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Metabolome analysis for pancreatic cancer risk in nested case-control study: Japan Public Health Center-based prospective Study

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Discovery of a high-risk group for pancreatic cancer is important for prevention of pancreatic cancer. The present study was conducted as a nested case-control study including 170 pancreatic cancer cases and 340 matched controls of our population-based cohort study involving 30 239 subjects who answered a baseline questionnaire and supplied blood samples. Twelve targeted metabolites were quantitatively analyzed by gas chromatography/tandem mass spectrometry. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated using conditional logistic regression models. Statistically significant *P*-value was defined as $P < .05$. Increasing 1,5-anhydro-D-glucitol (1,5-AG) levels were associated with a decreasing trend in pancreatic cancer risk (OR of quartile 4 [Q4], 0.50; 95% CI, 0.27-0.93; $P = .02$). Increasing methionine levels were also associated with an increasing trend of pancreatic cancer risk (OR of Q4, 1.79; 95% CI, 0.94-3.40: $P = .03$). Additional adjustment for potential confounders attenuated the observed associations of 1,5-AG and methionine (*P* for trend = .06 and .07, respectively). Comparing subjects diagnosed in the first 0-6 years, higher levels of 1,5-AG, asparagine, tyrosine and uric acid showed a decreasing trend for pancreatic cancer risk (*P* for trend = .04, .04, .04 and .02, respectively), even after adjustment for potential confounders. We found that the 12 target metabolites were not associated with pancreatic cancer risk. However, metabolic changes in the subjects diagnosed in the first 0-6 years showed a similar tendency to our previous reports. These results might suggest that these metabolites are useful for early detection but not for prediction of pancreatic cancer.

KEYWORDS
cohort study, JPHC, metabolomics, pancreatic cancer, risk

JPHC members are listed in the following website (as of April 2017): http://epi.ncc.go.jp/en/jphc/781/7951.html
Kobayashi, Nishiumi, and Hidaka contributed equally to this study.

1 | INTRODUCTION

Pancreatic cancer is one of the most common and deadly cancers. Surgical resection is the only potentially curative therapy, despite developments in detection and management of pancreatic cancer. However, more than 80% of patients have unresectable disease at the time of diagnosis, and its 5-year survival rate is less than 5%.[1-3] Some reports have indicated that there may be a broad time window for the development of pancreatic cancer. In a study by Yachida et al[4], a quantitative analysis at the time of the genetic evolution of pancreatic cancer was carried out, which indicated there is at least a decade between the occurrence of the initiating mutation and the birth of the parental, non-metastatic founder cell, and at least 5 more years are required for the acquisition of metastatic ability. Meza et al[5] reported the average sojourn time of premalignant pancreatic lesions to be about 50 years. These reports suggested that premalignant changes in a pancreatic lesion last for over 10 years before such a lesion becomes malignant and metastatic. If we can identify a high-risk group of pancreatic cancer with premalignant changes, we can undertake an intensive follow up to prevent them from developing an unresectable stage of pancreatic cancer.

The omics fields of study including genomics, transcriptomics, proteomics and metabolomics are used in life science research. Metabolomics is the latest field of the omics to be studied, and the last decade has focused on its development and application. Metabolite alterations mainly represent the endpoint of the omics cascade and are also close to the phenotype; therefore, metabolites serve as direct signatures of biochemical activity.[6-8] Some studies have shown the benefits of metabolomics for early detection of pancreatic cancer. [9-11] In addition, metabolomics offers measurement of environmental and exogenous exposures with far less measurement error than with questionnaires.[12] These studies showed the potential utility of metabolomics in the detection of metabolic changes as a result of premalignant pancreatic changes and evaluation of environmental factors. We reported that the levels of some metabolites differ significantly between pancreatic cancer patients and healthy volunteers, and the serum levels of valine, 2-aminoethanol, threonine, creatinine, asparagine, xylitol, 1,5-anhydro-D-glucitol (1,5-AG), lysine, histidine, tyrosine and inositol in pancreatic cancer patients were significantly lower than in healthy volunteers.[13,14] Changes of these metabolites may reflect metabolic changes as a result of pancreatic cancer or environmental factors or both. If some of these metabolite changes start before the occurrence of pancreatic cancer, we can apply these metabolites to predictive markers to identify the high-risk group of pancreatic cancer. In the present study, we investigated the plasma of a large number of pancreatic cancer patients, which was collected before the existence of clinical pancreatic cancer, drawn from a prospective cohort study to evaluate 12 metabolites for their association with pancreatic cancer risk.

