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## Gene Variants That Affect Levels of Circulating Tumor Markers Increase Identification of Patients with Pancreatic Cancer

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### Abstract

**Background & Aims:** Levels of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and CA-125 in blood are used as markers to determine response of patients with cancer to therapy, but are not used to identify patients with pancreatic cancer.

**Methods:** We obtained blood samples from 504 patients undergoing pancreatic surveillance from 2002 through 2018 who did not develop pancreatic cancer and measured levels of the tumor markers CA19-9, CEA, CA125, and thrombospondin-2. Single nucleotide polymorphisms (SNPs) in *FUT3*, *FUT2*, *ABO*, *GAL3ST2* that have been associated with levels of tumor markers were used to establish SNP-defined ranges for each tumor marker. We also tested the association between additional SNPs (in *FUT6*, *MUC16*, *B3GNT3*, *FAM3B* and *THBS2*) with levels of tumor markers. To calculate the diagnostic specificity of each SNP-defined range, we assigned the patients under surveillance into training and validation sets. After determining the SNP-defined ranges, we determined the sensitivity of SNP-adjusted tests for the tumor markers, measuring

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levels in blood samples from 245 patients who underwent resection for pancreatic ductal adenocarcinoma (PDAC) from 2010 through 2017.

**Results:** A level of CA19-9 that identified patients with PDAC with 99% specificity had 52.7% sensitivity. When we set the cutoff levels of CA19-9 based on each SNP, the test for CA19-9 identified patients with PDAC with 60.8% sensitivity and 98.8% specificity. Among patients with *FUT3* alleles that encode a functional protein, levels of CA19-9 above the SNP-determined cutoff values identified 66.4% of patients with PDAC, with 99.3% specificity. In the validation set, levels of CEA varied among patients with vs without SNP in *FUT2*, by blood group, and among smokers vs non-smokers; levels of CA125 varied among patients with vs without the SNP in *GAL3ST2*. Using the SNPs to define the ranges of CEA and CA125 did not significantly increase the diagnostic accuracy of the assays for these proteins. Combining data on levels of CA19-9 and CEA, CA19-9 and CA125, or CA19-9 and thrombospondin-2 increased the sensitivity of detection of PDAC but slightly reduced specificity.

**Conclusions:** Including information on SNPs associated with levels of CA19-9, CEA, and CA125 can improve the diagnostic accuracy of assays for these tumor markers in identification of patients with PDAC.

### Keywords

early detection; diagnosis; genotype; screening

## INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the 3rd most common cause of cancer death in the United States with a 5-year survival of only ~8%<sup>1</sup>. Most patients with pancreatic cancer are diagnosed with advanced stage disease. Improving early detection is likely to be the most effective way of reducing pancreatic cancer mortality. Existing pancreatic surveillance protocols for individuals with an inherited/familial susceptibility to pancreatic cancer can increase the proportion of patients diagnosed with Stage I disease<sup>2, 3</sup>. However, patients that develop pancreatic cancer on a pancreatic screening protocol are often diagnosed with more advanced stage disease<sup>2-8</sup>. One reason is that current pancreatic imaging tests can miss small cancers<sup>9</sup>, another is that the window of time to detect pancreatic cancer clinically while at its most curative stage is short<sup>10</sup> so some patients present with interval cancers even with annual surveillance. Some patients also find compliance with annual pancreatic imaging difficult<sup>11</sup>. Better non-invasive diagnostic tests are needed for patients under pancreatic surveillance.

Circulating biomarkers continue to be evaluated for their potential diagnostic utility to detect early pancreatic cancer<sup>12-14</sup>, but most biomarkers lack the needed performance characteristics. For example, CA19-9 is not expressed in ~10% of individuals who lack functional *FUT3* (fucosyltransferase-3-null (*FUT3*-/-)<sup>15</sup> and *FUT2* status influences CA199 and CEA levels<sup>16-19</sup>. Despite the evidence that genetic factors influence tumor marker levels<sup>20, 21</sup>, the potential utility of using a genetic test to improve the interpretation of tumor markers has drawn limited attention. Recent studies have identified three genetic subgroups with respect to CA19-9 levels; *FUT3*-null, those without functional *FUT2* (*FUT2*-null or

FUT2<sup>-/-</sup>), and a third group with intact *FUT2* and *FUT3*<sup>17, 22, 23</sup>. Luo et al reported this approach improved CA19-9's diagnostic performance for pancreatic cancer, though the authors did not describe marker performance using high-specificity cut-offs<sup>17</sup>.

