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The comprehensive analysis of UGT1A genetic polymorphisms in patients with metastatic gastrointestinal cancer treated with irinotecan chemotherapy

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Background: Uridine diphosphate-glucuronosyltransferases (*UGT*)*1A1*28* and *UGT1A1*6* have been reported to associate with irinotecan (CPT-11)-induced neutropenia. We analyzed the association among *UGT1A* genetic polymorphisms comprehensively in patients (pts) with metastatic gastrointestinal cancer enrolled in the UGT0601 genotype-directed dose finding study.
Material and Methods: Pts received prior chemotherapies except for CPT-11 for metastatic gastrointestinal cancer were enrolled. CPT-11 was administered biweekly. PK sampling was evaluated during the first cycle. The polymorphisms of *UGT1A1*28* and *UGT1A1*6* was detected by Invader Assay®. Those of *UGT1A7* (*387T>G*, *391C>A*, *392G>A*, *622T>C*), and *UGT1A9*22* were detected by direct PCR sequencing kit BigDye®. Those of *UGT1A1*27*, and **60* were detected by TaqMan® SNP Genotyping. We examined the association of *UGT1A* genotypes with adverse events (AE), and PK profile. This study is registered with UMIN Clinical Trial Registry, number UMIN000000618 and supported by Yakult Honsha Co., Ltd.
Results: Of 82 pts enrolled, 76 provided informed consent for this analysis. Allele frequency of *UGT1A1*28*, *UGT1A1*6*, *UGT1A9*22*, *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), and *UGT1A7* (*622T>C*) were 0.118, 0.237, 0.368, 0.388, 0.289, respectively. UGT1A7 (*387T>G*, *391C>A*, *392G>A*) was highly linked with *UGT1A9* ($r^2 = 0.92$). We also found the linkage association between *UGT1A7* (*387T>G*, *391C>A*, *392G>A*) and *UGT1A7* (*622T>C*), and *UGT1A7* (*622T>C*) and *UGT1A1*6* (r^2=0.64, 0.59, respectively). In the AE analysis, the incidence of grade 3 or 4 hematological toxicity was higher in pts with *UGT1A1*28*, *UGT1A1*6*, *UGT1A9*22*, *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), and *UGT1A7* (*622T>C*) allele (neutropenia: P = 0.01, P = 0.01, P = 0.007, P = 0.002, P = 0.001; respectively). Compared the wild-type and heterozygote with homozygote, the incidence of grade 1 or more anorexia was higher in pts with *UGT1A1*6*, *UGT1A9*22*, *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), and *UGT1A7* (*622T>C*) (P = 0.028, P = 0.046, P = 0.012, P = 0.001, respectively). In the PK analysis, pts with *UGT1A1*28*, *UGT1A1*6*, *UGT1A9*22* and *UGT1A7* (*387T>G*, *391C>A*, *392G>A*) allele showed the similar trend for SN-38 $AUC_{0-24\,h}$ (P = 0.012, P = 0.012, P = 0.018, P = 0.005; respectively).
Conclusions: Our results indicate that *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), *UGT1A7* (*622T>C*) and *UGT1A9*22* may also be predictive marker for safety in CPT-11 therapy.

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A new duplex real-time PCR assay for detection of the mSEPT9 biomarker for colorectal cancer screening using blood plasma