2 | MATERIALS AND METHODS

2.1 | Study population and baseline survey

The Japan Public Health Center-based prospective Study (JPHC Study) is a continuing cohort study with a total of 140 420 subjects (68 722 men and 71 698 women), and consists of 2 cohorts in 11 public health centers (PHC) in Japan. Cohort I (aged 40-59 years) and cohort II (aged 40-69 years) of this study were started in January 1990 and 1993, respectively.[15] This study has been approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

For the present case-control study, we defined a cohort of 39 143 subjects who answered a self-administered questionnaire and provided a 10-mL blood sample at the baseline survey (cohort I: 1990-1992, cohort II: 1993-1995). The questionnaire included various lifestyle factors, such as sociodemographic characteristics, personal medical history, family history, smoking, drinking habits. Blood samples were stored at −80°C until analysis. After exclusion of subjects who reported a history of cancer (n = 830), subjects whose blood samples were already used for other measurements (n = 6462), subjects who did not give information regarding smoking, frequency of alcohol consumption, coffee consumption, diabetes mellitus (DM), and body mass index (BMI) (n = 1612), the remaining 30 239 subjects were eligible for this study.

2.2 | Selection of pancreatic cancer cases and controls (including follow up)

Anatomical site and cancer histological type were classified according to the International Classification of Diseases for Oncology, Third edition (ICD-O-3).[16] Among 30 239 eligible subjects, we identified 170 newly diagnosed pancreatic cancer cases (C25.0-C25.9) until 31 December 2009 (median follow-up period: 16.4 years) through local major hospitals and population-based cancer registries. Death certificate information was also used as a supplementary information source, but descriptions in Part II of the death certificate (other significant conditions contributing to death but not resulting in the underlying) were not used for identification of pancreatic cancer cases. We did not include pancreatic tumors located in the islets of Langerhans (C25.4) from our analysis because the etiology of these tumors is considered to be different. Two controls were randomly selected from those of 30 239 eligible subjects and were matched to a case for age (5-year age group), gender, PHC area, and fasting time at blood donation (less than 7 hours, 7 hours or more, or unknown). Our analysis was done on 170 pancreatic cancer cases and 340 matched controls.

2.3 | Chemicals and standards

We focused on 12 metabolites that have previously been reported to be biomarker candidates;[13,14] ie, threonine, methionine, arabinose,

asparagine, xylitol, glutamine, 1,5-AG, lysine, histidine, tyrosine, inositol and uric acid. Stable isotope products for each metabolite; ie, L-threonine [13C4, 97%-99%], L-methionine [13C5, 99%], D-arabinose [U-13C5, 99%], L-asparagine:H_2O [13C4, 99%], D-xylitol [U-13C5, 99%], L-glutamine [13C5, 99%], 1,5-AG [U-13C6, 98%+], L-lysine:2HCl [13C6, 99%], L-histidine:HCl:H_2O [13C6, 97%-99%], L-tyrosine [ring-13C6, 99%], myo-inositol [1,2,3,4,5,6-D6, 98%], and uric acid [1,3-15N2, 98%], were obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Methanol was acquired from Kanto Chemical Co., Inc. (Tokyo, Japan). 2-Isopropylmalic acid and methoxyamine hydrochloride were purchased from Sigma-Aldrich (St Louis, MO, USA). Pyridine was obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) was acquired from GL Sciences Inc. (Tokyo, Japan). Human standard plasma (as a quality control) was obtained from Kohjin Bio Co., Ltd (Osaka, Japan).

2.4 | Sample preparation

Every batch included 2 quality control samples of pooled standard plasma, blank sample (distilled water) and 6 calibration samples. Calibration samples were made by mixing a native and a stable isotope, the concentration ratios of which (native/stable isotope) were 0, 0.1, 0.5, 1, 2 and 5.

Extraction and derivatization of low molecular weight metabolites were carried out as follows: Each sample (20 μL) was dispensed into a 1.5-mL Eppendorf tube. The samples were then extracted with 250 μL methanol containing stable isotopes and shaken in a vortex. Next, 10 μL of 2-isopropylmalic acid (0.5 mg/mL) was added as an internal standard. The mixture was incubated at 1200 rpm for 30 minutes at 37°C. After the mixture had been centrifuged at 19 300 *g* for 5 minutes at 4°C, 200 μL of the supernatant was transferred to a new Eppendorf tube capped with a pierced cap. After centrifugation for 40 minutes in a vacuum concentrator (Thermo SpeedVac), the mixture was freeze-dried overnight. For the derivatization, 80 μL of methoxyamine hydrochloride in pyridine (20 mg/mL) was added as the first derivatizing agent. The mixture was then incubated at 1200 rpm for 90 minutes at 30°C. The second derivatizing agent, 40 μL MSTFA, was added, and then the mixture was incubated at 1200 rpm for 30 minutes at 37°C. After the mixture had been centrifuged at 19 300 *g* for 5 minutes at room temperature, the supernatant was transferred to a vial for analysis by gas chromatography/tandem mass spectrometry (GC/MS/MS).

