A Vascular Endothelial Growth Factor Gene Polymorphism Predicts Malignant Potential in Intraductal Papillary Mucinous Neoplasm

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Objectives: The aim of this study was to evaluate the clinical relevance of vascular endothelial growth factor (VEGF) single nucleotide polymorphisms (SNPs) in intraductal papillary mucinous neoplasms (IPMNs).
Methods: A total of 169 IPMN and 108 pancreatic ductal adenocarcinoma patients who underwent curative resection were enrolled, and VEGF +405G/C and −460C/T SNPs were investigated.
Results: Vascular endothelial growth factor +405C/C was found more frequently in malignant IPMNs compared with +405G/G (odds ratio [OR], 2.7; $P = 0.04$), and +405C allele was associated with malignant IPMNs compared with +405G ($P = 0.055$). In branch duct IPMNs, VEGF +405C/C was significantly associated with malignant transformation (CC vs GG: OR, 4.0; $P = 0.03$; CC vs CG + GG: OR, 3.3; $P = 0.04$), and there was a trend of VEGF +405C/C associated with malignant transformation of gastric-type IPMNs (CC vs GG: OR, 3.0; $P = 0.07$). When the survival outcomes were analyzed based on VEGF +405G/C SNPs, however, there was no relationship between VEGF SNPs and overall survival in patients with both IPMNs and pancreatic ductal adenocarcinomas.
Conclusions: Vascular endothelial growth factor +405G/C SNP was significantly associated with malignant transformation in IPMNs, especially branch duct and gastric-type IPMNs. Vascular endothelial growth factor +405G/C SNP might be helpful in predicting clinical course in pancreatic disease with potential for malignant transformation.

Key Words: IPMN, PDAC, VEGF SNP

Abbreviations: IPMN - intraductal papillary mucinous neoplasm, PDAC - pancreatic ductal adenocarcinoma, VEGF - vascular endothelial growth factor

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Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas are morphologically characterized by an intraductal mucin-producing neoplasm with cystic dilatation of either the main pancreatic duct or branch duct.[1] Intraductal papillary mucinous neoplasms are well known to be premalignant lesions that sequentially progress from adenoma to carcinoma.[2,3] Although benign IPMNs can be followed-up because of an indolent natural course, some authors reported poor prognosis for invasive IPMNs comparable with ductal carcinoma.[4,5] From a macroscopic point of view, radiological type, size of cystic mass, and the presence of mural nodules are widely accepted as predictive malignant factors[3]; however, some reports showed that morphological subtypes, which are defined as gastric, intestinal, pancreatobiliary, or oncocytic types,[6] are conflicting survival outcomes from a microscopic regard.[7–9]

Angiogenesis plays an important role in the progression and metastasis of various tumor types, and vascular endothelial growth factor (VEGF) is a critical component. Vascular endothelial growth factor is a heparin-binding glycoprotein with potent angiogenic, mitogenic, and vascular permeability-enhancing activities specific to endothelial cells.[10,11] Vascular endothelial growth factor belongs to the platelet-derived growth factor supergene family and includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, and placenta growth factor. Among them, VEGF-A is the most important ligand; its gene is located on chromosome 6p21.3 and consists of 8 exons exhibiting alternate splicing to encode a family of proteins.[12,13] In previous studies, at least 30 single nucleotide polymorphisms (SNPs) of the *VEGF* gene have been reported.[14]

Vascular endothelial growth factor +405G/C SNP (rs2010963) is located within the 5′-untranslated region and may affect transcription factor binding affinity.[14,15] In contrast, VEGF −460C/T SNP (rs833061) is located in the promoter region and may influence promoter activity.[15] These 2 SNPs have been investigated, and their association with the risk or prognosis of several cancers has been explored.[16–24] Recent studies reported that serum VEGF levels were significantly higher in pancreatic ductal adenocarcinoma (PDAC) patients compared with control subjects, and VEGF +405G/C SNP was strongly associated with the carcinogenesis of PDAC patients.[25,26]

The aim of this study was to investigate the distribution of VEGF SNPs with regard to malignant transformation in IPMN patients. Furthermore, correlations between VEGF SNPs and clinicopathological parameters including the morphological subtypes were statistically analyzed. To our knowledge, this study is the first to investigate the association between VEGF SNPs and IPMNs.

