Impact of genetic variants involved in the lipid metabolism pathway on progression free survival in patients receiving bevacizumab-based chemotherapy in metastatic colorectal cancer: a retrospective analysis of FIRE-3 and MAVERICC trials

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Summary

Background Antiangiogenic drug (AAD)-triggered oxygen and nutrient depletion through suppression of angiogenesis switches glucose-dependent to lipid-dependent metabolism. Blocking fatty acid oxidation can enhance AAD-mediated anti-tumor effects in colorectal cancer (CRC). Therefore, we hypothesised that genetic variants in the lipid metabolism pathway may predict clinical outcomes [overall response rate (ORR), overall survival (OS) and progression-free survival (PFS)] in metastatic CRC (mCRC) patients receiving bevacizumab-based first-line treatment.

Methods Genomic DNA from blood samples of patients enrolled in FIRE-3 (a global, randomised, open-label, phase 3 trial, between 2007-6-23 and 2012-9-19, discovery cohort: FOLFIRI/bevacizumab arm, n = 107; control cohort: FOLFIRI/cetuximab arm, n = 129) and MAVERICC (a global, randomised, open-label, phase II study, between 2011–8 and 2015–7, in United States, Canada, Estonia, Ireland, Switzerland, Norway, and Portugal. Validation cohort: FOLFIRI/bevacizumab arm, n = 163) trials, was genotyped using the OncoArray-500 K beadchip panel. The impact on OS and PFS of 17 selected SNPs in 7 genes involved in the lipid metabolism pathway (CD36, FABP4, LPCAT1/2, CPT1A, FASN, ACACA) was analysed using Kaplan–Meier curves, the log-rank test for univariate analyses and likelihood ratio tests of Cox proportional hazards regression parameters for multivariable analyses. ORR and SNP associations were evaluated using Chi-square or Fisher's exact tests.

Findings In the discovery cohort, patients with *FASN* rs4485435 any C allele (n = 21) showed significantly shorter PFS (median PFS: 8.69 vs 13.48 months) compared to carriers of G/G (n = 62) in multivariable (HR = 2.87; 95%CI 1.4–5.9; $p = 0.00675$) analysis. These data were confirmed in the validation cohort in multivariable analysis (HR = 2.07, 95%CI: 1.15–3.74; $p = 0.02$), but no association was observed in the cetuximab cohort of FIRE-3. In the comparison of bevacizumab vs cetuximab arm in FIRE-3, a significant interaction was shown with *FASN* rs4485435 ($p = 0.017$) on PFS.

Abbreviations: ADD, antiangiogenic drug; CRC, colorectal cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; mCRC, metastatic colorectal cancer; FAO, fatty acids β-oxidation; SNP, single nucleotide polymorphisms; MAF, minor allele frequency; ECOG PS, Eastern Cooperative Oncology Group performance status; AIM, ancestry informative markers; MUFA, monounsaturated fatty acids; 3' UTR, 3′ untranslated regions; CORECT, Colorectal Cancer Transdisciplinary; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CPT1A, carnitine palmitoyl transferase 1A; LPCAT1, lysolecithin acyltransferase 1; LPCAT2, lysolecithin acyltransferase 2; ACACA, acetyl-coA carboxylase; FASN, fatty acid synthase

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Interpretation Our study demonstrates for the first time, to our knowledge, that *FASN* polymorphisms may predict outcome of bevacizumab-based treatment in patients with mCRC. These findings support a possible role of the lipid metabolism pathway in contributing to resistance to anti-VEGF treatment.

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Keywords: Lipid metabolism; Colorectal cancer; Bevacizumab; Biomarker

Research in context

Evidence before this study

Colorectal cancer (CRC) could rely on fatty acids β-oxidation (FAO), the most energetically efficient way to provide sufficient energy for the proliferation and spread of cancer cells, through the elevated exogenous FAs uptake or de novo FA synthesis. We searched PubMed for original studies (with no start date and up to March 31, 2022) to explore the association of single nucleotide polymorphisms (SNPs) in the lipid metabolism (involving FA uptake, FA synthesis, FAO and lipid membrane remodeling) and clinical outcomes in patients with metastatic CRC (mCRC) receiving bevacizumab-based first-line treatment. Terms used in this search were "lipid metabolism", "bevacizumab", "colorectal cancer" and "efficacy". There was a paucity of published literature in this area. *CD36* A52C polymorphism was reported to be related to the elevated risk of CRC. Hence, the impact of SNPs involved in the lipid metabolism on the efficacy of bevacizumab in mCRC remains unknown.

Added value of this study

The current study explored the association of SNPs in the lipid metabolism and clinical outcomes in mCRC patients receiving bevacizumab-based first-line treatment, using genetic and clinical data from FOLFIRI-bevacizumab cohorts in randomised trials, FIRE-3 and MAVERICC. It provides evidence that *FASN* rs4485435 may predict the efficacy of bevacizumab-based treatment in patients with mCRC.

