Original Article

Validation of a genotype-based algorithm that identifies individuals with low, intermediate, and high serum CA19-9 levels in cancer-free individuals and in patients with colorectal cancer

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Background: Serum levels of Carbohydrate antigen CA19-9 are determined by the genotype of fucosyltransferases 2 and 3. To validate, possibly modify, and improve a grouping algorithm based on these genotypes.

Methods: CA19-9 levels genotypes and of fucosyltransferase 2 and 3 were analyzed in cancer-free and colorectal cancer patients. Patients were assigned to groups with low (group A), intermediate (B), or high (C) CA19-9 biosynthetic activity based on a previously developed grouping algorithm based on genotype of fucosyltransferases 2 and 3.

Results: Three hundred thirty-eight patients were included (n=177 cancer-free). Of cancer-free patients 7.9%, 75.7%, and 16.4% were assigned to groups A, B, and C, respectively. In colorectal cancer patients it 7.5%, 77.0%, and 15.5%, respectively. There were significant differences between median CA19-9 levels in the three groups (P<0.001) in both cohorts. The T59G single-nucleotid polymorphism in fucosyltransferase 3 had a significant influence on CA19-9 levels in cancer-free group B patients, which led to establishment of subgroups B1 and B2. However, no difference in CA19-9 levels between these subgroups was found in colorectal cancer patients. A receiver-operating characteristic showed similar areas under the curve for original group B as well as for subgroups B1 and B2.

Conclusions: The grouping algorithm based on genotype of fucosyltransferases 2 and 3, which defines groups with distinct CA19-9 serum levels, was validated in cancer-free patients and in colorectal cancer patients. No clinically relevant improvement to the grouping algorithm was identified.

Keywords: Fucosyltransferase; colorectal cancer; primary sclerosing cholangitis; cholangiocarcinoma

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Introduction

The carbohydrate antigen, CA19-9 has been evaluated and is used as tumor marker for gastrointestinal cancers (1,2). It is a carbohydrate epitope, which is present on mucins and it might be secreted by plasma cells into the blood plasma by cancer cells (1,3).

CA19-9 biosynthesis depends on the enzymatic activity of fucosyltransferase-2 (FUT2, also known as Secretor) and fucosyltransferase-3 (FUT3, also known as Lewis) (4,5). Individuals who do not have FUT3 activity are unable to express the CA19-9 epitope irrespective of FUT2 activity. Contrary, inactivity of FUT2 results in higher CA19-9 serum levels (4-6). The activity of both enzymes is determined by the *FUT2* and *FUT3* genotype (5), and several single nucleotide polymorphisms (SNPs) affecting enzyme activity are known (6).

In a large study on patients with primary sclerosing cholangitis (PSC), we developed a grouping algorithm based on the results of the *FUT2* and *FUT3* genotyping, which classified patients as having either low, intermediate, or high CA19-9 biosynthetic activity. In these PSC patients, the CA19-9 levels in serum were considerably different between these groups, and screening for biliary tract cancer was improved (6). Similar evidence has recently been reported for pancreatic adenocarcinoma (7). CA19-9 was further linked to the *FUT3-FUT6* gene cluster and the *FUT2* gene in a genome-wide association study (8). Additionally, FUT2 was identified as a risk gene in PSC and a FUT2 knockout caused liver disease in mice (9-11).

This study was set up to (I) validate our previously developed grouping algorithm in cancer-free controls, (II) to investigate further improvements of the grouping algorithm, and (III) to evaluate influence of *FUT2* and *FUT3* genotype on serum CA19-9 levels in patients with colorectal cancer (CRC). We present the following article in accordance with the MDAR reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-310/rc).

Methods

Study design

Patients with and without colorectal cancer were included in this study. These patients were selected from two large cohort studies evaluating colorectal cancer screening in southern Germany. Cancer-free individuals were selected from the the BliTz study (Begleitende Evaluierung innovativer Testverfahren zur Darmkrebsfrüherkennung), which included patients who underwent screening colonoscopy. Patients with colorectal cancer were identified from the DACHS+ study, a substudy of the DACHS study (Darmkrebs: Chancen der Verhütung durch Screening) that included patients with confirmed colorectal cancer.

Serum CA19-9 levels were measured, and *FUT* genotyping was performed for the included patients. Additionally, basic demographic and health characteristics of all patients were included.

During course of the study, inclusion of a cohort of patients with PSC for the validation of results was decided. These patients were identified from a local study database, that already served as basis for previous studies on *FUT* genotype and its association with CA19-9 and carcinoembryonic antigen (6,12-14).