MATERIALS AND METHODS

Patient Selection

A total of 169 consecutive patients with IPMN and 108 patients with PDAC, who underwent surgical resection between January 1993 and December 2012 at the Department of Gastroenterological Surgery, Nagoya University Hospital, were enrolled in this study. All patients were confirmed to have histological diagnosis of either IPMN or PDAC.

Histological grading of IPMNs was performed according to the criteria defined by the international consensus guidelines 2012.[27] Each IPMN was classified as any of following: low-grade

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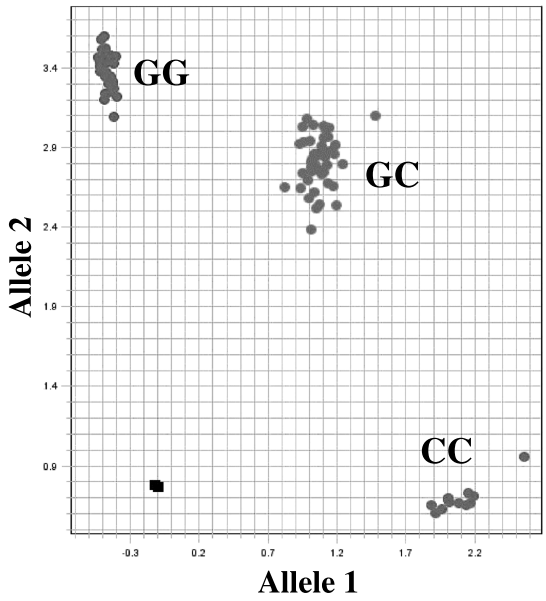

FIGURE 1. Allelic discrimination plot of VEGF +405G/C. Clusters in the allelic discrimination plot show the 3 genotypes of VEGF +405G/C.

dysplasia, moderate dysplasia, high-grade dysplasia, and invasive carcinoma. Low-grade dysplasia and moderate dysplasia were defined as benign IPMNs; on the other hand, high-grade dysplasia and invasive carcinoma were defined as malignant IPMNs.

Intraductal papillary mucinous neoplasm was morphologically classified into main duct-type (MD-IPMN) or branch duct-type (BD-IPMN) according to which component of the pancreatic duct system was predominantly involved. The classification was made based on clinical imaging studies and macroscopic investigations of the resected specimens.[28] We classified all patients into either of these 2 types and not to the mixed type because the definition of this entity remains unclear and controversial.

The intraductal components were classified into 4 distinct epithelial subtypes (gastric, intestinal, pancreatobiliary, and oncocytic) based on their epithelial morphology on routine (hematoxylin and eosin) staining and, for those suspected of being pancreatobiliary or oncocytic, immunoreactivity against mucin glycoproteins according to previously described criteria for confirmation.[6] In patients with multiple morphologically distinct IPMNs, each neoplasm was classified depending on the papilla type of highest histological grade or the most dominant component present in areas of the same histological grade.

Genotyping

Genomic DNA was isolated from paraffin-embedded pancreatic tissue using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Hilden, Germany). Vascular endothelial growth factor +405G/C and −460C/T polymorphisms were examined by TaqMan Allele Discrimination assay (Applied Biosystems, Foster City, Calif), and real-time polymerase chain reactions (PCRs) were used (Applied Biosystems). The VEGF +405G/C (rs2010963) and −460C/T (rs833061) genotypes were determined by real-time PCR with commercially available primers labeled with FAM or vasoactive intestinecontractor (VIC) (Applied Biosystems). Polymerase chain reaction were performed in a 96-well plate format in a reaction volume of 10 μL containing 2 μL DNA, 0.5 μL of each primer, 5 μL TaqMan Universal PCR Master Mix (Applied Biosystems), and 2.5 μL of DNA-free water. The PCR program was as follows: an initial denaturation step at 95°C for 20 seconds followed by 40 cycles at 95°C for 3 seconds and 60°C for 20 seconds. The 2 allele-specific probes were labeled with reporter dyes VIC (allele 1) and 6-carboxyfluorescein (FAM) (allele 2), respectively, which contain a nonfluorescent quencher. SDS software was used to record the results of the allelic discrimination run on a scatter plot of allele 1 versus allele 2. For samples homozygous for allele 1, the VIC probe was cleaved, but the FAM probe remained intact. Similarly, in samples homozygous for allele 2, the FAM probe was cleaved, and the VIC probe remained intact. For heterozygous alleles, both probes were cleaved, and both VIC and FAM fluorescence were high. Each well of the 96-well reaction plate was represented as an individual point on the plot (Fig. 1).