2.5 | GC/MS/MS procedure

Gas chromatography/MS/MS analysis was carried out on a GCMS-TQ8040 GC/MS/MS system (Shimadzu Co., Kyoto, Japan). Each sample was injected in a split ratio of 1:10, and then separation was carried out on a fused silica capillary column (BPX5; inner diameter: 30 m × 0.25 mm, film thickness: 0.25 μm; SGE Analytical Science). The front inlet temperature was 250°C. Helium gas was used as the GC carrier gas, and argon gas was used as the MS/MS collision gas. Flow rate of helium gas through the column was 39.0 cm/s. Column temperature was maintained at 80°C for 2 minutes and then raised by 15°C/min to 330°C, before being maintained for 3 minutes. Transfer line and ice-source temperatures were 280°C and 200°C, respectively. Multiple reaction monitoring optimization was done as follows. Each stable isotope was analyzed by GC/MS/MS and the optimal transition, which consisted of the precursor ion, collision energy and product ion, was selected. The transitions for all stable isotopes were added to the software, and target and reference ions were selected. The method file created by the software was used to analyze the samples.

2.6 | Data processing

Mass spectrometry data were exported to a personal computer with GCMSsolution software (Shimadzu Co.), and the peaks for the targeted metabolites and stable isotopes were detected by the software and then checked manually. Concentrations of the targeted metabolites in each sample were calculated based on the calibration sample's peak area ratios of the targeted metabolites.

2.7 | Statistical analysis

Chi-squared test for categorical variables and Wilcoxon rank-sum test for continuous variables were used to compare baseline characteristics between cases and controls. Spearman's rank correlation was used to evaluate the associations among plasma xylitol, 1,5-AG, histidine, inositol, threonine, methionine, arabinose, asparagine, glutamine, lysine, tyrosine, and uric acid levels. We calculated odds ratios (OR) and 95% confidence intervals (CI) for the association between these metabolites and pancreatic cancer risk using conditional logistic regression models after adjustment for age (5-year age group), gender, PHC area, and fasting time at blood donation (less than 7 hours, 7 hours or more, or unknown). Additional adjustment was made for smoking, BMI, and past history of DM to evaluate their confounding effect on the observed associations. We also conducted those of associations among pancreatic cancer cases diagnosed in the first 0-6 years of follow up (n = 48). There were no missing values in the analyzed variables. Statistical analyses were conducted with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Reported *P*-values are two-sided, and the statistically significant level was basically set at less than .05.

3 | RESULTS

Clinical characteristics of subjects and concentrations of 12 metabolites for cases and corresponding controls are shown in Table 1. Family history of pancreatic cancer and past history of DM or drug use of DM showed significant differences between cases and controls ($P = .03$ and $P = .008$, respectively). For the 12 metabolites, difference in plasma levels between cases and controls was observed for 1,5-AG ($P = .02$).

TABLE 1 Baseline characteristics of cases and controls in the present study

Characteristics	Cases	Controls	*P*-value[a]
n	170	340	
Age, y, mean (SD)	57.5 (7.77)	57.4 (7.68)	Matching value
Males (%)	80 (47.1)	160 (47.1)	Matching value
Smoking status			.15
Never smoked (%)	100 (58.8)	217 (63.8)	
Previous smoker (%)	24 (14.1)	52 (15.3)	
Current smoker, <20 cigarettes/d (%)	16 (9.4)	15 (4.4)	
Current smoker, ≥20 cigarettes/d (%)	30 (17.7)	56 (16.5)	
Frequency of alcohol drinking			.80
Never or occasional (%)	103 (61.7)	215 (63.6)	
≥1 d/wk, <150 g/wk (%)	29 (17.3)	51 (15.1)	
≥1 d/wk, ≥150 g/wk (%)	35 (21.0)	72 (21.3)	
BMI (kg/m^2)			.26
<22 (%)	53 (31.2)	115 (33.8)	
22 ≤ BMI <25 (%)	65 (38.2)	144 (42.4)	
25 ≤ BMI (%)	52 (30.6)	81 (23.8)	
Physical activity ≥1 d/wk, (%)[b]	40 (23.8)	70 (20.7)	.49
Coffee consumption ≥1 cup/d, (%)	57 (33.5)	116 (34.1)	.89
Family history of pancreatic cancer (%)	8 (4.7)	4 (1.2)	.03
History of DM or DM-related drug use (%)	18 (10.6)	14 (4.1)	.008
Plasma metabolites			
Xylitol (μg/mL), mean (SD)	0.2 (0.1)	0.2 (0.2)	.84
1,5-AG (μg/mL), mean (SD)	24.1 (10.3)	26.6 (10.8)	.02
Histidine (nmol/mL), mean (SD)	86.9 (15.6)	84.8 (15.1)	.27
Inositol (μg/mL), mean (SD)	4.3 (1.4)	4.1 (1.3)	.31
Threonine (nmol/mL), mean (SD)	242.6 (62.7)	238.1 (68.4)	.37
Methionine (nmol/mL), mean (SD)	27.8 (7.1)	27.2 (7.8)	.12
Arabinose (μg/mL), mean (SD)	0.3 (0.2)	0.3 (0.2)	.79
Asparagine (nmol/mL), mean (SD)	58.6 (13.0)	59.2 (13.7)	.67
Glutamine (nmol/mL), mean (SD)	642.9 (108.5)	641.2 (111.7)	.80
Lysine (nmol/mL), mean (SD)	222.7 (48.7)	215.5 (48.4)	.14
Tyrosine (nmol/mL), mean (SD)	132.3 (33.1)	127.6 (31.0)	.15
Uric acid (mg/dL), mean (SD)	5.4 (1.6)	5.5 (1.8)	.66