In this study, we evaluated genetic variants for their association with tumor marker levels in a cohort of high-risk individuals (HRIs) undergoing pancreatic cancer surveillance. We then determined if diagnostic characteristics of a tumor marker panel for pancreatic cancer (diagnostic sensitivity at high specificity) could be improved by using a tumor marker SNP genotype test to create SNP-defined diagnostic cut-offs for each tumor marker.

## MATERIALS AND METHODS

### Subjects

This study included 504 HRIs prospectively enrolled in the Cancer of the Pancreas Screening (CAPS) studies between 2002 and 2018 (NCT00438906, NCT00714701, NCT02000089)<sup>2, 24-28</sup> without evidence of pancreatic cancer after >1 year since their blood-draw, as well as 245 patients with blood samples collected prior to pancreatic resection for pancreatic ductal adenocarcinoma at the Johns Hopkins Hospital (2010-2017). Patients who underwent neoadjuvant chemotherapy were excluded. A detailed description of CAPS enrollment criteria has been published<sup>2</sup>. Twenty-four HRIs that had previous surgery for pancreatic cancer or high-grade dysplasia or had worrisome imaging findings were excluded. The final diagnoses were made by surgical pathology using World Health Organization criteria. All pathological diagnoses were made by an expert pathologist (R.H.H)<sup>29</sup>. Tumor stage was defined by American Joint Committee on Cancer (AJCC) 8<sup>th</sup> edition. All authors had access to study data and reviewed and approved the final manuscript. This study was approved by the Johns Hopkins Institutional Review Board and written informed consent was provided from all enrolled patients.

### Genotyping and ELISA

Sixteen SNPs (in *FUT3*, *FUT2*, *FUT6*, *ABO*, *GAL3ST2*, *MUC16*, *B3GNT3*, *FAM3B* and *THBS2*) associated with tumor marker levels (CA19-9, CEA, CA125, thrombospondin-2) from prior studies<sup>16-18, 20</sup> were genotyped (Table S1).

All serum samples were assayed in duplicate and analyzed randomly. New serum aliquots were used for all measurements. Tumor marker levels were measured by ELISA; CA19-9 (EIA-1474R, DRG International, NJ, USA), CEA (EIA-1868R, DRG International), CA125 (CA125, Quantikine; R&D Systems, Minneapolis, MN, USA), thrombospondin-2 (DTSP20, Quantikine; R&D Systems). Since CA125 levels are higher prior to menopause<sup>30</sup>, CA125 levels in women were measured only in those age >55 (n=174). An internal reference serum was measured in duplicate for each ELISA plate. Coefficients of variation calculated using a serum reference sample were 9.0%, 6.9% and 7.9%, for CA19-9, CA125 and thrombospondin-2, respectively. Further description of methods are provided in Supplemental Materials.

## Statistical analysis

Patient characteristics were compared and differences between groups were evaluated using Fisher's exact test for categorical variables and Student t-tests for continuous measures. Control subjects were split randomly into discovery and validation sets. The training and validation study design was aimed at setting strict 99% specificity cut-offs that could be applied to the PDAC cases. Diagnostic cut-offs for each marker and corresponding SNP groups were set using data from the controls in the discovery set. The estimated 99th percentile cut-off for each subgroup in the discovery set controls was set as the mean+3SD. Diagnostic sensitivity was then estimated in the PDAC patients and specificity at the threshold established in the discovery set was estimated from the validation set controls. The area under the receiver operator characteristic curve (AUC) calculations were included for descriptive purposes (validation set controls versus PDAC cases).

Statistical analysis were conducted using R v3.4.2 and JMP-13 software (SAS Institute, Cary, NC, USA). *P* values <0.05 were considered statistically significant.

## RESULTS

The characteristics of the pancreatic cancer cases and the discovery and validation set CAPS controls are described in Table 1.

We compared mean CA19-9 levels by SNP group (Figure 1) within the control discovery set. The SNP(s) used to classify each tumor marker SNP group is summarized in Supplemental Table S1. As expected, *FUT3*-null subjects had minimal or absent CA19-9 levels and *FUT2*-null subjects had significantly higher CA19-9 levels than *FUT2*-intact subjects (Figure 2, Table 2). Among controls with intact *FUT3*, those with only one functional *FUT3* allele (*FUT3*+/-) had lower CA19-9 levels than controls with two functional *FUT3* alleles (*FUT3*+/+) in both the training (*p*=0.0038) and validation set (*p*<0.01)(Figure 2). Other *FUT2*/*FUT3* SNP subgroups were not significantly different.