Implications of all the available evidence

Our study suggested that bevacizumab might be a better choice for patients with mCRC with *FASN* rs4485435 G/G allele, identified in the discovery and validation cohorts, while cetuximab for *FASN* rs4485435 C allele, observed in the control cohort. These findings support a possible role of the lipid metabolism pathway in contributing to resistance to anti-VEGF treatment.

Introduction

Metabolic adaption is a hallmark of cancer.[1] Beyond the Warburg effect (aerobic glycolysis), certain cancers, including colorectal cancer (CRC), also rely on fatty acids β-oxidation (FAO), the most energetically efficient way to provide sufficient energy for the proliferation and spread of cancer cells, through the elevated exogenous FAs uptake or de novo FA synthesis.[2–5] In details, cancer cells could enhance the acquisition of exogenous FAs through the upregulation of cell surface fatty acid translocase (e.g., CD36) or fatty acid-binding proteins (e.g., FABP4). The activation of de novo FA synthesis could be mediated by the upregulation of key rate-limiting enzymes of FA synthesis, such as acetyl-coA carboxylase (ACACA) and fatty acid synthase (FASN). ACACA carboxylates acetyl-CoA into malonyl-CoA, which is further catalysed to the saturated FA palmitate by FASN at the terminal step of FA synthesis.[6] Palmitate could be further converted into long-chain FAs and monounsaturated FAs, which are transported into mitochondria by carnitine palmitoyl transferase 1 A (CPT1A) for FAO-mediated ATP production.[2] Besides, accumulated FAs in cancer cells can also be esterified into saturated phospholipids by lysolecithin acyltransferase 1/2 (LPCAT1/2), incorporated into membrane lipids by proliferating tumor cells.[7,8] Saturated phospholipids are packed more densely, accompanied with the alteration of membrane fluidity, the decrease of receptor internalization and the limitation of drug uptake, leading to the tumor dissemination and the resistance to oxidative stress-induced cell death. The high expression of these molecules (CD36, FABP4, FASN, ACACA, CPT1A, LPCAT1/2) was reported to be associated with malignant phenotype of tumor cells in CRC and could be used as biomarkers for poor prognosis and chemoresistance.[2,8–12]

Bevacizumab, as an anti-VEGF antibody inhibiting tumor angiogenesis, combined with standard chemotherapy is the one of the cornerstones in the treatment of metastatic colorectal cancer (mCRC). However, not all patients with mCRC could benefit from bevacizumab-based treatment. Identifying predictive biomarkers and reversing resistance to bevacizumab are clinically critical. Iwamoto et al. revealed that lipid metabolism confers antiangiogenic drug (AAD) resistance in CRC.[4] Mechanically, AAD exacerbated the depletion of oxygen and nutrient in the tumor environment, switching the glucose-dependent to lipid-dependent metabolism, via the release of free FA, increased FA uptake and the activation of FAO pathway. Inhibition of these processes of lipid metabolism may overcome the resistance to AAD in CRC. Incio et al. suggested that hypoxia induced by AAD may also contribute to AAD resistance via the increased secretion of inflammatory cytokines, such as IL-6 and FGF2.[13] Accordingly, accumulating clinical studies show an inverse correlation between obesity and clinical benefits in patients who received AAD.[14,15]

Single nucleotide polymorphisms (SNPs) are stable biomarkers of germline background of the host (patient), which may have impacts on the structure, stability and expression of mRNA or protein. For example, the G allele of FBAP4 rs1054135, located on its 3′ untranslated regions (3′ UTRs), was significantly associated with increased tumor recurrence risk of triple-negative breast cancer, probably due to the increased plasma FABP4 levels.[16] CD36 A52C Polymorphism was related to the elevated risk of CRC, which might be linked to its function as not only a long-chain fatty acid translocase but also a scavenger of oxidised low-density lipoprotein.[17] Therefore, we aimed to investigate the association between SNPs in the lipid metabolism (involving FA uptake, FA synthesis, FAO and lipid membrane remodeling) and clinical outcomes [overall response rate (ORR), progression Free Survival (PFS) and overall survival (OS)] using genetic and clinical data from FOLFIRI-bevacizumab cohorts in FIRE-3 and MAVERICC. We also examined these associations in the FOLFIRI-cetuximab arm of FIRE-3 as control.

