, Individualized Therapy, Polymorphism/,UGT1A1*28.

INTRODUCTION

Glucuronidation is a main mechanism of cellular detoxification of many exogenous and endogenous compounds by generating products that are more polar and, thus, more readily excreted in bile or urine.Glucuronidation is catalyzed by Uridine Diphosphate (UDP)-glucuronosyltransferase enzymes (UGT) [1]. UGT1A1 is the major UGT1 gene product of alternative splicing from UGT1A locus located on chromosome 2q37 that catalyzes the glucuronidation of bilirubin. Expression of UGT1A1 is, in part, controlled by a polymorphic dinucleotide repeat within the UGT1A1promoter TATA element consisting of between five and eight copies of a TA repeat with A $(TA)_6$TAA the most common considered the wild typeReduced expression of UGT1A1 is primarily caused by an insertion of two extra bases (TA) in TATAA element of the 5'promoter region creating A $(TA)_7$TAA (usually denoted as*UGT1A1*28).*

Mutations in the UGT1A1 gene would decrease the enzymatic activity and leads to hyperbilirubinemia [2], neonatal jaundice and acetaminophen hepatotoxicity[3].

UGT1A1 are also involved in the glucuronidation of many drugs. A typical example is given by irinotecan, an anticancer drug. Irinotecan is a prodrug; its hydrolysis by carboxylesterases (CES) produces active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38)[4].SN-38 is 100-1000 times more potent than irinotecan. SN-38 is inactivated through glucuronidation via UDP-Glucuronosyl Transferase (UGT) family predominantly UGT1A1 enzymes[5].The reduced level of functional UGT1A1, called Gilbert's syndrome or *28 allele, is associated with a high risk of irinotecan adverse drug reactions due to the relatively high level and prolonged exposure to the cytotoxic active form of the parent drug, SN-38.Patients homozygous for TA_7 are 3.5 times more likely to develop severe neutropenia compared with individuals homozygous for TA_6. Up to 35% of patients receiving irinotecan for metastatic colon cancer have been reported to experience dose-limiting toxicities[6,7]. In 2005, the FDA amended the product label of irinotecan to include a precautionary note warning of increased neutropenia and toxicity for patients who are homozygous for UGT1A1*28 allele. In the same year, FDA approved a molecular assay for the detection of Gilbert syndrome. In Japan, for example, the Ministry of Health has also approved the use of UGT1A1*28 to predict toxicity[8].The prevalence of UGT1A1*28 gene polymorphisms have been investigated in different populations and associated with irinotecan toxicities and dosing modifications, and at present, clinicians use UGT1A1 pharmacogenetic testing to consider alternative treatment options or a weekly dosing schedule instead of the 2- or 3- weekly dose if the testing reveals TA_7/TA_7.

However, pharmacogenetic tests currently used in genotyping *UGT1A1* to reduce the incidence ofsevere toxicity of irinotecan were developed withoutinitial identification of inter population differences. To our knowledge, there has not been any report investigating the UGT1A1*28 gene polymorphisms inter-ethnic differences. In this study, we aimedat genotyping new population samples and exploring for the first time the global genetic variation of UGT1A1. In addition, the study examined the cost effectiveness of using UGT1A1 in the application of irinotecan in personalized cancer treatment.

MATERIALS AND METHODS

UGT1A1 genotype frequency determination in Lebanese subjects

A total of 146 unrelated healthy Lebanese volunteers were recruited from the Lebanese population. All subjects signed a consent form. Included were non-obese subjects (body mass index (BMI) < $29.5Kg/m^2$), with no history or clinical evidence of diabetes, cardiovascular problems, hypertension, renal insufficiency, and/or depression. All study subjects are of Lebanese origin, and living in Lebanon at the time of study. Exclusion criteria were set to achieve parity with other studies.

DNA was isolated from cheek swabs by a method previously described[9].DNA isolated from buccal cells samples was used for the analysis of the variants of UGT1A1*28.