The study was previously approved by the Ethics Committee of the Medical Faculty of University Heidelberg (study ID: S-043/2011), and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided written informed consent prior to inclusion in the BliTz or the DACHS+ study or the PSC patient database.

Patient selection

The cancer-free control patients were selected from the BliTz study, which is aimed to evaluate new tests for the early detection of CRC, as described in previous publications (15-21). As part of the German screening colonoscopy program, a screening colonoscopy is offered to persons who are 55 years of age or older with average risk. Individuals who underwent the screening colonoscopy as part of the program were invited to participate in the study. Participants provided samples of blood and stool for evaluation of novel screening tests for CRC. Patients were screened and recruited during a preparatory visit for screening colonoscopy. The study was conducted in 20 gastroenterology institutions in southern Germany. Cancer-free patients without a pre-colonoscopy blood sample and CRC patients without a pre-operative blood sample were excluded. Those cancer-free patients with neoplastic or hyperplastic polyps, which were detected during colonoscopy and those patients with incomplete colonoscopy or inadequate bowel preparation were further excluded.

The colorectal cancer patients were recruited from the DACHS+ study, a substudy of the DACHS study (20-24). DACHS is an ongoing case-control study that focuses on

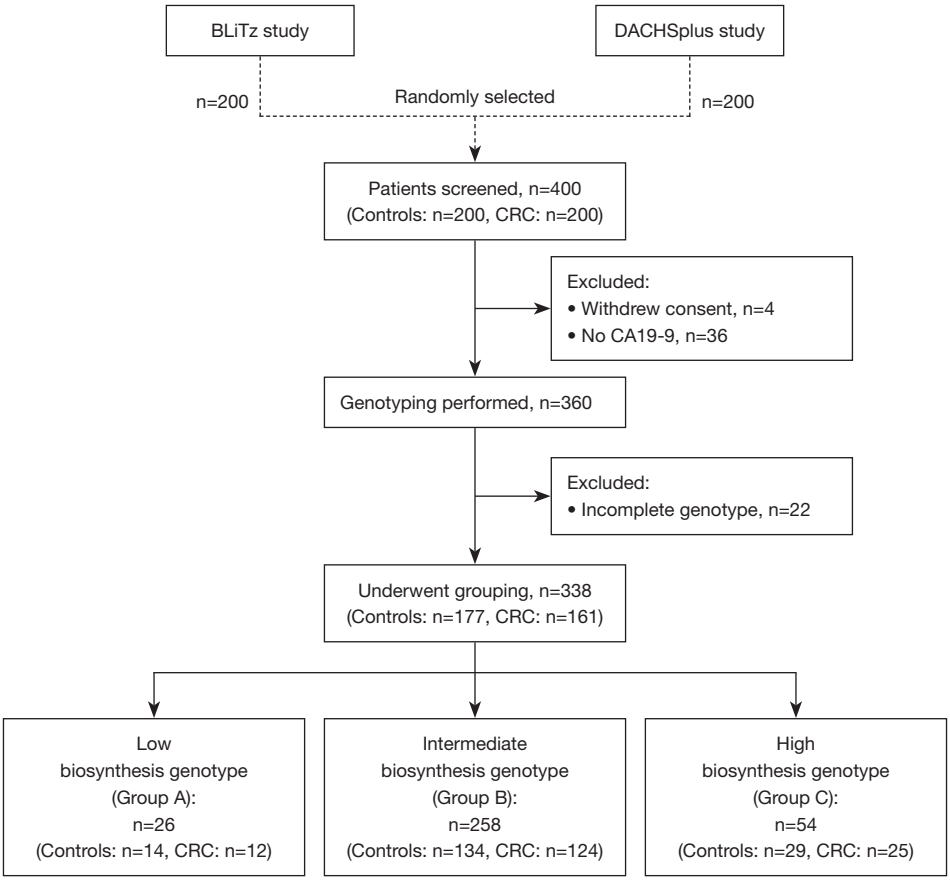

Figure 1 Selection of the study population. This figure shows the way, by which patients were selected for the study, including the number of patients and reasons for exclusion. CRC, colorectal cancer.

the role of colonoscopy in CRC prevention. The substudy, DACHS+, included patients with CRC, who were referred by general practitioners or gastroenterologists for surgery at one of four participating hospitals between 2006 and 2016. Patients who received neoadjuvant therapy before sample collection were excluded from this study.

From the remaining study subjects, patients were randomly selected for inclusion in the current analysis. Cancer free patients were not matched to the CRC patients.