Methods

Study design and patient population

A total of 968 patients with mCRC were enrolled in randomised, open-label FIRE-3 (NCT00433927, from June 23, 2007 to Sept 19, 2012)[18] and MAVERICC (NCT01374425, from August, 2011 to July, 2015).[19] The FOLFIRI regimen was comparable among the two studies as described previously.[20] Treatment cycles were 14 days. On day 1, patients received irinotecan at a dose of 180 mg/m^2 of body-surface area over 1 or 2 h; leucovorin at a dose of 400 mg/m^2 of body-surface area over 2 h; fluorouracil as an intravenous bolus of 400 mg/m^2 of body-surface area, and then a continuous 46 h infusion of 2400 mg/m^2 of body surface area. Bevacizumab was administered at a dose of 5 mg/kg on day 1 every 14 days with no differences between the two trials. Cetuximab was given weekly at the initial dose 400 mg/m^2 of body-surface area over 2 h, followed by a weekly dose of 250 mg/m^2 of body surface area over an hour. In both trials, treatments continued until disease progression, unacceptable toxic effects, complete response, surgical resection, or patient-requested or physician-decided withdrawal of therapy.

Only 399 patients with mCRC receiving FOLFIRI-based treatment with sufficient samples and SNPs data were analysed in our study. Patients treated with FOLFIRI plus bevacizumab in FIRE-3 and MAVERICC were selected as the discovery cohort (n = 107) and the validation cohort (n = 163) respectively, while those treated with FOLFIRI plus cetuximab in FIRE-3 as the negative control cohort (n = 129). FIRE-3 and MAVERICC trials were approved by the institutional review committees at each centre and written informed consent was obtained from all participants.

The study protocols were approved by the institutional review board of each participating institution and was conducted in accordance with the tenets of the Declaration of Helsinki as well as the Good Clinical Practice and the reporting recommendations for tumor marker prognostic studies (REMARK) guidelines. The protocols for FIRE-3 and MAVERICC are available on the websites (https://clinicaltrials.gov/ct2/show/NCT00433927 or http://www.klinikum.uni-muenchen.de/CCCLMU-Krebszentrum-Muenchen/download/inhalt/studien/fire3/en/FIRE3_EN_translation_protocolTLO.pdf for FIRE-3; and https://clinicaltrials.gov/ct2/show/NCT01374425 for MAVERICC).

Genotyping and selecting polymorphisms

Genomic DNA was genotyped through the OncoArray including 530 K SNP markers (Illumina, San Diego, CA, USA) as described previously.[20] SNP data quality control and imputation were conducted within the Colorectal Cancer Transdisciplinary (CORECT) Study.[21] Stringent quality control procedures were applied at both the individual and SNP levels as described. Prior imputation, SNPs with call rate below 98% and minor allele frequency (MAF) < 0.01 in either European or Asian samples were excluded. The imputation panel reported in the prior CORECT study was updated to the 1000 Genomes Project Phase 3.[22] Seventeen candidate SNPs of genes in FAs uptake (*CD36* rs1761667, rs7755, rs1194197; *FABP4* rs1054135, rs16909187, rs2290201), lipid synthesis (*FASN* rs4246444, rs4485435, rs6502051; *ACACA* rs7211875, rs1714987), FAO (*CPT1A* rs2278908, rs11228373) and phosphatidylcholine remodeling (*LPCAT1* rs7737692; *LPCAT2* rs9302667, rs893260, rs837550) were selected using the following criteria as previously described[23] (Table S1): (1) MAF in Caucasians should be equal or greater than 10%, based

on Ensemble Genome Browser (https://www.ensembl.org/index.html). (2) SNPs with potential biological functions based on published papers or public databases (snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). (3) Tag SNPs with R^2 threshold of 0.8 based on HapMap genotype data within the CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) population.

Clinical outcomes

ORR was defined as the percentage of patients in a cohort who achieved completed response and partial response to treatment. PFS was defined as time from randomization until disease progression, death or until last follow-up in patients who were alive and remained free of disease progression. OS was defined as time from randomization until death. Patients still alive were censored at the last date of follow-up.

Statistical analysis

The aim of this study was to identify associations between SNPs within genes involved in the lipid metabolism and clinical outcome in patients with mCRC receiving bevacizumab/cetuximab-based first line treatment, using the dominant model. Possible associations between patient characteristics, ORR and selected SNPs were evaluated using the Chi-square or Fisher's exact test. To assess associations between these SNPs and PFS or OS, the log-rank test was conducted in univariable analyses and likelihood ratio tests of parameters from Cox proportional hazards regression in multivariable analyses. Study-specific adjustment covariates in the Cox models included sex, age, Eastern Cooperative Oncology Group performance status (ECOG PS), primary tumor site, liver-limited disease, and RAS/BRAF status for FIRE-3; ethnicity, sex, age, ECOG PS, primary tumor site, primary tumor resected, number of metastases, and RAS status were included for MAVERICC. The first three principal components computed from ancestry informative markers (AIMs) included on the OncoArray were also included as adjustment covariates for both cohorts. A two-sided $P < 0.05$ was considered statistically significant, and *P*-values are reported without adjustment for multiplicity. The Cox PH assumptions were evaluated using Schoenfeld residuals. No major violation of the PH assumption was observed. All analyses were performed in R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). Our study was constructed in accordance with REMARK reporting guidelines.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors confirm that they had full access to all the analysed data in the study and accept the responsibility to submit for publication.