Genotyping of *UGT1A1*28* promoter (TA repeats) polymorphismwas performed by PCR followed by Pyrosequencing[10]. Genotyping analysis was carried out using a PSQ HS 96 System (Biotage, Uppsala, Sweden). The PCR and sequencing primers were designed based on previously published Pyrosequencing assay[11]. The Pyrosequencing assays was performed according to the manufacturer's recommendations. Pyrosequencing assays results are depicted in figure 1 showing the TA_6/TA_7genotype. Volunteers could be divided into three different groups:TA_6/TA_6(wild type), TA_6/TA_7(heterozygous mutant), or TA_7/TA_7(homozygous mutant).

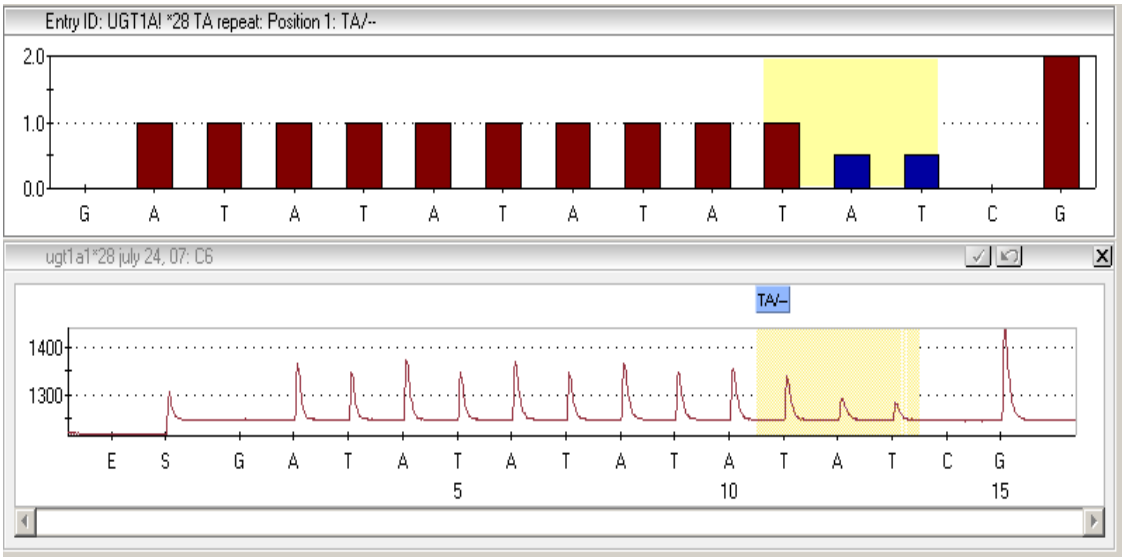

Fig. 1: UGT1A1*28 TA6/TA7 genotype: a Pyrosequencing sample of the results

UGT1A1 gene global mapping

We identified literature, published in English between 1984 and 2013, reporting UGT1A1 gene polymorphisms. The extracted data are summarized and tabulated in Table 2. Excluded were studies of a small sample size (<40), studies where the subjects' origins were unknown and where subjects were known to be suffering from a disease.

The exception was the Bangladeshi and Tunisian samples, which comprisea small sample size of 26 and 28 individuals, respectively. Both samples were included due to the shortage of available samples from Southern Asia, and North Africa different countries, but appear to display allele and genotype frequencies consistent with those observed in the region.

UGT use and Cost effectiveness

The use of pharmacogenetic tests prior to or during the treatment with irinotecan was investigated. Accredited public and/ or private hospitals that have an oncology department were identified through a search of the Lebanese Ministry Of Public Health website[12]. Included were teaching hospitals that serve cancer patients. Irinotecan dosing based on any pharmacogenetic/ clinical test conducted prior or during the irinotecan treatment was examined.

The amount and cost of irinotecan use in Lebanon for the year 2011 (January 1st, 2011 till December 31st, 2011) were determined from the Lebanese Order of Pharmacists and the Ministry of Public Health databases. The cost effectiveness analysis included the calculated cost of irinotecan use in the year 2011 on one hand, and an estimation of the irinotecan cost taking into account the study results' allele frequency of TA_7on the other hand; i.e., the cost of irinotecan with and without the use of the pharmacogenetic testing. Test performance, chemotherapy toxicity, additional hospitalization expenses and quality of life, were not included.