Statistical Analyses

The genotype distributions of patients with IPMNs were analyzed with logistic regression analysis and presented as odds ratio (OR) with 95% confidence intervals (95% CIs). The deviations from Hardy-Weinberg equilibrium were analyzed using the χ^2 test. The association of VEGF genotypes with clinicopathological parameters was examined using χ^2 and Fisher exact tests. Disease-specific survival was assessed to determine the presence of any correlations among the groups. The survival rates were calculated by the Kaplan-Meier method, and the differences in survival curves were analyzed using the log-rank test. Data were analyzed using JMP v10 software (JMP; SAS Institute, Cary, NC). The level of statistical significance was set at $P < 0.05$.

RESULTS

Patient Demographics

The demographics of patients with IPMNs in this study are summarized in Table 1. There were 105 males and 64 females. The age of the patients ranged from 37 to 85 years (median, 66.5 years). Macroscopic type was identified as MD-IPMN in 57 patients (33.7%) and BD-IPMN in 112 patients (66.3%). With regard to histological grade, 85 patients (50.3%) were pathologically classified as having low-grade dysplasia, 0 (0%) as moderate dysplasia, 45 (26.6%) as high-grade dysplasia, and 39 (23.1%) as invasive carcinoma, and a total of 84 patients were diagnosed with malignant IPMNs. The morphological subtypes of the study subjects consisted of 123 gastric type (72.8%), 42 intestinal

TABLE 1. Patient Demographics

	Total (N = 169)
Age, median (SD, range), y	66.5 (8.4, 37-85)
Sex, male:female	105:64
Macroscopic type, n (%)	
MD-IPMN	57 (33.7)
BD-IPMN	112 (66.3)
Histological grade, n (%)	
Low-grade dysplasia	85 (50.3)
Moderate dysplasia	0 (0)
High-grade dysplasia	45 (26.6)
Invasive carcinoma	39 (23.1)
Morphological subtype	
Gastric type	123 (72.8)
Intestinal type	42 (24.9)
Pancreatobiliary type	3 (1.7)
Oncocytic type	1 (0.6)

TABLE 2. The Genotype Distribution of *VEGF* Gene Polymorphisms +405G/C and –460C/T Between Patients With Malignant IPMNs and Benign IPMNs

		Malignancy (n = 80)	Benign (n = 85)	*P*
VEGF +405G/C, n (%)	GG	22 (27.5)	33 (38.8)	
	GC	42 (52.5)	43 (50.6)	
	CC	16 (20.0)	9 (10.6)	
Logistic regression analysis, OR (95% CI)	CC vs GG	2.7 (1.0-7.3)		0.04*
	CC vs CG	1.8 (0.7-4.7)		0.20
	CG vs GG	1.4 (0.7-2.9)		0.27
	CC vs CG + GG	2.1 (0.9-5.3)		0.09
VEGF −460C/T, n (%)	CC	5 (6.2)	13 (15.3)	
	CT	39 (48.8)	34 (40.0)	
	TT	36 (45.0)	38 (44.7)	
Logistic regression analysis, OR (95% CI)	CT vs CC	3.0 (1.0-10.1)		0.05*
	CT vs TT	1.2 (0.6-2.3)		0.56
	TT vs CC	2.5 (0.8-8.3)		0.10
	CT vs CC + TT	1.4 (0.3-2.7)		0.26

*Statistically significant.

type (24.9%), 3 pancreatobiliary type (1.7%), and 1 oncocytic type (0.6%). The pancreatobiliary and oncocytic types were excluded from the subsequent analysis because of their rarity.