Thus, in both training and validation sets results indicated *FUT2* and *FUT3* SNP status yielded 4 functional SNP subgroups each with a significantly different mean CA19-9 levels (Figure 2, Table 2). (Other SNP subgroups did not have evidence of effect on CA19-9). The four groups (*FUT3*-null, *FUT3*+/-, *FUT3*+/+ and *FUT2*-null) represented ~10%, 35%, 33% and 22% of the control population, respectively. SNP-stratified mean/SD CA19-9 levels, varied by ~3-fold between the lowest and highest SNP subgroups with intact *FUT3* (Table 2). There was no significant difference in CA19-9 levels by age, diabetes status, presence or absence of cysts or worrisome features in controls (data not shown).

Having defined the SNP-defined subgroups, we set the diagnostic cut-off for each tumor marker using the observed 99<sup>th</sup> percentile of tumor marker in the discovery control set (Table 2). We then applied these cut-offs (i) to patients undergoing pancreatic resection for PDAC, (ii) to the control validation set, (iii) to another blood sample collected at a later time from controls.

The diagnostic sensitivity of CA19-9 applied to 245 (49 Stage 1, 196 Stage 2) localized PDAC cases using a uniform (not SNP-stratified) 99% specificity cut-off was 52.7% (95% CI: 46.2%, 59.0%), AUC 0.8827 (Table 3)(Figure 3). The diagnostic sensitivity of SNP-stratified CA19-9 at the 99% specificity cut-off was 60.8% (95% CI: 54.4, 67.0%). the AUC using SNP-stratified CA19-9 cut-offs was 0.9182. Applying SNP-stratified CA19-9 cut-offs to the validation set controls yielded 98.8% specificity (95% CI: 96.6%, 99.8%)(Table S2). The combined CA19-9-SNP test reclassified 28 of the 245 PDAC cases (11.4%) compared to the CA19-9 test alone (24 from negative-to-positive, 4 positive-to-negative)(Table S2).

Diagnostic specificity of CA19-9 was further evaluated using a 2<sup>nd</sup> longitudinal blood collected at a later time point from 217 of the 504 controls. Twenty of these 217 controls were *FUT3*-null; 2 of these 20 had elevated CA19-9 levels for this group. None of the 197 subjects with intact *FUT3* had an elevated SNP-stratified CA19-9 level on repeat testing.

For CA19-9, genotyping is most helpful for the ~90% of subjects with functionally intact *FUT3* genes. For patients with localized PDAC and intact *FUT3*, diagnostic sensitivity of SNP-stratified CA19-9 was 66.4% (95% CI: 59.6%, 72.6%). The specificity among intact *FUT3* validation set controls was 99.2% (95% CI: 97.0%, 99.9%). The AUC for SNP-stratified CA19-9 in *FUT3*-intact subjects was 0.9315. The sensitivity of SNP-stratified CA19-9 in the 49 patients with Stage I PDAC was 36.7% (95% CI: 23.4%, 51.7%); among Stage I PDAC cases with intact *FUT3*, it was 40% (95% CI: 25.7%, 55.7%)(Table 3)(Figure 3). Among PDAC patients with similar-sized tumors (e.g. tumors of 2-4 cm diameter) and intact *FUT3*, the proportion with an elevated CA19-9 level was significantly higher if they had lymph node metastases and/or local invasion (Stage II) than if they did not (Stage IB disease)(79 of 107 versus 13 of 30 patients) (mean/SD tumor size, 3.07/0.6 versus 3.0/0.6 cm,  $p=0.016$ ).

For CEA, levels were associated with *FUT2* status and ABO blood group. *FUT2*-null controls had higher levels than those with intact *FUT2* ( $p<0.001$ ) and within the same *FUT2* subgroup, CEA levels differed by blood-group B status ( $p=0.001$ ), yielding 4 genetic subgroups (Table 2). CEA levels were higher in active smokers, as previously described<sup>18</sup>, so active smokers were grouped separately (Figure 2). For CEA, the overall specificity using SNP-stratified cut-offs in the validation set was >98% (Table S2).