Statistical Analysis

Sample size was determined a priori by use of statistical power analysis with SAS software (SAS Institute, Cary, NC). Calculations revealed that a minimum of 120 samples are required for the genotype representation in the Lebanese population[13]. Statistical analyses were performed using the SPSS® version 19 for Windows. The study samples alleles and genotypes frequencies were estimated by gene counting method. The agreement with Hardy-Weinberg equilibrium of the observed genotypic distribution was tested by chi-square tests. A P value of < 0.05 was considered statistically significant. The distribution of genetic variation among different populations was analyzed by descriptive statistics and population comparisons were performed by Chi-square test of population differentiation. The cost effectiveness was determined based on the irinotecan dose reduction in patients with TA_7 allele.

Reproducibility was assessed by analyzing 42 samples in duplicate DNA. The study was approved by the School of Pharmacy Research Committee at the Lebanese American University.

Table 1: Distribution of UGT1A128* genotypes and allele frequencies in Lebanese**

Genotype	Observed Genotype N (%)	Observed Allele Frequency	Expected Genotype N (%)	Chi square P
TA7/TA7	23 (16)	TA7: 0.38	21 (14.5)	0.699
TA6/TA6	59 (40)	TA6: 0.62	56 (38.5)	
TA6/TA7	64 (44)		69 (47)	
All	146 (100)		146 (100)	

TA6/TA6 (wild type), TA6/TA7 (heterozygous mutant), or TA7/TA7 (homozygous mutant

RESULTS

Subjects Demographic Characteristics and UGT1A1 gene polymorphisms

A total of 146 Lebanese subjects were included in the study, which intended to determine the *UGT1A1* gene polymorphismprevalence in the Lebanese population. The study samples consisted of 51.9% and 48.1% males and females, respectively. The mean age was 20. 63 years (range: 18-69 years) and the average BMI was 21.04 Kg/m^2 (range: 17.15- 28.41 Kg/m^2).

UGT1A1*28 allele and genotype frequencies did not deviate from Hardy-Weinberg equilibrium (Chi square test P=0.699). The genotype and frequency distribution in Lebanese include TA_7/TA_7(16%), TA_6/TA_6(40%) and TA_6/TA_7(44%) as shown in table 1.

UGT1A1 allele frequencies global mapping

Table 2 shows *UGT1A1*28 TA7* allele frequencies (AF) among different populations of different ethnic groups and based on different geographic regions. The results showed a new finding; i.e., UGT1A1*28 diversity reflects not only interethnic differences, but also a geographic distribution. In particular, decreased-function variants UGT1A1*28 were common in African and Southern Asian populations (AF= 0.41 and 0.43, respectively). The highest frequency of the active gene (AF= 0.88 and 0.87) was found in Eastern and South Eastern Asian populations, respectively. Moreover, AFs distribution in Lebanese was found close to that of other Caucasian groups (P=0.156-0.564).

Table 2: Distribution of UGT1A1 genotypes in different ethnic groups as compared to Lebanese