Results

Patient characteristics

Baseline characteristics of patients in the discovery (FIRE-3 FOLFIRI+bevacizumab), validation (MAVERICC FOLFIRI+bevacizumab), and control (FIRE-3 FOLFIRI+cetuximab) cohorts are outlined in Fig. 1 and Table S2. Right-sided primary tumors ($p < 0.0001$), metastases in >2 organs ($p = 0.003$), RAS mutation rates ($p < 0.0001$) are more prevalent in the bevacizumab arm of MAVERICC, while the resection rate of primary tumors was lowest, compared to those in both bevacizumab and cetuximab arms of FIRE-3. Patients in cetuximab arm had more males than the other two cohorts ($p = 0.037$). The median PFS and OS were 11.5 months and 31.4 months in the discovery cohort, 12.5 months and 27.4 months in the validation cohort, and 12.8 months and 49.8 months in the control cohort, respectively.

Predictive and prognostic values of the SNPs involved in the lipid metabolism in the discovery cohort

The association between the selected SNPs involved in the lipid metabolism and clinical outcomes in the discovery cohort was shown in Table S3 and Fig. 2. Among them, *FASN* rs4485435, *FASN* rs6502051 and *ACACA* rs1714987 were significantly associated with the efficacy of bevacizumab in both uni- and multi-variate analyses (Table 1). Patients with any C allele in *FASN* rs4485435 (n = 21) had significantly shorter median PFS [8.69 months vs 13.48 months, univariate: hazard ratio (HR): 2.88, 95% confidence intervals (CI): 1.57–5.29, $p = 0.00039$; multivariate HR: 2.87, 95%CI: 1.4–5.9, $p = 0.0068$.] than those with the G/G genotype (n = 62) (Fig. 3A). No significant association between *FASN* rs4485435 and OS was observed after adjustment for covariates (median OS: 24.69 months vs 41.77 months, univariate: HR: 2.29, 95%CI: 1.15–4.54, $p = 0.015$, multivariate: HR: 1.87, 95%CI: 0.79–4.43, $p = 0.17$, Fig. 3B). Patients with any A allele in *FASN* 6502051 (n = 72) had significantly shorter median PFS (10.23 months vs 14.89 months, univariate: HR:1.78, 95%CI: 1.07–2.94, $p = 0.024$; multivariate: HR: 2.01, 95%CI: 1.11–3.66, $p = 0.02$) than those with the C/C genotype (n = 34) (Fig. S1A). Patients carrying any G allele in *ACACA* rs1714987 (n = 32) showed a significantly longer PFS (13.54 months vs 10.49 months, univariate: HR: 0.56, 95%CI : 0.34–0.93, $p = 0.023$; multivariate: HR: 0.53, 95%CI: 0.3–0.94, $p = 0.02$) than carriers of C/C (n = 75) (Fig. S2A).

Confirmation of the predictive values of the SNPs involved in the lipid metabolism in the validation cohort

Among those SNPs discovered in the bevacizumab cohort of FIRE-3, *FASN* rs4485435 remained the significant association with PFS in the validation cohort (Table 1, Fig. 3C–D, Fig. S1B, Fig. S2B). Patients with any C allele in *FASN* rs4485435 (n = 39) had

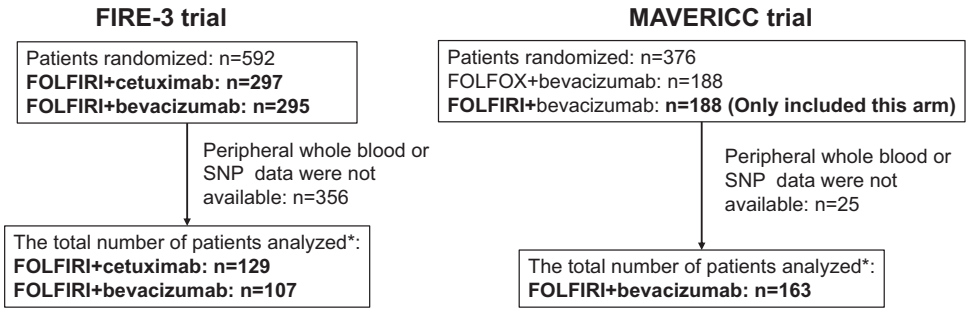

***Fig. 1:* Consort diagram.** SNP, single nucleotide polymorphism. * The number of patients in the SNP analyses varied due to variation in the SNP call rate from SNP to SNP. The call rate for a given SNP is defined as the proportion of individuals in the study for which the corresponding SNP information is not missing. Here, we filter using a call rate of 98% during the process of imputation, meaning we retain SNPs for which there is less than 2% missing data.