This diagnostic sensitivity of CEA for PDAC using a uniform cut-off was 13.8% (9.1%, 17.9%); SNP-stratified CEA sensitivity was 15.9% (95% CI: 11.6%, 21.1%). The specificity of SNP-stratified cut-off for CEA levels in controls in the validation set was 98.1% (95% CI: 95.55% to 99.37%), a non-SNP cut-off had a similar specificity of 98.8% (95% CI: 96.65% to 99.76%). CEA's AUC was 0.7849, uniform diagnostic cut-off; 0.7724 using SNP-stratified cut-offs. With genotyping, 13 PDAC cases (5.3%) had their CEA levels reclassified compared to the positive/negative results set with the uniform cut-off (3.9 ng/ml)(Table S3).

CA125 levels in controls were associated with one variant (rs12469459) in *GAL3ST2*<sup>21</sup>. In both discovery and validation control sets, there were significant differences in CA125 levels between *GAL3ST2* rs12469459-AA subjects and rs12469459-AT and between rs12469459-AA compared to rs12469459-TT subjects ( $p=0.0007$ ,  $p<0.0001$ , respectively for the

discovery set). CA125 levels in discovery set controls trended higher in rs12469459-AT subjects compared to rs12469459-TT subjects ( $p=0.062$ ); in the combined discovery/validation sets, rs12469459-AT subjects had higher CA125 levels than rs12469459-TT subjects ( $p=0.028$ ). The specificity of SNP-stratified and non-SNP-stratified CA125 in the validation set were similar (97.8%, 97.4%)(Table S2).

The diagnostic sensitivity of CA125 using a uniform cut-off was 15.5% (95% CI: 11.2%, 20.8%); for SNP-stratified CA125 it was 17.6% (95% CI: 11.9%, 23.3%). The specificity of SNP-stratified CA125 in the validation set was 97.8% (95% CI: 94.47% to 99.40%), whereas a non-SNP cut-off had a specificity of 97.4% (95% CI: 93.71% to 99.10%). CA125's AUC was 0.7943 using the uniform cut-off; 0.7752 with SNP-stratified cut-offs. Genotyping reclassified the CA125 results of 6.3% of PDAC cases compared with a uniform cut-off (19.2 U/ml)(Table S3).

Combining tumor markers modestly improved diagnostic sensitivity but reduced specificity to a similar degree. Thus, overall sensitivity of combined CA19-9/CEA, CA19-9/CA125, and CA19-9/CEA/CA125 was 64.5%, 64.1%, and 66.1%, respectively, which yielded specificities in the validation set controls of 96.9%, 97.3%, and 95.4%, respectively (Table S4). Combining CA19-9 and CEA in patients with Stage I disease with intact *FUT3* cases yielded a sensitivity of 44.4% (95% CI: 29.6%, 60.0%)(Table S4).

The diagnostic sensitivity of thrombospondin-2 was 18.0% (95% CI: 13.0%, 23.9%), and overall specificity (discovery and validation set controls) was 97.4% (95% CI: 95.4%, 98.6%). Within validation set controls, thrombospondin-2 had a specificity of 96.7% (95% CI: 93.2%, 98.6%). Adding thrombospondin-2 to SNP-stratified CA19-9 increased sensitivity to a similar degree as CEA or CA125 but reduced specificity more than combinations of SNP-stratified CA19-9 with CEA and/or CA-125 (Table S4). Additional results in Supplemental Materials.

## DISCUSSION

We find that a CA19-9 SNP test improved CA19-9's performance as a tumor marker, converting it into a test with improved diagnostic performance, especially in the ~90% of subjects with intact *FUT3*, achieving a 66.4% sensitivity for patients with localized PDAC at a specificity 99.3%. Most reclassified PDAC cases were *FUT3* heterozygotes (who have a low CA19-9 reference range) classified as having elevated CA19-9 by SNP-stratified cut-offs but normal CA19-9 using the uniform diagnostic cut-off. Setting uniformly high diagnostic cut-offs for biomarkers can improve diagnostic specificity, but at the expense of reduced diagnostic sensitivity especially for small cancers<sup>14, 31</sup>; (in our study a uniform 99% specificity cut-off for CA19-9 yielded a sensitivity of 52.7%). We identify four genetic subgroups with respect to CA19-9; this four-group classification improves CA19-9's performance as a diagnostic test compared to a three-group classification suggested in recent studies<sup>1722, 32</sup>. The diagnostic value of SNP stratification for CA19-9 is particularly evident when diagnostic sensitivity is evaluated at 99% specificity.