	Reference	Population	TA7	N	P
Asians					
Eastern			**12.08**	2635	0.0032
	[23-26]	Japan	11	634	
	[27, 28]	Korea	11.5	405	
	[29-31]	China	12.7	1378	
	[32]	Taiwan	12.4	218	
South-Eastern			**13.13**	530	0.0001
	[31-33]	Thailand	13.7	172	
	[31]	Vietnam	8.4	83	
	[34]	Philippines	12	72	
	[35, 36]	Malaysia	13.2	143	
	[31]	Indonesia	19.2	60	
Southern			**43.18**	824	0.2434
	[31]	Bangladesh	48.1	26	
	[31, 37]	India	40.5	569	
	[31]	Sri-Lanka	49.3	229	0.03
Middle Eastern			**33.25**	254	0.3381
	[31]	Yemen	25.4	61	
	[Current Study]	Lebanese	38	146	1
	[31]	Cyprus	28.7	47	
Europeans			**32.43**		
Northern			**31.56**	5656	0.1565
	[31]	Iceland	34.1	69	
	[31]	UK	27.1	59	
	[38]	Sweden	32.3	248	
	[20]	Scotland	36	77	
	[39]	Norway	30.5	70	
	[40]	Denmark	31.5	5133	
Western			**32.34**	1254	0.1687
	[41]	France	32.5	75	
	[42-44]	Germany	33.7	567	
	[45]	Netherland	33	399	
	[46]	Portugal	28	98	
	[47]	Spain	27	115	
Southern			**33.54**	1219	0.2827
	[48]	Greece	40.1	186	
	[25,49, 50]	Italy	32.4	937	
	[51]	Macedonia	32	96	
Eastern			**35.57**	1154	0.5641
	[52, 53]	Czech	38	986	
	[54]	Russian	31.8	118	
	[55]	Slovenia	40	50	
Africans			**40.88**	224	0.5804
Northern	[56]	Tunis	37.5	28	
Middle	[25]	Central Africa	56	42	0.0386
Eastern	[57]	Kenya	44.4	80	
Western	[31]	Ivory Coast	35.8	74	
Northern America	[58]	Canada	**34.5**	656	0.4233
African- Americans	[23,57,59, 60]		**40**	489	0.6646
African-Brazilian	[61, 62]		**40.7**	54	0.7282
Hispanics	[25]		**37.5**	50	0.9499
Caucasians	[23,57,59, 61,62, 63,64]		**33.61**	786	0.3051
White Europeans	[64]		**32.5**	1780	0.1743

Table 3: Irinotecan use in Lebanon in the year of 2011: products, quantity, price, and total cost

Name	Dosage	Agent	Laboratory	Country of origin	Price in LBP	Quantity	Total Price
	1 vial of concentrate solution for infusion				1USD=1510 LBP		
Campto Concentrate For Infusion	100mg/ 5mL	Food & Drug Corporation FDC	Pfizer (perth) pty Ltd	Australia	320,893	3150	LBP 1,010,812,950
Campto Concentrate For Infusion	40mg/ 2mL	Food & Drug Corporation FDC	Pfizer (perth) pty Ltd	Australia	149,109	1350	LBP 201,297,150
Irinogen	100mg/ 5mL	UPO S.A.L.	Bioprofarma S.A.	Argentine	355,422	0	LBP 0.00
Irinotecan Ebewe	300mg/ 15mL	Codipha	EbewePharma GesmbH.NFG.KG	Austria	847,873	0	LBP 0.00
Irinotecan Ebewe	40mg/ 2mL	Codipha	EbewePharma GesmbH.NFG.KG	Austria	137,957	590	LBP 81,394,630
Irinotecan Ebewe	100mg/ 5mL	Codipha	EbewePharma GesmbH.NFG.KG	Austria	292,370	950	LBP 277,751,500
Irinotecan Thymogran	40mg/ 2mL	Hikma-Liban S.A.R.L.	ThymoogranPharmazie GmbH	Germany	110,840	320	LBP 35,468,800
Irinotecan Thymogran	100mg/ 5mL	Hikma-Liban S.A.R.L.	ThymoogranPharmazie GmbH	Germany	224,798	1350	LBP 303,477,300
Trinotecan	100mg/ 5mL	Macromed S.A.R.L.	Filaxis SA	Argentine	355,310	1300	LBP 461,903,000
			Total Cost use in LBP				LBP2,372,105,330
			Total Cost in USD				$1,570,930
			Total decrease in Cost with Pharmacogenetic use testing				$172,802

Pharmacogenetic biomarker use and cost effectiveness

A total of eight accredited hospitals by the Ministry of Public Health that include an oncology department were included in this study survey. Seven out of eight are teaching hospitals. The number of beds ranges from 200 to 600 per hospital. Reported cancer cases according to the last published National Cancer Registry arean approximate average of 7500 cases per year in the Lebanese population that accounts for almost 4M[14]. The study results showed that irinotecan is used in all the hospitals that serve cancer patients, but there is no implication for any pharmacogenetic test use in irinotecan dosing. Oncologists mainly rely on patients' laboratories tests values i.e., liver and kidney function, Body Surface Areaand demographic (e.g. age) characteristics in irinotecan dosing.