significantly shorter median PFS (11.17 months vs 14.06 months) than those with G/G genotype (n = 91) in both univariable (HR: 1.56, 95%CI: 1–2.41, p = 0.047) and multivariable (HR: 2.07, 95%CI: 1.15–3.74, p = 0.02) analyses (Fig. 3C and D). No significant association between *FASN* rs6502051 (median PFS: 12.75 months vs 11.07 months, multivariate HR: 1.04, 95%CI: 0.62–1.77, p = 0.88), *ACACA* rs1714987 (median PFS: 14.98 months vs 12.32 months, multivariate HR: 0.94, 95%CI: 0.53–1.66, p = 0.83) and PFS in patients with mCRC recieving FOLFIRI/bevacizumab in the MAVERICC trial (Table 1, Fig. S1B, Fig. S2B).

Evaluation of the predictive values of the SNPs involved in the lipid metabolism in the control cohort

In the control cohort, there was no evidence for associations of *FASN* rs4485435 (median PFS: 13.54 months vs 10.82 months, multivariate HR: 0.86, 95%CI: 0.51–1.45, p = 0.56), *FASN* rs6502051 (median PFS: 12.26 months vs 13.28 months, multivariate HR: 1.36, 95%CI: 0.82–2.25, p = 0.23), and *ACACA* rs1714987 (median PFS: 13.54 months vs 11.77 months, multivariate HR: 0.92, 95%CI: 0.57–1.51, p = 0.75) with PFS in patients with mCRC receiving FOLFIRI/cetuximab (Table 1, Table S5, Fig. 3E–F, Fig. S1C, Fig. S2C). Treatment-SNP interaction test confirmed the poor predictive value of *FASN* rs4485435 for the efficacy of bevacizumab vs cetuximab ($P_{\text{interaction-for-PFS}}$ = 0.017, $P_{\text{interaction-for-OS}}$ = 0.09) in the FIRE-3 trial (Fig. S3).

Discussion

The application of bevacizumab suppressed tumor angiogenesis, accompanied by the severe depletion of glucose supply, resulting in a metabolic shift toward FA uptake or de novo lipogenesis to fuel cancer cells.[4,5] These preclinical data highlight the vital role of the enhanced lipid metabolism in the resistance to anti-angiogenesis treatment. However, the predictive value of genes involved in the lipid metabolism for the efficacy of bevacizumab has never been examined in patients with mCRC. To the best of our knowledge, this is the first study to identify genetic variants involved in lipid

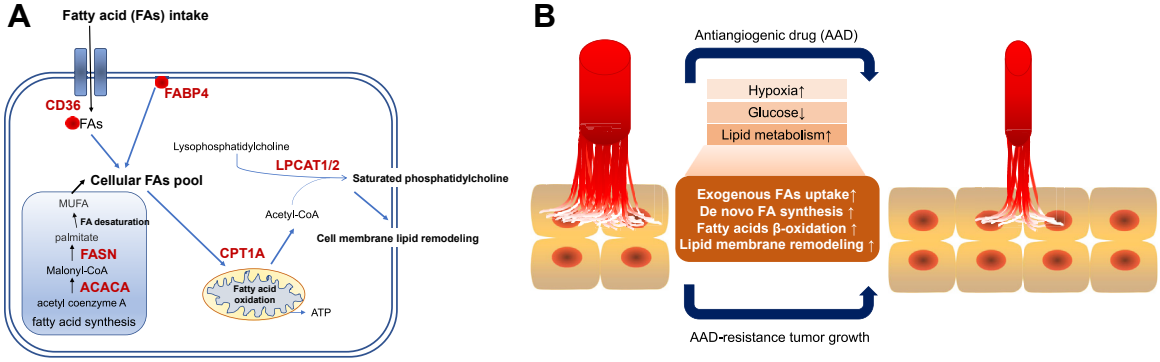

***Fig. 2:* The potential relationship between the lipid metabolism pathway and the efficacy of bevacizumab.** A. Overview of the major molecules participating in the lipid metabolism, leading to the tumor progression. B. The metabolic shift towards the lipid metabolism due to the severe deletion of oxygen and nutrients, is the potential resistant mechanism to bevacizumab. Abbreviation: FA, fatty acid; AAD, anti-angiogenic drug.

metabolisms that may predict the efficacy of bevacizumab-based first line treatment in patients with mCRC.