Setting high specificity cut-offs to limit the number of false-positive tests and understanding causes of false-positive tests are required elements of a diagnostic test offered to an asymptomatic high-risk population such as those in the CAPS program. Although the average lifetime risk of pancreatic cancer in this cohort is relatively high (~5-20%, depending on the extent of familial and inherited susceptibility), the probability of having pancreatic cancer at any one point in time is low, perhaps 0.2 to 1% so diagnostic specificity is especially important. One strength of this study is that we selected individuals in the CAPS program as a control group. This group is one of the main targets of a pancreatic cancer detection blood test.

Our results show that tumor SNP-defined normal reference ranges can reduce the number of false positive results. Once the normal reference ranges of these tumor markers have been determined, one still has to account for non-neoplastic causes of elevated CA19-9 or tumor other markers. Most diseases that cause elevated CA19-9 cause symptoms (e.g. cholangitis, acute pancreatitis)<sup>12</sup>, are uncommon, and require diagnostic evaluation anyway, so these clinical scenarios are very different to the scenario of an asymptomatic high-risk individual undergoing tumor marker surveillance who presents with a positive tumor marker test. Studies have shown some *FUT3*-null subjects have detectable CA19-9 levels, perhaps from cross reactivity with other antigens<sup>33</sup>. Identifying other rare causes of elevated CA19-9, (e.g. polycystic or large liver/renal cysts)<sup>34</sup>, can usually be achieved with abdominal imaging undertaken during pancreatic surveillance.

A prior GWAS identified the *GAL3ST2* rs12469459 variant as influencing CA125 levels<sup>21</sup>. Our results provide evidence that this variant influences CA125 levels enough to improve its diagnostic performance. A CA125 SNP test could have diagnostic value for patients undergoing surveillance for ovarian cancer but this possibility requires further investigation.

Although our test performed well at detecting cases with localized pancreatic cancers, the toughest hurdle for a pancreatic cancer detection blood test is the detection of Stage I disease. Cancers generally shed biomarkers in proportion to their size and small Stage I pancreatic cancers shed fewer diagnostic biomarkers into the circulation making diagnosis more difficult. Evaluating biomarkers of Stage I pancreatic cancers is challenging because very few patients are diagnosed with Stage I disease. Such patients are usually asymptomatic and found through surveillance. We did enrich our study population with Stage I cases (20% of the localized PDAC cases) and found SNP-stratified CA19-9 had 40% sensitivity for detecting Stage I disease PDAC cases with intact *FUT3*.

Our results demonstrate that a tumor marker SNP test can improve the diagnostic accuracy of CA19-9 and to a lesser extent CEA and CA125, but further work is needed to improve the diagnostic accuracy of our panel for the detection of early-stage pancreatic cancer.

The precision of the specificity cut-offs was set using a large number of controls. Our sample size was adequate for defining diagnostic cut-offs for common genetic subgroups, but larger sample sizes are needed to define better tumor marker reference ranges in rarer genetic subgroups.

Overall, the diagnostic sensitivity and specificity achieved using SNP-stratified reference ranges for CA19-9 justifies further evaluation of a CA19-9 SNP test in a prospective study for subjects undergoing pancreatic surveillance.

In conclusion, a tumor marker SNP test can be used to define SNP-stratified reference ranges for CA19-9, CEA and CA125. Evaluation of the use of SNP-stratified CA19-9 measurements as an early pancreatic cancer detection test for high-risk individuals with intact *FUT3* undergoing pancreatic surveillance appears warranted.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations used in this paper:

<b>PDAC</b>	pancreatic ductal adenocarcinoma
<b>IPMN</b>	intraductal papillary mucinous neoplasm
<b>PanIN</b>	pancreatic intraepithelial neoplasia
<b>GWAS</b>	Genome-wide association studies
<b>HRI</b> s	high-risk individuals