Final study cohort

Of the 400 patients initially screened for eligibility, 338 were finally included in the current analysis (*Figure 1*). Of these patients, 177 (52.4%) were assigned to the control cohort, while 161 (47.6%) were diagnosed with CRC. The cancer stages [according to diseases stages defined by the Union internationale contre le cancer (UICC)] were stage 0 in 1 (0.6%), stage I in 42 (26.1%), stage II in 46 (28.6), stage III in 53 (32.9%) and stage IV in 19 (11.8%) of the patients (*Table 1*).

Measurement of CA19-9 levels

An enzyme-linked immunosorbent assay (ELISA) test (Abnova, Taipei, Taiwan) was used for measuring CA19-9 levels in serum samples from the primary study cohort. It was used according to the manufacturer's instructions.

Genotyping for FUT2 and FUT3

In all patients, genotyping was performed for the following allelic variants: *rs601338* (G428A) in *FUT2* and *rs778986* (C314T), *rs812936* (T202C), *rs3894326* (T1067A), and *rs28362459* (T59G) in *FUT3*. The following methods were used in the central laboratory of the Heidelberg

Table 1 Baseline characteristics of patients in the two study cohorts

Characteristics	Cancer-free (n=177)	CRC (n=161)	P
Age, years (±SD)	62.5 (±6.8)	68.4 (±11.4)	<0.001
Sex, male, n (%)	76 (42.9)	94 (58.4)	0.005
Regular cigarette smoking, n (%)			0.014
Never	109 (61.9)	79 (56.4)	
Former	46 (26.1)	54 (38.6)	
Currently	21 (11.9)	7 (5.0)	

Information on smoking behavior was not available for all patients.

University Hospital. Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). DNA was analyzed using LightCycler 2.0 (Roche, Mannheim, Germany) with LightSNiP assays from TibMolBiol (Berlin, Germany) for *rs601338*, *rs812936*, *rs778986*, and *rs28362459* according to the manufacturer's instructions. The analysis of *rs3894326* was performed by Sanger sequencing using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Darmstadt, Germany), and the sequences of the forward and reverse primers used are 5'ACCTGAGCTACTTTCGCTGG3' and 5'CAAAGGACTCCAGCAGGTGA3', respectively. For individual cases, 5'GCCCCAGGCAGATGAGG3' was used as an alternative reverse primer.

Patient classification based on the FUT genotypes

Patients were assigned to one of the three groups based on the results of the *FUT2* and *FUT3* genotyping, as described previously (6,13). These groups represented patients with low (group A), intermediate (group B), or high (group C) CA19-9 biosynthetic activity that was genetically determined. For grouping purposes, the enzyme activity of FUT2 and FUT3 in a patient was estimated based on his *FUT2* and *FUT3* genotype. All patients with an expected loss of FUT3 enzyme activity were assigned to group A. Patients with expected normal or only partly reduced FUT3 enzyme activity were assigned to group C in case of an expected loss of FUT2 activity, or to group B in case of normal or partly reduced activity of FUT2.

Group A comprised all those patients carrying a homozygous *FUT3* mutation (C314T, T202C, or T1067A) that results in the loss of enzymatic activity and consequently, very low serum CA19-9 levels (25,26). Individuals who were heterozygous for more than one *FUT3* SNP were assumed to be heterozygous and to have *FUT3* enzymatic activity provided that their allele status matched one of the seven *FUT3* alleles described by Orntoft *et al.* (27). Therefore, these patients were assigned to groups B or C depending on the *FUT2* status. If the *FUT2* status did not match any one of these alleles, the patients were considered to be compound heterozygous and have loss of enzyme activity, and were assigned to group A. Patients with homozygous mutations for T59G in *FUT3* were only assigned to group A, if at least one concomitant heterozygous mutation for one of the four other *FUT3* SNPs was present, since only this results in the loss of enzymatic activity (28). The *FUT2* genotype was not needed for the grouping of patients to group A.

Patients who did not meet the criteria for allocation to group A based on the *FUT3* genotype were assigned to groups B or C depending on the *FUT2* gene. Of these patients, group C consisted of all those patients with a homozygous *FUT2* mutation, that resulted in higher serum CA19-9 levels (4,29), while those with either a wild-type or heterozygous *FUT2* allele status were assigned to group B (*Figure 2*).