Our data suggest that bevacizumab might be a better choice for patients with mCRC carrying *FASN* rs4485435 G/G allele, identified in the discovery and validation cohorts, while cetuximab for *FASN* rs4485435 C allele, observed in the control cohort. The association between *FASN* rs4485425 C allele and shorter PFS remained significant in FOLFIRI/bevacizumab cohorts, when BMI was added as a categorical variable, with categories <25, 25–30, and >30 kg/m^2 (multivariate: discovery cohort: HR 3.13, 95%CI 1.51–6.52, $p = 0.0039$; validation cohort: HR 2.14, 95%CI 1.17–3.9, $p = 0.015$; control cohort: HR: 0.9, 95%CI 0.53–1.53, $p = 0.7$). Similar to our results, *FASN* rs4485435 any C allele also exhibited a poor prognosticator for recurrence-free survival in non-small cell lung cancer and hepatocellular carcinoma.[24,25] Mechanically, rs4485435, located in the exon 21 of *FASN*, may participate in alternative splicing of mRNA, via modifying the binding affinity for splicing factors SF2ASF1/2, SC35, and SRp55, which is described on the SNP website (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). No significant association between *FASN* rs4485435 and OS in the multivariate analysis in both FIRE-3 and MAVERICC trials. However, the potential effect of *FASN* rs4485435 on OS in the bevacizumab-based chemotherapy can be noticed. This difference in the effect of *FASN* rs4485435 between PFS and OS could be due to the subsequent therapies, in consideration that patients receiving bevacizumab-based chemotherapy may further received cetuximab or immune checkpoint inhibitors. Rs6502051, located in the 3′UTR region of *FASN*, may regulate gene transcription by affecting binding activity of transcription factor (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). It was reported that rs6502051 was associated with lower incidence of prostate cancer, which may be associated with the lower levels of monounsaturated fatty acids (MUFAs) (25). Conversely, our data showed that rs6502051 any A genotype was associated with shorter PFS in patients with mCRC receiving bevacizumab-based chemotherapy in the FIRE-3, probably due to the context-dependent role of *FASN* rs6502051 in the tumor progression. *ACACA* rs1714987 is a missense SNP, located on splicing abolish domain, were predicted to be associated with benign disease (http://genetics.bwh.harvard.edu/pph/). Consistent with this function, we found *ACACA* rs1714987C allele was associated with longer PFS in the discovery cohort. However, the impact of *FASN* rs6502051 and *ACACA* rs1714987 on the efficacy of bevacizumab was not observed in the validation cohort.

SNP	Genotype	TR			Progression-free Survival					Overall Survival				
					Median PFS	Univariable analysis		Multivariable analysis		Median OS	Univariable analysis		Multivariable analysis	
		N	ORR	P	(months)	HR(95%CI)	P	HR(95%CI)	P	(months)	HR(95%CI)	P	HR(95%CI)	P
FASNrs4485435														
Discovery cohort	G/G	62	65.6%	0.79	13.48	1	**0.00039**	1	**0.0068**	41.77	1	**0.015**	1	0.17
	Any C	21	60.0%		8.69	2.88 (1.57,5.29)		2.87 (1.4,5.9)		24.69	2.29 (1.15,4.54)		1.87 (0.79,4.43)	
Validation cohort	G/G	91	68.9%	1	14.06	1	**0.047**	1	**0.02**	27.93	1	0.19	1	0.31
	Any C	39	68.4%		11.17	1.56 (1,2.41)		2.07 (1.15,3.74)		27.5	1.48 (0.82,2.67)		1.53 (0.68,3.44)	
Control cohort	G/G	74	74.6%	0.44	10.82	1	0.38	1	0.56	42.69	1	0.44	1	0.67
	Any C	27	66.7%		13.54	0.8 (0.49,1.3)		0.86 (0.51,1.45)		51.9	0.72 (0.31,1.65)		0.83 (0.34,2)	
FASNrs6502051														
Discovery cohort	C/C	34	64.7%	1	14.89	1	**0.024**	1	**0.02**	49.18	1	0.073	1	**0.04**
	Any A	72	63.8%		10.23	1.78 (1.07,2.94)		2.01 (1.11,3.66)		28.03	1.82 (0.94,3.54)		2.33 (0.99,5.48)	
Validation cohort	C/C	44	68.2%	1	11.07	1	0.61	1	0.88	27.47	1	0.58	1	0.7
	Any A	118	67.0%		12.75	0.9 (0.59,1.36)		1.04 (0.62,1.77)		31.28	1.17 (0.66,2.07)		1.15 (0.56,2.36)	
Control cohort	C/C	34	75.0%	0.81	13.28	1	0.063	1	0.23	60.62	1	0.32	1	0.53
	Any A	95	77.5%		12.26	1.52 (0.96,2.4)		1.36 (0.82,2.25)		46.43	1.44 (0.7,2.93)		1.3 (0.58,2.91)	
ACACArs1714987														
Discovery cohort	C/C	75	65.3%	0.83	10.49	1	**0.023**	1	**0.02**	31.41	1	0.35	1	0.49
	Any G	32	62.5%		13.54	0.56 (0.34,0.93)		0.53 (0.3,0.94)		28.75	1.37 (0.71,2.63)		1.28 (0.63,2.59)	
Validation cohort	C/C	108	68.9%	0.6	12.32	1	0.52	1	0.83	27.47	1	0.72	1	0.84
	Any G	55	64.8%		14.98	0.88 (0.59,1.31)		0.94 (0.53,1.66)		31.28	0.91 (0.53,1.54)		1.08 (0.52,2.23)	
Control cohort	C/C	93	76.7%	1	11.77	1	0.44	1	0.75	46.43	1	0.31	1	0.91
	Any G	36	77.4%		13.54	0.85 (0.56,1.3)		0.92 (0.57,1.51)		51.9	0.68 (0.32,1.43)		0.95 (0.41,2.24)	