Distribution of *VEGF* +405G/C and –460C/T Gene Polymorphisms in Patients With Malignant IPMNs and Benign IPMNs

Vascular endothelial growth factor +405G/C and −460C/T genotypes were successfully determined in all 169 enrolled subjects (Fig. 1); the frequencies of each genotype are shown in Table 2. The distributions of both +405G/C and −460C/T genotypes were followed by the Hardy-Weinberg equilibrium. The homozygous +405C/C genotype was found to be more frequent in patients with malignant IPMNs (20.0%) compared with those with benign IPMNs (10.6%). The OR for being malignant in the patients with +405C/C genotype compared with those with +405G/G was 2.7, which was statistically significant ($P = 0.04$). When the VEGF +405G/C and −460C/T polymorphisms were analyzed in terms of the allele distribution, +405C allele in the patients with IPMNs was weakly associated with the malignant transformation ($P = 0.055$). On the other hand, there was no correlation between VEGF −460C/T allele and malignant transformation in IPMNs ($P = 0.37$; Table 3).

Correlation Between VEGF +405G/C Polymorphism and Clinicopathological Parameters of IPMNs

Next, the correlation between VEGF +405G/C polymorphism and the clinicopathological parameters of IPMNs was statistically analyzed. There was no significant association between VEGF +405G/C genotype and age, sex, tumor location, main pancreatic duct diameter, cystic diameter, mural nodule, and serum carbohydrate antigen 19-9 levels. No significant difference in VEGF +405G/C genotype was found between the 2 macroscopic types. As for the morphological types, the proportion of patients with VEGF +405C/C was slightly higher in the intestinal group than in the gastric group, although not significantly so ($P = 0.07$; Table 4).

The clinical impact of VEGF +405G/C genotypes was then separately analyzed in the MD-IPMN and BD-IPMN subgroups. As a result, the homozygous 405C/C genotype was found more frequently in malignant BD-IPMNs than in benign BD-IPMNs (CC vs GG, $P = 0.03$; CC vs CG + GG, $P = 0.04$). There was no such difference in the incidence of the homozygous 405C/C genotype for the MD-IPMN subgroup (Table 5).

The association of the VEGF +405G/C genotype with histological grade was also examined in the subgroups with gastric type and intestinal type. Although not statistically significant, the homozygous 405C/C genotype was found more frequently in malignant gastric-type IPMNs than in benign gastric-type IPMNs ($P = 0.07$). However, there was no significant association in patients with intestinal-type IPMNs (Table 6).

Distribution of *VEGF* +405G/C and –460C/T Gene Polymorphisms in Patients With PDAC and IPMN and Survival

VEGF +405G/C and −460C/T gene polymorphisms were also examined in 108 resected PDAC patients and compared with those

TABLE 3. The Allele Distribution of *VEGF* Gene Polymorphisms 405 G/C and –460C/T Between Malignant IPMNs and Benign IPMNs

VEGF +405G/C	Allele C	Allele G	OR (C/G)	95% CI	*P*
Malignancy	74 (46.3%)	86 (53.7%)	1.5	1.0–2.4	0.055
Benign	61 (35.9%)	109 (64.1%)			
VEGF −460C/T	**Allele C**	**Allele T**	**OR (C/T)**	**95% CI**	***P***
Malignancy	49 (30.6%)	111 (69.4%)	0.8	0.5–1.3	0.37
Benign	60 (35.3%)	110 (64.7%)			

TABLE 4. Correlation Between VEGF +405G/C Polymorphism and Clinicopathological Parameters of IPMNs

Variables	G/G (%)	G/C (%)	C/C (%)	*P*
Age, y				
≧67	28 (31.5)	49 (55.0)	12 (13.5)	0.62
≦66	29 (36.3)	38 (47.5)	13 (16.2)	
Sex				
Male	33 (31.4)	55 (52.4)	17 (16.2)	0.66
Female	24 (37.5)	32 (50.0)	8 (12.5)	
Tumor location				
Head	38 (32.8)	63 (54.3)	15 (12.9)	0.46
Body/tail	19 (35.8)	24 (45.3)	10 (18.9)	
Main pancreatic duct diameter, mm				
≧5	30 (34.1)	45 (51.1)	13 (14.8)	0.97
<5	23 (32.4)	37 (52.1)	11 (15.5)	
Cystic diameter, mm				
≧30	26 (31.7)	45 (54.9)	11 (13.4)	0.69
<30	29 (35.8)	39 (48.1)	13 (16.1)	
Mural nodule				
Yes	40 (32.3)	65 (52.4)	19 (15.3)	0.79
No	17 (37.8)	22 (48.9)	6 (13.3)	
Carbohydrate antigen 19-9, U/mL				
≧37	12 (30.0)	23 (57.5)	5 (12.5)	0.67
<37	43 (34.4)	62 (49.6)	20 (16.0)	
Macroscopic type				
MD-IPMN	17 (29.8)	29 (50.9)	11 (19.3)	0.46
BD-IPMN	40 (35.7)	58 (51.8)	14 (12.5)	
Morphological subtype				
Gastric type	43 (35.0)	66 (53.6)	14 (11.4)	0.07
Intestinal type	12 (28.6)	19 (45.2)	11 (26.2)	