Statistical analysis

Results are presented either as ordinary numbers or as median with interquartile range (IQR). Chi-square test was used for comparison of categorical data, and Mann-Whitney-U and Kruskal-Wallis tests were used for the comparison of non-normally distributed data. A receiver operating characteristic (ROC) analysis with calculation of the area under the curve (AUC) was done. A P value <0.05 was considered to be statistically significant. All analyses were performed using SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA), and graphs were plotted using Prism version 5 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Results of the FUT2 and FUT3 genotyping and allocation to groups based on CA19-9 biosynthesis

Of the cancer-free patients, n=14 (7.9%) of the patients were assigned to group A, n=134 (75.7%), to group B, and the remaining n=29 (16.4%), to group C. Patients with colorectal cancer were grouped in a similar manner. Of these patients, n=12 (7.5%) were assigned to group A,

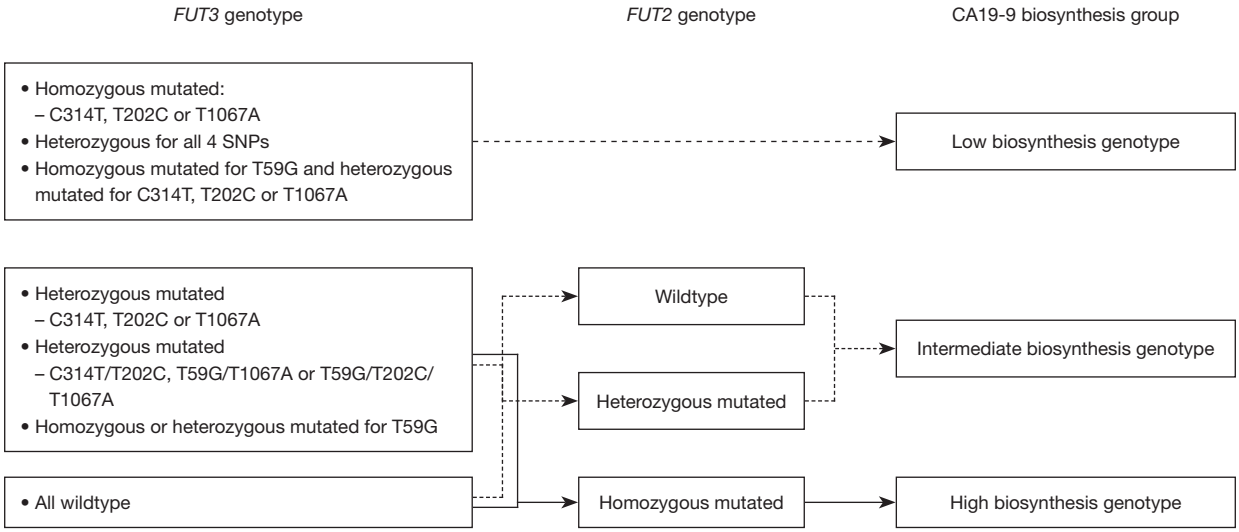

Figure 2 Grouping algorithm used for assigning the patients to either one of the three groups based on genetically determined CA19-9 biosynthetic activity. Algorithm representing how we used *FUT2* and *FUT3* genotypes for assigning the patients to either one of the three groups based on expected enzyme activity: A, no FUT3 activity regardless of FUT2 activity; B, all other FUT2/ FUT3 combinations; and C, no FUT2 activity. These groups represented individuals with genotypes determining low (group A), intermediate (group B), and high (group C) CA19-9 biosynthetic activity, respectively. Based upon an algorithm by Wannhoff *et al.* (6), permission obtained.

n=124 (77.0%) to group B, and n=25 (15.5%) to group C (P=0.961 between the two cohorts). Detailed results of the genotyping for the single *FUT2* SNP and the 4 *FUT3* SNPs are provided in *Table 2*.

Serum levels of CA19-9 levels in the control cohort

The overall median serum level of CA19-9 was 11.67 U/mL in the cancer-free patients (IQR: 4.33–21.99). The median CA19-9 levels were 1.41 U/mL (IQR: 0.00–5.75) in the group A patients, 10.09 U/mL (IQR: 4.30–20.50) in the group B patients, and 23.12 U/mL (IQR: 16.12–32.90) in the group C patients (P<0.001).