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Background: Despite the good prognosis for colorectal cancer patients when disease is detected early, compliance to screening programs remains low. Concomitants and inconvenience of available methods point to the need of convenient and reliable tests with the potential to increase patient adherence. Previously we have shown in 3,000 patient plasma samples that the detection of methylated DNA of the SEPT9 gene (mSEPT9) is strongly associated with the presence of colorectal cancer. Here, we present data generated with a new workflow that utilizes a duplex real-time greatly simplifying reliable mSEPT9 detection in human plasma.
Material and Methods: DNA from human plasma is extracted, bisulfite converted, and finally purified with the workflow developed by Epigenomics. The output DNA is suited for detection via real-time PCR. Detection of DNA is accomplished via a duplex PCR combining a highly sensitive methylation specific SEPT9 DNA detection assay with a beta-actin assay used as an internal control. The entire workflow has been optimized to increase robustness and improve the ease-of use. Comparative data were generated on technical samples prepared from human plasma and spiked with concentrations of methylated DNA (mDNA). Plasma aliquots with a dilution series of mDNA spikes were measured repeatedly. In addition, 100 sample aliquots derived from clinical patient material were measured. The results were compared to data generated by the published reference method (mSEPT9 Detection Assay, Epigenomics).
Results: Both workflows consistently detected a mSEPT9 signal ($\geqslant$95% of replicates) in aliquots with a 30 pg/ml spike of mDNA. The observed standard deviation for repeated measurements was slightly lower for the new duplex workflow when compared to the reference method. For the clinical samples we observed excellent agreement of results between the two methods in more than 85% of the valid cases.
Conclusions: The biomarker mSEPT9 has been established in several independent studies to be ~70% sensitive to detect colorectal cancer at ~90% specificity. The newly developed duplex workflow for mSEPT9 detection in human plasma shows excellent agreement with, and therefore is considered substantially equivalent to, the reference method. This new assay, which has been demonstrated to accurately detect mSEPT9 in a standard blood specimen, is expected to significantly improve patient. The design and robustness of the assay will enable its use in standard routine laboratory procedures.

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Microsatellite instability in sporadic colorectal cancer: correlation with novel clinical parameters

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Background: Microsatellite instability (MSI), present in up to 15% of sporadic colorectal cancers (CRC), is associated with clinico-pathologic features including right sided cancers and increased age. Associations with other clinical parameters have yet to be fully explored.
Methods: MSI, using Bethesda 5-panel markers, was evaluated in primary CRC tumors. Samples were selected to provide an even spread of tumor location (right colon, left colon and rectum) and patient age. Clinico-pathologic features of patients with microsatellite stable (MSS) versus MSI tumours were compared using a prospectively collected clinical database.

Table 1: Clinico-pathologic parameters, MSI versus MSS tumors

	MSI-H (n = 78)	MSS (n = 493)	P value
Median age	76.5	69	0.001
Site of tumor	n (%)	n (%)	
Right colon	57 (73.08)	163 (33.06)	<0.0001
Left colon	12 (15.38)	167 (33.87)	
Rectum	9 (11.54)	163 (33.06)	
Stage at presentation	N = 78	N = 492	
A	4 (5.13)	41 (8.33)	Stage D vs other = 0.0009
B	21 (26.92)	91 (18.5)	
C	46 (58.97)	231 (46.95)	
D	7 (8.97)	129 (26.22)	
Median lymph node yield	N = 78	N = 485	0.007
	17.5	14	
BMI (median, kg/m^2)	N = 43	N = 279	
	28.3	26.6	0.07
Grade of differentiation	(n = 77)	(n = 485)	
well	0 (0)	4 (0.82)	
moderate	31 (40.26)	300 (61.86)	
poor	41 (53.25)	174 (35.88)	Poor vs other = 0.0009
not reported	5 (6.49)	7 (1.44)	
Gender			
Male	38 (48.05)	249 (50.51)	0.71
Female	40 (51.95)	244 (49.49)	
Smoking status	(n = 77)	(n = 485)	
current	10 (12.99)	61 (12.58)	0.87
ex	22 (28.57)	127 (26.19)	
non	45 (58.44)	297 (61.24)	
Type I/II diabetes	(n = 77)	(n = 488)	
yes	19 (24.68)	103 (21.11)	0.46
no	58 (75.32)	385 (78.89)	

Results: Tumors from 571 patients were evaluated. Results are presented in Table 1. In total 78 tumors (13.7%) were MSI and 493 (86.3%) were MSS. The majority of MSI tumors occurred in older patients, were right sided, and poorly differentiated. Patients with MSI were significantly less likely to