of IPMNs patients. Interestingly, PDAC had higher incidence of the VEGF +405G/G genotype than that of malignant IPMNs patients (49.6% in PDAC, 27.5% in malignant IPMNs GG vs CG + CC, $P = 0.002$; 38.8% in benign IPMNs GG vs CG + CC, $P = 0.14$; Table 7).

Finally, survival outcomes based on VEGF +405G/C genotypes were statistically analyzed. There was no significance among PDAC and malignant IPMNs patients (Fig. 2).

DISCUSSION

Vascular endothelial growth factor has been actively investigated as a critical angiogenic factor in tumor growth, invasiveness, and metastasis. Several studies have reported that some VEGF SNPs are associated not only with VEGF expression, but also with increased risk for solid tumors.[29] Stevens et al[15] showed that VEGF +405/−460 SNPs significantly altered VEGF promoter activity in response to phorbol esters in an in vivo study. Recent literature has reported the association of VEGF SNPs, especially +405G/C and −460C/T, with risk or prognosis of various cancer types.[16–24,30]

Talar-Wojnarowska et al[25] reported that the +405CC genotype contributed to pancreatic angiogenesis in a Polish population, whereas Sivaprasad et al found a significant association of the +405GG genotype with the carcinogenesis of PDAC in South Indian patients.[26] Previously, some studies reported that the distribution of the SNPs varied by ethnicity.[31–33] With regard to the pancreatic disease, several authors reported that the incidence of pancreatic cancer was different between the races, and this fact was considered attributable to the genetic disparity.[34,35] The difference in the distribution and contribution of VEGF SNPs in carcinogenesis of pancreatic tumors observed in the current study most likely reflect the ethnic difference, but further studies in various regions of the world are needed to prove this.

To the best of our knowledge, no previous study has assessed the distribution of VEGF SNPs in patients with IPMNs. Therefore, we examined the functional activity of VEGF SNPs in 169 resected patients with IPMNs. Our study revealed that the +405CC genotype was significantly associated with malignant transformation in IPMNs. Furthermore, the +405C allele was found more frequently in the malignant IPMNs group, although no associations with most clinicopathological characteristics

TABLE 5. The Genotype Distribution of *VEGF* Gene Polymorphisms +405G/C and −460C/T Between Patients With Malignant IPMNs and Benign IPMNs Based on Macroscopic Type

		MD-IPMNs			BD-IPMNs		
		Malignancy (n = 37)	Benign (n = 18)	*P*	Malignancy (n = 43)	Benign (n = 67)	*P*
VEGF +405G/C, n (%)	GG	10 (27.1)	6 (33.3)		12 (27.9)	27 (40.3)	
	GC	20 (54.0)	8 (44.4)		22 (51.2)	35 (52.2)	
	CC	7 (18.9)	4 (22.3)		9 (20.9)	5 (7.5)	
Logistic regression analysis, OR (95% CI)	CC vs GG	1.0 (0.4–5.6)		0.95	4.0 (1.2–15.7)		0.03*
	CC vs CG	0.7 (0.2–2.5)		0.64	2.9 (0.9–10.4)		0.08
	CG vs GG	1.5 (0.4–5.6)		0.54	1.4 (0.6–3.4)		0.43
	CC vs CG + GG	0.8 (0.2–3.5)		0.78	3.3 (1.0–11.4)		0.04*
VEGF −460C/T, n (%)	CC	1 (2.8)	2 (11.2)		4 (9.3)	11 (16.4)	
	CT	18 (48.6)	8 (44.4)		21 (48.8)	26 (38.8)	
	TT	18 (48.6)	8 (44.4)		18 (41.9)	30 (44.8)	
Logistic regression analysis, OR (95% CI)	CT vs CC	4.5 (0.4–105.4)		0.23	2.2 (0.7–8.9)		0.21
	CT vs TT	1.0 (0.3–3.3)		1.00	1.3 (0.6–3.1)		0.48
	TT vs CC	4.5 (0.4–105.4)		0.23	1.6 (0.5–6.7)		0.58

*Statistically significant.