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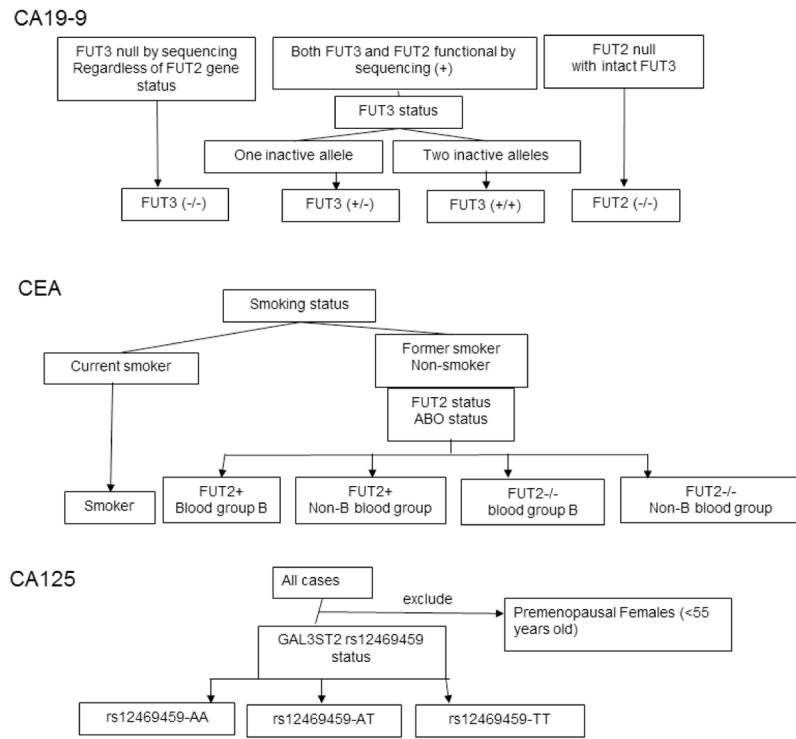
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### Need to Know

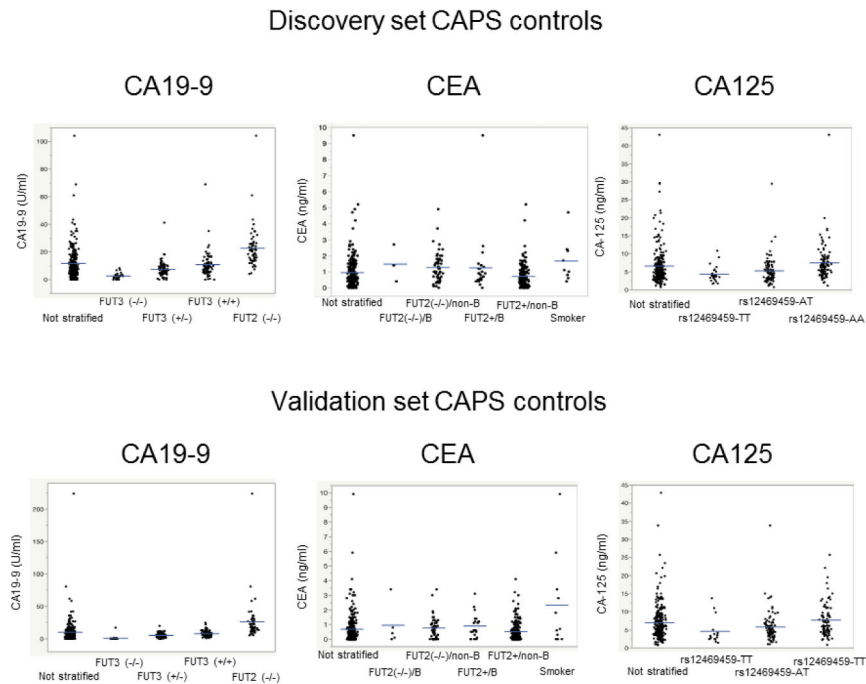
Background: Single-nucleotide polymorphisms (SNPs) have been associated with blood levels of tumor proteins. These SNPs can affect accuracy of assays for these proteins in detection of pancreatic cancer.

Findings: Ranges of carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), CA-125 in blood of patients without cancer vary based on SNPs in 4 genes. When we adjusted the cutoff values for these proteins based on the SNP information, these assays identified patients with pancreatic cancer with higher levels of specificity.

Implications for patient care: This blood test might be used to aid in detection of patients with early-stage pancreatic cancer.