Analysis of the influence of the SNPs in FUT2 and FUT3 on serum levels of CA19-9 in each of the three groups organized on the basis of CA19-9 biosynthetic activity

In patients with intermediate CA19-9 biosynthetic activity (group B), a tendency of the influence of the T202C and C314T variants on CA19-9 levels was observed. Higher serum CA19-9 levels were found in those patients with the wild-type genotype than those with a heterozygous mutation (P=0.097 and P=0.099, respectively). In group B, the T59G variant further exerted a substantial influence on serum CA19-9 levels. In group B patients, who possessed the wild-type genotype for T59G, the median CA19-9 level was 10.42 U/mL (IQR: 4.80–20.92) and in those patients with a heterozygous mutation for the T59G variant, the value was 5.32 U/mL (IQR: 0.19–13.23, P=0.014) (*Figure 3*). No such differences were observed in serum CA19-9 levels of patients in groups A and C with regard to any of the included *FUT2* and *FUT3* SNPs,

Modification of the grouping algorithm

Based on the aforementioned findings, the initial grouping algorithm was modified, by which group B was subdivided into two groups. Patients, who were originally assigned to group B, and heterozygous for T202C, C314T, or T59G, were now assigned to group B_1, while those with the wild-type alleles of the three SNPs were assigned to group B_2. The median CA19-9 level in the patients of group B_1 was 7.24 U/mL (IQR: 3.16–15.00) and that in the patients of group B_2 was 13.29 U/mL (IQR: 6.14–24.46); the difference was statistically significant (P=0.005).

Serum CA19-9 levels in patients with CRC

The median serum CA19-9 level in patients with colorectal

Table 2 Distribution of the *FUT2* and *FUT3* SNPs among the patients

SNP	Healthy controls (n=177)	Cancer patients (n=161)	P
rs601338 *FUT2* (G428A)			0.780
Wild-type	58 (32.8%)	52 (32.3%)	
Heterozygous mutated	87 (49.2%)	84 (52.2%)	
Homozygous mutated	32 (18.1%)	25 (15.5%)	
rs812936 *FUT3* (T202C)			0.529
Wild-type	97 (54.8%)	98 (60.9%)	
Heterozygous mutated	70 (39.5%)	55 (34.2%)	
Homozygous mutated	10 (5.6%)	8 (5.0%)	
rs778986 *FUT3* (C314T)			0.635
Wild-type	101 (57.1%)	100 (62.1%)	
Heterozygous mutated	68 (38.4%)	55 (34.2%)	
Homozygous mutated	8 (4.5%)	6 (3.7%)	
rs3894326 *FUT3* (T1067A)			0.065
Wild-type	162 (91.5%)	137 (85.1%)	
Heterozygous mutated	15 (8.5%)	24 (14.9%)	
Homozygous mutated	0	0	
rs28362459 *FUT3* (T59G)			0.037
Wild-type	151 (85.3%)	123 (76.4%)	
Heterozygous mutated	26 (14.7%)	38 (23.6%)	
Homozygous mutated	0	0	

SNP, single nucleotide polymorphism.

cancer was 15.52 U/mL (IQR: 9.10–34.09), which was significantly lower than that of the controls (P<0.001). In groups A, B, and C, the median C19-9 levels were 1.52 U/mL (IQR: 0.59–13.71), 13.92 U/mL (IQR: 9.09–32.07), and 26.83 U/mL (IQR: 16.61–47.57), respectively (P<0.001 between groups). There was no difference in CA19-9 level between the controls and the CRC patients within groups A (P=0.404) or C (P=0.152); however, a significant difference was present in group B patients (P<0.001).

An AUC of 0.622 (95% CI: 0.563–0.682, P<0.001) for CA19-9 level was shown by the ROC analysis to distinguish the CRC patients from the cancer-free patients (*Table 3*). By applying the modified grouping algorithm to the patients with colorectal cancer, the medians were found to be 12.78 U/mL (IQR: 7.47–31.28) in group B_1 patients and 15.72 U/mL (10.03–43.60) in group B_2 patients, respectively. The difference was not statistically significant; however, there was a trend towards higher CA9-9 levels in the patients of group B_2 (P=0.101). Using the modified grouping algorithm, an AUC of 0.665 (0.575–0.756. P=0001) for group B_1, and an AUC of 0.625 (0.526–0.725. P=0.018) for group B_2 were obtained (*Figure 4A*).

Application of the modified grouping algorithm to patients with primary sclerosing cholangitis

After the exclusion of patients with missing genotype data (n=53) and extrabiliary malignancies (n=18), and without an available CA19-9 value (n=19), 212 PSC patients were finally included, among which 17 patients were diagnosed with biliary tract cancer. Allocation of patients to the three groups according to the genotype determining CA19-9 biosynthetic activity was as follows: group A with n=20 (9.4%), group B with n=148 (69.8%), and group C with n=44 (20.8%). By applying the modified grouping