TABLE 6. The Genotype Distribution of *VEGF* Gene Polymorphisms +405G/C and –460C/T Between Patients With Malignant IPMNs and Benign IPMNs Based on Morphological Subtype

		Gastric Type			Intestinal Type		
		Malignancy (n = 48)	Benign (n = 75)	*P*	Malignancy (n = 32)	Benign (n = 10)	*P*
VEGF +405G/C, n (%)	GG	13 (27.0)	30 (40.0)		9 (28.1)	3 (30.0)	
	GC	27 (56.3)	39 (52.0)		15 (46.9)	4 (40.0)	
	CC	8 (16.7)	6 (8.0)		8 (20.9)	3 (30.0)	
Logistic regression analysis, OR (95% CI)	CC vs GG	3.0 (0.9–11.1)		0.07	0.9 (0.1–6.1)		0.90
	CC vs CG	1.9 (0.6–6.5)		0.27	0.7 (0.1–4.4)		0.70
	CG vs GG	1.6 (0.7–3.7)		0.26	1.2 (0.2–7.0)		0.80
	CC vs CG + GG	2.3 (0.7–7.4)		0.15	0.8 (0.2–4.3)		0.76
VEGF −460C/T	CC	4 (8.4)	12 (16.0)		1 (3.1)	1 (10.0)	
	CT	22 (45.8)	31 (41.3)		17 (53.1)	3 (30.0)	
	TT	22 (45.8)	32 (42.7)		14 (43.8)	6 (60.0)	
Logistic regression analysis, OR (95% CI)	CT vs CC	2.1 (0.6–8.4)		0.22	5.7 (0.2–174.4)		0.27
	CT vs TT	1.0 (0.5–2.2)		0.93	2.4 (0.5–13.2)		0.25
	TT vs CC	2.1 (0.6–8.1)		0.24	2.3 (0.1–66.0)		0.58

could be found. Therefore, our results suggest that VEGF +405G/C SNP might affect malignant transformation in IPMNs. However, further studies are needed to verify this result.

The genotypic frequencies for VEGF +405CC were also prominent in BD-IPMNs and gastric-type IPMNs. Histologically, gastric-type IPMNs are generally found in BD-IPMNs, and intestinal-type IPMNs are usually found in MD-IPMNs.[9,36–38] We reported that gastric-type IPMNs often had benign behavior, but invasive carcinomas derived from gastric-type IPMNs had poorer prognoses than those derived from intestinal-type IPMNs.[28] In this study, VEGF +405C/C was strongly associated with malignant transformation, especially in gastric type. In fact, although not statistically significant, patients with VEGF +405C/C had worse overall survival in gastric type (data not shown); hence, VEGF +405C/C might be a prognostic factor in gastric-type IPMNs and BD-IPMNs.

A similar genotypic analysis was conducted in 108 resected PDAC patients to enable a comparison with the results of IPMNs. In PDAC, an increased frequency of VEGF +405G/G was observed compared with malignant IPMN patients, which was consistent with the genotyping result of PDAC in South India.[26] In this sense, the *VEGF* gene in Japanese patients might be similar to that of Indian rather than that of Polish populations.[26] Intraductal papillary mucinous neoplasm is essentially a different disease from PDAC; hence, the process of malignant transformation may be different between IPMN and PDAC, presumably leading to the difference in the incidence of the VEGF +405G/G genotype between the malignant IPMN and PDAC.