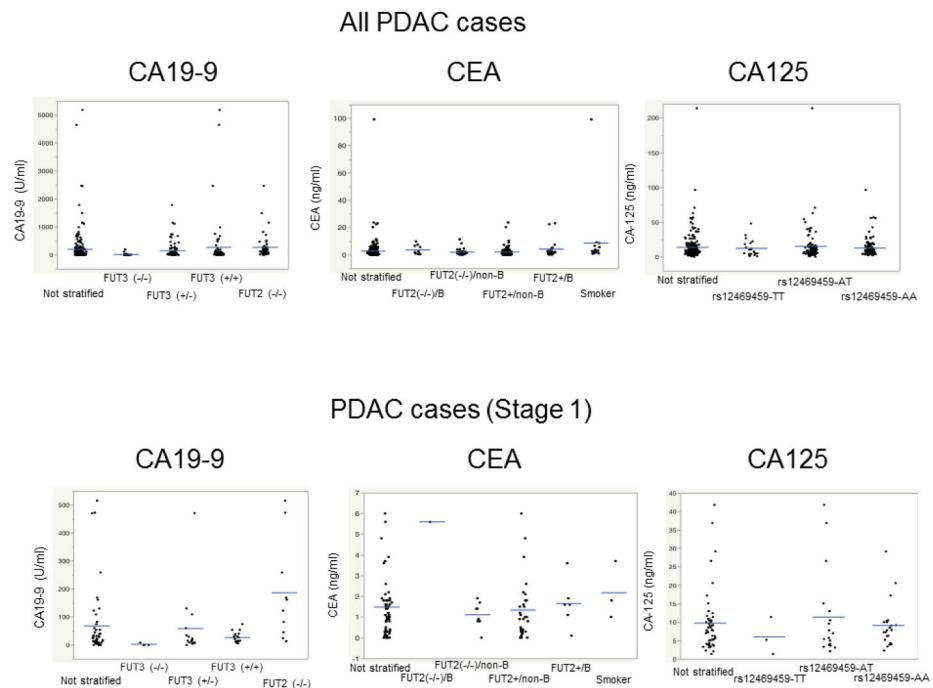


**Figure 1.** Schematic outline of SNP subgroups for CA19-9, CEA and CA125.



**Figure 2.**

SNP-stratified tumor marker levels in (A) discovery set, (B) validation set controls. For CA19-9, discovery set controls; *FUT3*<sup>+/+</sup> versus *FUT3*<sup>+/-</sup>,  $p=0.0038$ ; all other comparisons,  $p<0.0001$ ; for validation set controls all comparisons were  $p<0.001$ . CEA levels in discovery set controls were lower in *FUT2*<sup>+/non-blood group B</sup> (*FUT2*<sup>+/non-B</sup>) than all other groups; in the validation set, levels in the *FUT2*<sup>+/non-B</sup> group were lower than *FUT2*<sup>-/non-B</sup> and *FUT2*<sup>+/B</sup> groups controls ( $p=0.04$  for both). For CA125, *GAL3ST2* rs12469459-AA subjects had higher levels than rs12469459-TT and rs12469459-AT carriers ( $P=0.0007$  and  $<0.0001$ , respectively, discovery set; both  $p=0.001$ , validation set). Rs12469459-TT carriers had lower levels than rs12469459-AT carriers ( $p=0.062$  validation set,  $p=0.028$  combined set). *FUT2*<sup>+</sup> subjects include *FUT2*<sup>+/+</sup> and *FUT2*<sup>+/-</sup>.



**Figure 3.** SNP-stratified tumor marker levels in patients with localized pancreatic ductal adenocarcinoma: All PDAC cases (top panel), Stage I cases only (bottom panel). CA19-9 levels differed within SNP subgroups (*FUT2*<sup>-/-</sup> versus other groups and *FUT3*<sup>-/-</sup> versus other groups,  $p < 0.00001$ , Mann-Whitney).