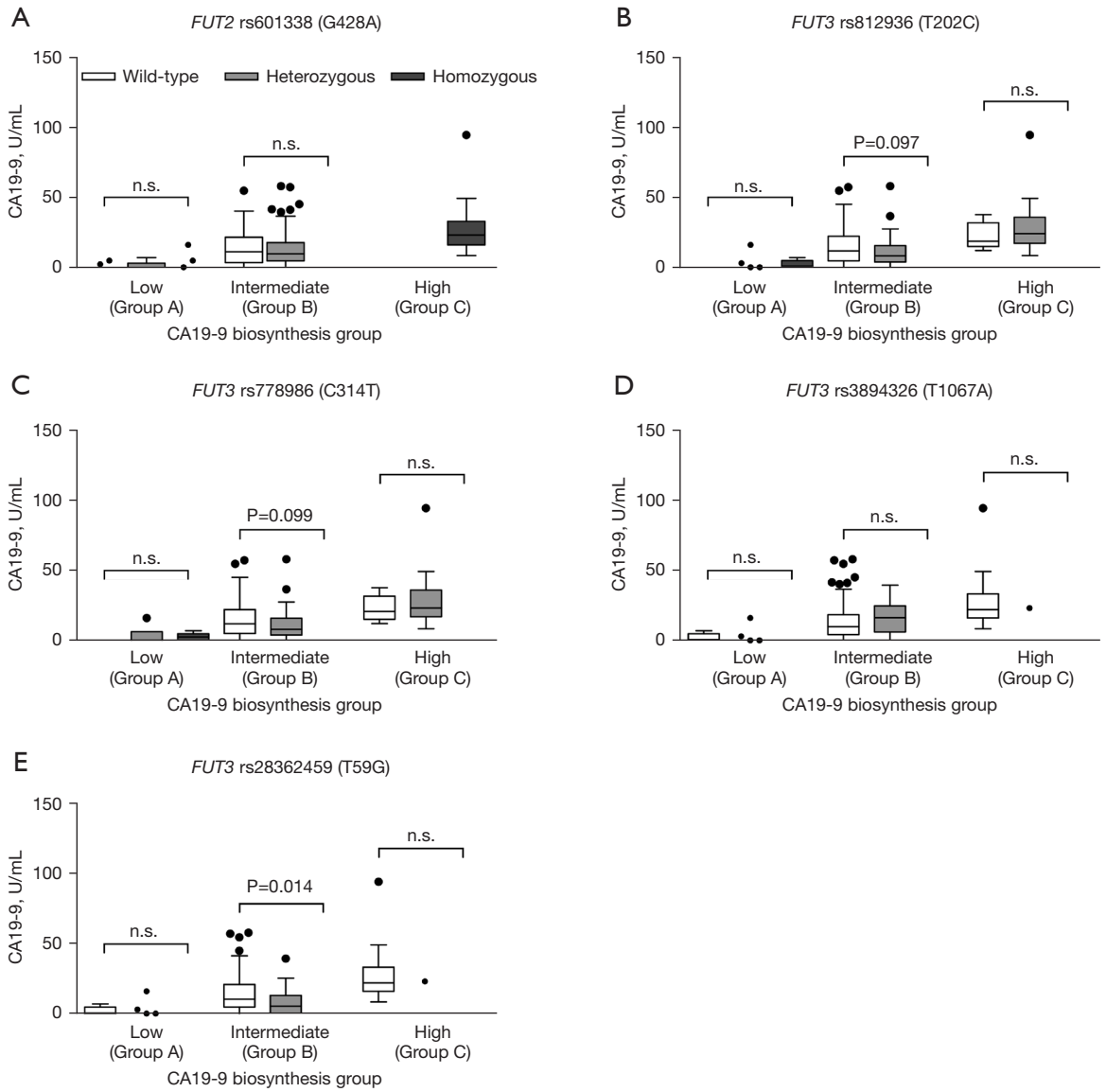

Figure 3 Influence of the *FUT2* and *FUT3* SNPs on serum CA19-9 levels within the three groups in the cancer-free patients. The *FUT2* variant G428A was not associated with CA19-9 levels in any of the three groups (A). For the *FUT3* variants T202C (B) and C314T (C), there was a trend towards lower CA19-9 levels in heterozygous mutated than the patients with wild-type genotype in the group corresponding to intermediate biosynthetic activity. For the T1067A variant of *FUT3*, no difference in CA19-9 level was found (D), while in the group corresponding to intermediate biosynthetic activity, those patients with a heterozygous mutation in the T59G *FUT3* variant had significantly lower levels of CA19-9 than the patients with wild-type genotype (E).

algorithm, 84 (39.6%) patients were allocated to group B_1, and 64 (30.2%) patients were allocated to group B_2.