Significant P values (< 0.05) are in bold. In Univariate analysis, P-values were based on log-rank test. Meanwhile, in multivariate analysis, P-values were based on Wald test in the multivariate Cox proportional hazards regression model.

Table 1: **Association between SNPs involved in lipid synthesis pathway and clinical outcomes in FOLFIRI + bevacizumab/cetuximab cohort.**

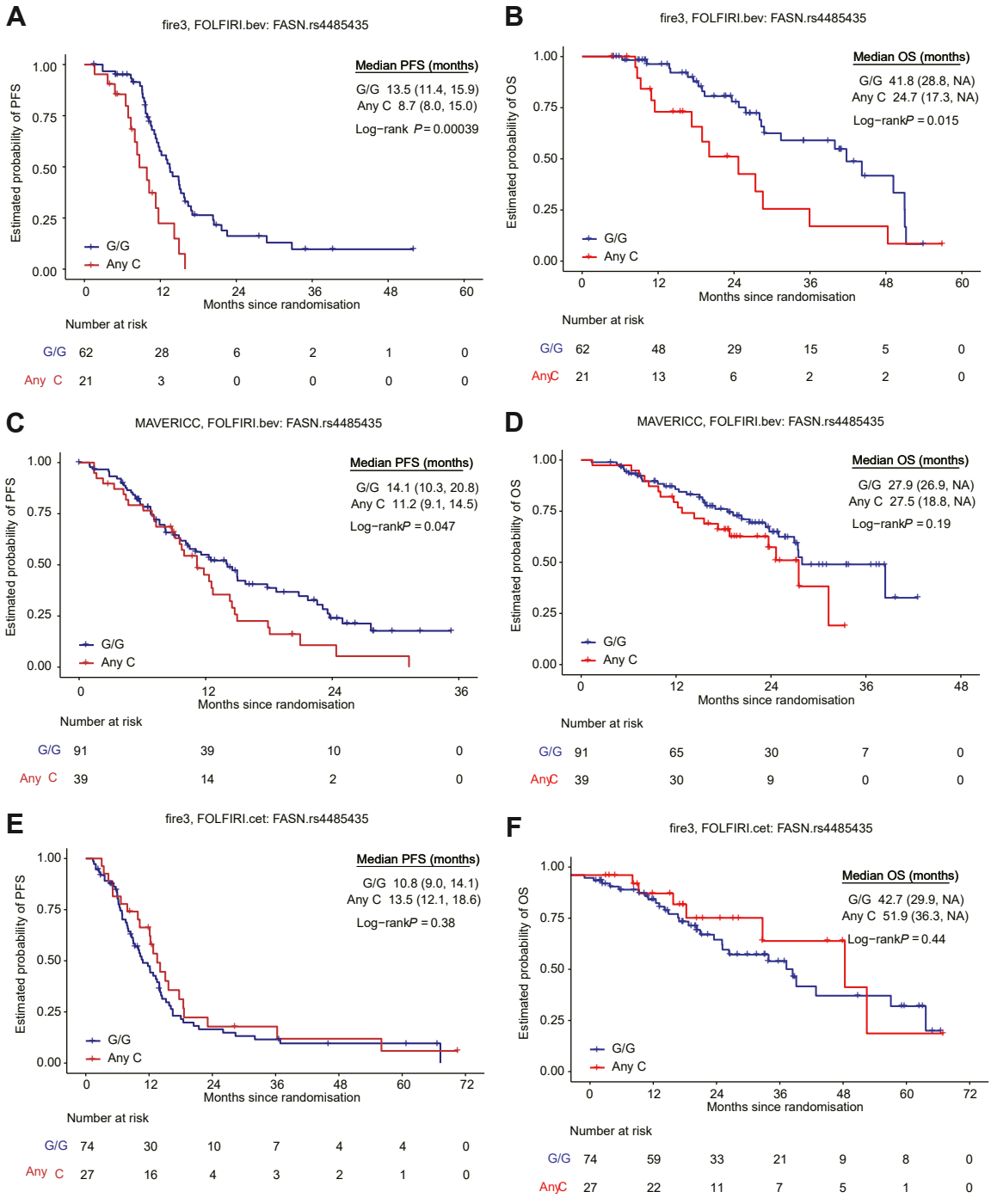

***Fig. 3*: The association of FASN rs4485425 with the efficacy of bevacizumab/cetuximab-based first-line treatment in mCRC.** A-B. The association of FASN rs4485425 with PFS and OS in the bevacizumab cohort in FIRE-3. C-D. The association of FASN rs4485425 with PFS and OS in the bevacizumab cohort in MAVERICC. E-F. The association of FASN rs4485425 with PFS and OS in the cetuximab cohorts of FIRE-3. Abbreviation: mCRC, metastatic CRC; PFS, progression-free survival; OS, overall survival.

Tumor adaptation to antiangiogenic therapy through a metabolic shift toward the lipid metabolism in tumors, indicated by the upregulation of FASN.[5] The inhibition of FASN could block tumor regrowth and metastatic dissemination after anti-angiogenesis treatment withdrawal. A phase I clinical trial about a FASN inhibitor, TVB-2640, combined with paclitaxel, has shown promising results, indicated by the disease control rate of 70% (NCT02223247).[26] Based on these findings, it would be reasonable to explore whether the combination of TVB-2640 and bevacizumab could produce the greater and more durable antitumor effects in preclinical models of CRC, such as patients derived xenografts, and clinical trials.

Cetuximab combined with standard chemotherapy is the one of the cornerstones in the treatment of RAS/BRAF wildtype and left-sided mCRC.[27] Metabolically, cetuximab blunts carbohydrate metabolism by blocking glucose uptake and glycolysis, with little impact on the microvessels and the supply of oxygen and nutrients.[28] Under the nonmonic condition, free FAs has no impact on cancer cell proliferation.[4] In addition, AMPK activation could overcome KRAS-induced resistance to cetuximab by the inhibition of MCL-1 translation via the suppression of the mTOR pathway.[29] Meanwhile, the upregulation of AMPK could activate FAO pathway to supply energy for the accelerated tumor growth.[4] Consistent with treatment-by-SNP interaction analysis, we speculated that patients with FASN rs4485435 any C allele might benefit from cetuximab, rather than bevacizumab.