**Table 1.**

Characteristics of patients with pancreatic cancer and controls

	Discovery set controls (n=245)	Validation set controls (n=259)	P Value	All Controls (n=504)	PDAC (n=245)	P Value
Age - mean (SD)	61.5 (9.5)	56.4 (10.6)	<0.0001	58.9 (10.4)	67.9 (10.6)	< 0.0001
Race - no. (%)			1			0.0002
White	232 (94.7)	244 (94.2)		476 (94.4)	211 (86.1)	
Other	13 (5.3)	15 (5.8)		28 (5.6)	34 (13.9)	
Sex - no. (%)			0.0157			0.0231
Female	121 (49.4)	156 (60.2)		277 (55.0)	113 (46.1)	
Male	124 (50.6)	103 (39.9)		227 (45.0)	132 (53.9)	
BMI - mean (SD)	27.4 (4.7)	27.5 (5.0)	0.5964	27.4 (4.8)	25.6 (4.1)	< 0.0001
Smoker - no. (%)			0.8216			0.0507
Not current smoker	236 (96.3)	248 (95.8)		484 (96.0)	224 (91.4)	
Current smoker	9 (3.7)	11 (4.2)		20 (4.0)	18 (7.3)	
Diabetic~ - no. (%)			1			< 0.0001
No	228 (93.1)	240 (92.7)		36 (7.1)	90 (54.5)	
Yes	17 (6.9)	19 (7.3)		468 (92.9)	75 (45.5)	
Stage (8th AJCC)						
I A					18 (7.3)	
I B					31 (12.6)	
IIA					4 (1.6)	
IIB					106 (43.3)	
III					86 (3.5)	

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; SD, standard deviation; BMI, body mass index; ~status not known for some cases

**Table 2.**

SNP stratified tumor marker levels in discovery set controls

<b>CA19-9</b>	<b>number</b>	<b>Mean (U/mL)</b>	<b>SD</b>	<b>Mean +/- 3 SD*</b>
FUT3 (-/-)	26	2.7	2.3	9.5
FUT3 (+/-)	85	7.4	5.4	23.5
FUT3 (+/+)	75	10.9	9.1	38.1
FUT2 (-/-)	59	22.8	14.6	66.6
Uniform diagnostic cut-off (Not SNP-stratified)	245	11.7	11.5	46.1
<b>CEA</b>	<b>number</b>	<b>Mean (ng/mL)</b>	<b>SD</b>	<b>Mean +/- 3 SD*</b>
FUT2+/blood B	26	1.2	1.8	6.6
FUT2+/non-blood B	151	0.7	0.7	2.8
FUT2 -/- /blood B	4	1.5	0.9	4.3
FUT2 -/- /non blood B	55	1.3	0.9	4.0
smoker	9	1.7	0.8	4.2
Uniform diagnostic cut-off (Not SNP-stratified)	245	0.9	1.0	3.9
<b>CA125</b>	<b>number</b>	<b>Mean (U/mL)</b>	<b>SD</b>	<b>Mean +/- 3 SD*</b>
GAL3ST2 rs12469459-AA	101	7.4	5.0	22.3
GAL3ST2 rs12469459-TT	90	5.2	3.7	16.2
GAL3ST2 rs12469459-TT	26	4.3	2.1	10.7
Uniform diagnostic cut-off (Not SNP-stratified)	217	6.1	4.4	19.2



**Table 3.**

Sensitivity for each tumor marker for localized PDAC

<b>CA19-9</b>	<b>u/ml</b>	<b>All PDAC (n=245)</b>	<b>Stage I (n=49)</b>
99th percentile by SNP		60.8% (95% CI: 54.40% to 66.97%)	36.7% (95% CI: 23.42% to 51.71%)
Uniform diagnostic cut-off	46.1	52.7% (46.20% to 59.04%)	28.6% (16.58% to 43.26%)
FUT3 intact only (n=217)		66.4% (59.65% to 72.61%)	40% (25.70% to 55.67%)
<b>CEA</b>	<b>ng/ml</b>	<b>All PDAC (n=245)</b>	<b>Stage I (n=49)</b>
99th percentile by SNP		15.9% (11.57% to 21.11%)	8.2% (2.27% to 19.60%)
Uniform diagnostic cut-off	3.9	13.1% (9.11% to 17.94%)	8.2% (2.27% to 19.60%)
<b>CA125</b>	<b>u/ml</b>	<b>All PDAC (n=238)</b>	<b>Stage I (n=45)</b>
99th percentile by SNP		17.6% (11.88% to 23.30%)	11.1% (3.71% to 24.05%)
Uniform diagnostic cut-off	19.2	15.5% (11.19% to 20.79%)	11.1% (3.71% to 24.05%)
<b>thrombospondin-2</b>	<b>u/ml</b>	<b>n=206</b>	<b>Stage I (n=13)</b>
Uniform diagnostic cut-off	42.2	18% (12.97% to 23.90%)	15.4% (1.92% to 45.45%)

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