Several studies suggested that VEGF +405G/C SNPs affect survival in gastric and ovarian cancers.[39–42] Some reports demonstrated a relationship between higher VEGF serum levels and larger tumor size, as well as lymph node involvement and the presence of distant metastasis in PDAC patients.[43] They also reported that serum levels of VEGF decreased significantly after radical tumor resection and was a prognostic factor for PDAC.[43,44] Talar-Wojnarowska et al[25] found on the other hand that there was no relationship between VEGF SNPs and VEGF serum levels. Thus, opinions regarding prognostic role of VEGF SNPs are rather mixed. Although our data suggest that VEGF SNPs can be involved in malignant transformation not only of PDAC but also of IPMN, there was no correlation between VEGF SNPs and survival outcome among patients who are already with malignant disease. At this time, this could only be explained by the fact that although +405C/C genotype was found more often among

TABLE 7. The Genotype Distribution of *VEGF* Gene Polymorphisms +405G/C and –460C/T Between Patients With PDAC and IPMNs

		PDAC (n = 108)	Malignant IPMNs (n = 80)	Benign IPMNs (n = 85)
VEGF +405G/C, n (%)	GG	53 (49.6)	22 (27.5)	33 (38.8)
	GC	33 (30.8)	42 (52.5)	43 (50.6)
	CC	21 (19.6)	16 (20.0)	9 (10.6)
Logistic regression analysis, OR (95% CI)	GG vs GC + CC	Reference group	0.39 (0.2–0.7)	0.65 (0.4–1.1)
			P = 0.002*	*P* = 0.14
VEGF −460C/T, n (%)	CC	7 (6.7)	5 (6.2)	13 (15.3)
	CT	39 (37.1)	39 (48.8)	34 (40.0)
	TT	59 (56.2)	36 (45.0)	38 (44.7)
Logistic regression analysis, OR (95% CI)	TT vs CT + CC	Reference group	0.64 (0.4–1.1)	0.63 (0.4–1.1)
			P = 0.13	*P* = 0.11

*Statistically significant.

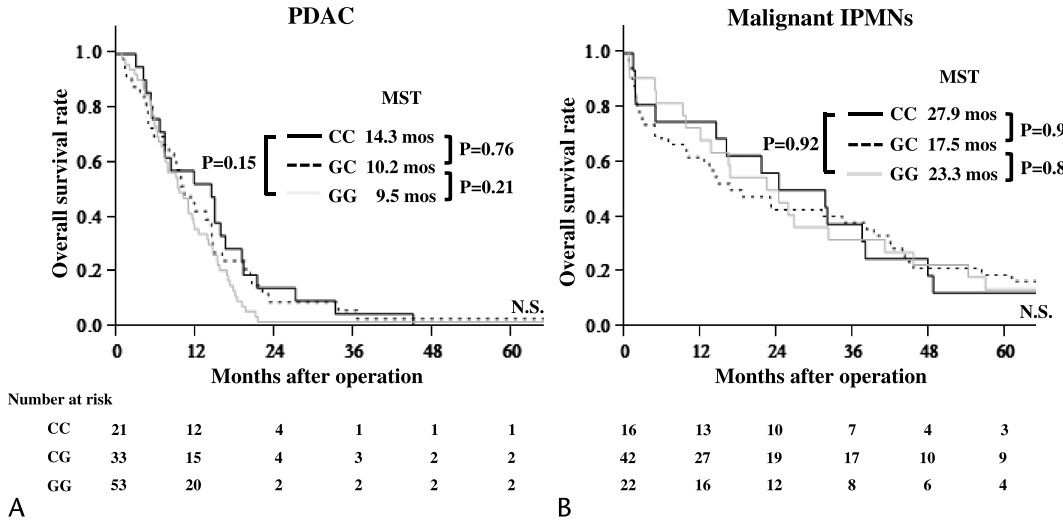

FIGURE 2. Survival curves after resection of (A) PDAC and (B) malignant IPMN based on VEGF +405G/C genotypes. There was no significant difference among PDAC and malignant IPMN patients.

malignant IPMNs than among the benign counterparts, it does not have any specific function that influences survival of the patients who harbor malignant IPMNs.

In conclusion, our study demonstrates that VEGF +405G/C SNPs can play an important role in the carcinogenesis also of IPMNs. Vascular endothelial growth factor +405C/C was significantly associated with malignant transformation of IPMNs, especially in patients with BD-IPMNs or gastric-type IPMNs. Therefore, VEGF SNPs might be helpful in predicting the clinical course of pancreatic disease with potential for malignant transformation.

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