Limitations of this work need to be mentioned. First, the retrospective setting of this study may introduce the selection bias, thus, these results need to be validated in prospective clinical trials, including more social and demographic factors, such as smoking and co-morbidities such as diabetes. Besides, ethnicity data was not provided in the FIRE-3, which may influence the multivariate analysis. However, majority of patients were white, as patients were recruited in Germany and Austria. Second, MMR status has not been tested in patients because these two trials were initiated before the publication of NCT01876511.[30] Therefore, MMR status was not accounted for the multivariate analysis. Third, the biological function of the identical SNPs, as well as the associations with the efficacy of bevacizumab, should be further confirmed *in vitro* and *in vivo*.

In conclusion, our study provides the first evidence, to our knowledge, that genetic variants in the lipid metabolism pathway could predict outcomes of bevacizumab-based treatment in patients with mCRC, which may potentially guide personalised treatment decisions. These finding may also provide insights for the combination of a FASN inhibitor and anti-angiogenesis therapy in patients with mCRC. Further *in vitro* and *in vivo* studies are warranted to validate our novel findings.

Contributors

J. Wang and H.-J. Lenz designed the study and wrote the manuscript. S. Stintzing, H. Arai, F. Battaglin, N. Kawanishi, S. Soni, W. Zhang, C. Mancao, C. Cremolini, T. Liu, V. Heinemann, A. Falcone, L. Shen and H.-J. Lenz acquired and managed patients, carried out the data collection and database creation. J. Millstein carried out the statistical analysis. Y. Yang verified the underlying data. J. Wang, J. Millstein, Y. Yang, H.-J. Lenz were responsible for manuscript preparation. All authors confirm that they had full access to all the analysed data in the study and accept the responsibility to submit for publication.

Data sharing statement

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declaration of interests

H.-J.L. reports receiving honoraria from consultant/advisory board membership for Merck Serono, Bayer, and Genentech. S.S. reports receiving honoraria for talks and advisory board role: AMGEN, Bayer, BMS, Pierre-Fabre, Merck KGaA; MSD, Leo-Pharma, Lilly, Sanofi, Servier, Roche, Takeda, Taiho. A.F reports receiving honoraria as consultant/advisory board for Amgen, Bayer, Bristol, Daiichi Sankyo, Incyte, Lilly, Merck, MSD, Pierre-Fabre, Roche, Servier. C.M was a full time employee of Roche/Genentech when the MAVERICC data was generated. All remaining authors have declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.101827.

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