Of the 148 patients of group B, 14 (9.5%) had diagnosis of biliary tract cancer. The median serum CA19-9 level in these patients was 95.35 U/mL (IQR: 38.45–405.73), which was significantly higher than the median CA19-9, 15.00 U/mL (IQR: 7.89–31.80) in the 134 cancer-free patients (P<0.001). No difference in CA19-9 level was found among the cancer-free patients of group B_1 (median: 14.60 U/mL, IQR: 7.50–29.00) compared to those of group B_2 (median: 15.40 U/mL, IQR: 8.00–33.40, P=0.487). Accordingly, only minor differences in the AUC values

obtained by the ROC analysis were seen in the original group B and the modified groups B_1 and B_2: the AUC values were 0.897 (95% CI: 0.787–0.975, P<0.001) in group B, 0.927 (95% CI: 0.860–0.993, P=0.001) in group B_1, and 0.846 (95% CI: 0.697–0.996, P=0.001) in group B_2 (*Figure 4B*).

Discussion

The main result of this study is that we were able to validate the previously published genotype-based grouping algorithm that identifies individuals with low, intermediate, or high CA19-9 biosynthetic activity (6). This was done in two further cohorts, namely, one consisting of colorectal cancer patients and, most importantly, another with cancer-free controls. In both cohorts, there were significant differences in serum CA19-9 levels among the three groups. After the initial evaluation of the algorithm in PSC patients with and without concomitant biliary tract cancer, similar results were recently reported for patients with pancreatic diseases including pancreatic adenocarcinoma (6,7). Based on those results and especially on the algorithm validation in the control cohort of cancer-free patients in the current study, we conclude that the grouping algorithm truly distinguishes between individuals with different serum CA19-9 levels.

In the current study, our aim was to improve the previously developed algorithm. Our analysis revealed that the T59G variant of the *FUT3* gene had a significant influence on serum levels of CA19-9 in the patients of the group with the genotype determining intermediate biosynthetic activity. While this finding resulted in the creation of two groups with distinct serum CA19-9 levels in the healthy controls (groups B_1 and B_2), the modified grouping algorithm including these two subgroups could not be validated in CRC patients and in PSC patients who were included additionally. Noteworthy, the majority of PSC patients included in this study were as well included in a previous analysis used to develop the grouping algorithm (6), however, the modified algorithm (including B_1 and B_2) had not been tested in these patients before. Nevertheless, an independent group of PSC patients to further validate this finding would be of scientific interest. The modified grouping algorithm did not help to further increase the AUC value obtained by the ROC analysis for the detection of cholangiocarcinoma (CCA) in PSC patients. Thus, we

Table 3 ROC analysis and AUC for CA19-9

Group	ROC	
	AUC (95% CI)	P
All patients	0.622 (0.563–0.682)	<0.001
Group A	0.595 (0.369–0.821)	0.411
Group B	0.640 (0.573–0.707)	<0.001
Group B_1	0.665 (0.575–0.756)	0.001
Group B_2	0.625 (0.526–0.725)	0.018
Group C	0.614 (0.459–0.768)	0.152

ROC, receiver operated characteristic; AUC, area under the curve; 95% CI, 95% confidence interval.

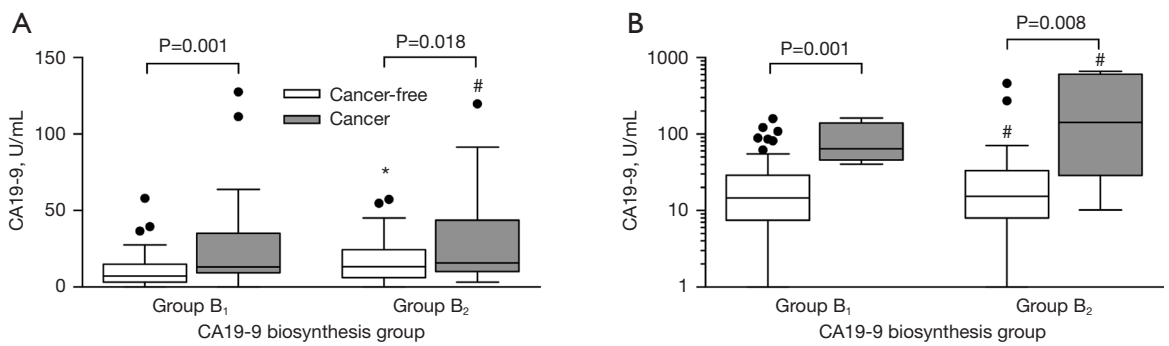

Figure 4 Values of CA19-9 levels in patients with and without cancer in groups B_1 and B_2. (A) In controls, the CA19-9 level was significantly higher in group B_2 than in group B_1 (P=0.005). CA19-9 levels in the colorectal cancer patients were higher in groups B_1 and B_2 than in the cancer-free patients. However, no difference in CA19-9 level was found among the patients with colorectal cancer in group B_1 compared to those of group B_2 (P=0.101). (B) No difference in serum CA19-9 level was observed between the patients with primary sclerosing cholangitis of groups B_1 and B_2 without biliary tract cancer (P=0.487) nor with biliary tract cancer (P=0.549). However, the CA19-9 levels in cancer patients were higher than those of the cancer-free patients in groups B_1 and B_2. *P<0.05 and #P>0.05 between groups B_1 and B_2.