Irinotecan is available in Lebanon under five different brands in three various dosages as shown in table 3. The cost of irinotecan uses in Lebanon for the year 2011 (January 1st, 2011 to December 31st, 2011) accounts for $1,570,930.68 USD.

Since dose reduction should be considered in a patient with TA7 allele (heterozygote and homozygote), estimated was the dose reduction based on of TA7 allele frequency in Lebanese. With the pharmacogenetic test, irinotecan dosage should have been reduced by 25% in patients with UGT1A1*28 homozygous polymorphism and 16% in heterozygotes[15], and then the cost could have been reduced to a total of 11% to account for $172,802.38 USD.

DISCUSSION

There is emerging evidence that UGT1A1 is a pharmacogenetic marker for drugs such as irinotecan and that it may be useful in assessing neonatal jaundice[2,16,17], and hepatotoxicity [3] suggesting future applications of this assay. Understanding the worldwide distribution of markers is useful in the strategic planning of the future implementationof pharmacogenetic testing for gene variants; thus optimizing the intake of drugs metabolized by UGT. The study, to our knowledge, is the first to examine the global variation of UGT polymorphisms plus a sample representative of the Lebanese general population.

The UGT1A1*28 allele frequency in control subjects in different populations of different countries was thoroughly examined and compared. Nevertheless, we accept that the comparison of the allele frequencies with different published studies has to be considered with some caution since published data might have been generated using slightly different methodologies, and thus there is a possibility of discrepancies in genotype classification [18]; thus data comprised and averaged different studies results on the same populations.

The genotype distribution in Lebanese did not significantly differ from that of Caucasians.Similarly, previous studies on genetic variants showed no significant difference in CYP 2C9 [19] and renin angiotensin gene polymorphism[9]distribution in Lebanese when compared to those of Caucasians.As for TA_7worldwide distribution, the study results showed a geographic distribution and clines of the mentioned allele; in individuals of African descent, TA_7/TA_7polymorphism is the most common variant[20]. A high $(TA)_7$ allele frequency has also been reported in Indian descent [21,22] but the prevalence of homozygous $TA_7/TA_7$was only 1.2%–5% in South-east Asian and Pacific populations[16]. No significant differences were observed in the genotype distribution among the Asian subgroups although, Japanese showed a high genotype frequency of homozygous TA_7/TA_7compared to other Asians.TA_7allele frequencies prevalence progressively decreases from South Africa to the Mediterranean region to Northern Europe and America. Similarly, TA7 progressively decreases moving from South (AF=49.3 in Srilanka) to East Asia (AF=12.08). This finding confirms the previous pharmacogenetic studies [9,16]that showedgeographic clines of Angiotensin Converting Enzyme ACE II and cytochrome P-450 CYP2C9*1*1 genotypes. Thus, the study recommends that the clinical phase in drug development and post marketing surveillance studies should include samples representing different populations of various ethnicities and from different geographic areas.

The study showed that although labeling of irinotecan has changed and a genetic test to identify Gilbert's symptom has received marketing FDA approval, the pharmacogenetic test is still not used in developing countries. A case in point is Lebanon;the use of pharmacogenetic testing of irinotecan could have decreased the amount of irinotecan use and consequently the cost by 11% per year. Testing would avoid neutropenia, and the efficacy for homozygotes after dose reduction can be achieved at more than 98% of full dose efficacy[15].

Thus the study recommendsthe future implementation of UGT1A1 pharmacogenetic marker aiming at an individual therapeutic approach and adverse drug effect profile prediction both, at the individual and regional country level

CONCLUSION

In conclusion, UGT1A1 polymorphisms have shown alleles and genotypes to be unequally distributed among different ethnic human populations, and showing a geographic cline. The significant relation between UGT1A1 *28 specifically TA7/7 and predicting severe side effects in patient receiving drugs metabolized by UGT1A1 is a fact that shall be taken into consideration when identifying patient genetically predisposed to severe toxic effects. Pre-clinical, clinical, and post marketing surveillance studies shall includerepresentative samples from different ethnicities and geographic regions to identify patientsat risk.

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