conclude that at least with the currently used selection of *FUT2* and *FUT3* SNPs, further clinically relevant improvements cannot be made to the grouping algorithm. We therefore advocate the further use and investigation of the current algorithm, if possible, in prospective studies.

The use of CA19-9 either alone or in conjunction with the grouping based on genotype was not sufficient to distinguish between the patients with and without CRC, as could be expected. This insufficiency in the use of CA19-9 for screening for CRC has previously been shown (30) and our study indicates that screening cannot be improved by inclusion of *FUT* genotyping. Regarding good scientific practice, this negative result is nevertheless worth publishing. In contrast to the carcinoembryonic antigen, the use of which is recommended for surveillance after primary diagnosis, the use of CA19-9 is not recommended in international guidelines (31-33). Unfortunately measurement of CEA, which was previously shown to be influenced by *FUT2* genotype as well (13), could not be conducted in the cohort of CRC patients do to limited sample volume available. Further, this study did not aim at investigating the application of CA19-9 in combination with *FUT* genotyping as a tool for screening for CRC, especially as colonoscopy is a well-established and efficient technique for CRC screening (34), but to validate the grouping algorithm per se. In contrast to previous studies on PSC and CCA, this study has the great advantage of including cancer-free individuals without an underlying diagnosis of PSC and CRC is a much more common cancer compared to biliary tract cancers. In contrast to CRC, the grouping algorithm might aid in the diagnosis (or even screening) of cancers such as pancreatic adenocarcinoma or CCA, especially in high-risk patients such as patients suffering from PSC or chronic pancreatitis (6,7,35).

Regarding results from this and from previous studies, the following clinically relevant conclusion can be drawn. In approximately 10% of patients (i.e., those belonging to Group A) CA19-9 is probably not useful as a tumor marker, neither for screening nor for follow-up surveillance, as these patients are genetically incapable of CA19-9 synthesis. However, a recent study indicated that raised CA19-9 values might be observed in case of pancreatic cancer in these patients as well. This might indicate that measurable CA19-9 values in these patients should raise even more suspicion (36). In contrast, patients belonging to Group C (approx. 15–20% of patients) show higher CA19-9 values than other patients, despite not having malignancy. Especially in patients with chronic pancreatico-biliary disease and an increased risk of either pancreatic adenocarcinoma or biliary tract cancer, higher cut-off values might be beneficial in these patients. Overall, these genetic determined inter-individual variance in CA19-9 indicates, that intra-individual changes are likely more important and reliable than absolute values (37). Data on the use of *FUT*-genotype-depended CA19-9 cut-off values in patients with chronic pancreatitis and PSC, indicate that their use might result in genotype-depended cut-off values with increases in sensitivity and specificity as well as a reduction in false-positive tests (6,7,35).

Conclusions

In summary, we validated a grouping algorithm that distinguishes patients with low, intermediate, and high CA19-9 biosynthetic activity based on the genotyping for *FUT2* and *FUT3* in this retrospective study. No further additional subgroup of clinical relevance could be identified. Even though the application of the grouping algorithm did not lead to an improvement in the accuracy of diagnosis using CA19-9 in the case of colorectal cancer detection, the validation performed in this study promotes its further application and investigation in patients with a high risk of gastrointestinal cancers such as pancreatic adenocarcinoma or biliary tract cancer.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-310/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-310/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-310/coif). AW is co-owner of a patent for a medical analysis system assessing

the risk of cancer based upon CA19-9 or CEA and FUT2- and FUT3 genotype. HB reports funding from German Research Council (DFG). DNG is co-owner of a patent for a medical analysis system assessing the risk of cancer based upon CA19-9 or CEA and FUT2- and FUT3 genotype. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). It was previously approved by the Ethics Committee of the Medical Faculty of University Heidelberg (study ID: S-043/2011). All participants provided written informed consent prior to inclusion in the BliTz or the DACHS+ study or the PSC patient database.

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