Analyses were based on 167 cases and 338 controls for frequency of alcohol drinking, and on 168 cases and 339 controls for physical activity.
[a]Based on the chi-squared test or Wilcoxon rank-sum test.
[b]Leisure time sports activity in terms of frequency of sports ≥1 d/wk.
BMI, body mass index; DM, diabetes mellitus; 1,5-AG, 1,5-anhydro-D-glucitol.

Results from Spearman's rank correlation among the 12 metabolites are presented in Table 2. Relatively strong correlations were seen between amino acids (ie, histidine, threonine, methionine, asparagine, glutamine, lysine and tyrosine).

To evaluate the magnitude of the risk of pancreatic cancer, we divided the subjects into the quartile of metabolites levels (Table 3). After adjustment for matching variables (namely, age, gender, PHC area, and fasting time at blood donation), increasing 1,5-AG levels were associated with a decreasing trend in the risk of pancreatic cancer. (OR of quartile 4 (Q4), 0.50; 95% CI, 0.27-0.93; *P* for trend = .02) Increasing methionine levels were also associated with an increasing trend in the risk of pancreatic cancer. (OR of Q4, 1.79; 95% CI, 0.94-3.40: *P* for trend = .03) An additional adjustment for smoking, BMI, and past history of DM attenuated the observed associations of 1,5-AG and methionine (*P* for trend = .06 and .07, respectively), implying their confounding effect on these associations. Periods of time from blood sample collection to diagnosis of pancreatic cancer were from 0 to 19 years. Previous report suggested that non-metastatic founder cell required at least 5 more years for the acquisition of metastatic ability. Therefore, we focused

TABLE 2 Spearman's rank correlation coefficients for the relationships among the 12 metabolites in the control population

	Xylitol	1,5-AG	Histidine	Inositol	Threonine	Methionine	Arabinose	Asparagine	Glutamine	Lysine	Tyrosine	Uric acid
Xylitol	1.00	−0.04 (.44)	−0.02 (.70)	0.09 (.09)	0.05 (.40)	0.05 (.37)	0.06 (.27)	0.15 (.006)	−0.03 (.56)	−0.06 (.27)	0.02 (.67)	−0.0005 (.99)
1,5-AG		1.00	0.16 (.003)	0.07 (.18)	0.15 (.005)	0.16 (.003)	0.25 (<.0001)	0.25 (<.0001)	0.17 (.002)	0.12 (.02)	0.18 (.0007)	0.24 (<.0001)
Histidine			1.00	0.41 (<.0001)	0.66 (<.0001)	0.68 (<.0001)	0.26 (<.0001)	0.70 (<.0001)	0.66 (<.0001)	0.71 (<.0001)	0.56 (<.0001)	0.30 (<.0001)
Inositol				1.00	0.20 (.0002)	0.28 (<.0001)	0.48 (<.0001)	0.37 (<.0001)	0.35 (<.0001)	0.33 (<.0001)	0.31 (<.0001)	0.29 (<.0001)
Threonine					1.00	0.76 (<.0001)	0.15 (.004)	0.70 (<.0001)	0.54 (<.0001)	0.60 (<.0001)	0.52 (<.0001)	0.23 (<.0001)
Methionine						1.00	0.23 (<.0001)	0.70 (<.0001)	0.53 (<.0001)	0.72 (<.0001)	0.69 (<.0001)	0.27 (<.0001)
Arabinose							1.00	0.30 (<.0001)	0.18 (.0008)	0.17 (.002)	0.31 (<.0001)	0.12 (.03)
Asparagine								1.00	0.60 (<.0001)	0.61 (<.0001)	0.50 (<.0001)	0.16 (.004)
Glutamine									1.00	0.56 (<.0001)	0.49 (<.0001)	0.21 (.0001)
Lysine										1.00	0.58 (<.0001)	0.37 (<.0001)
Tyrosine											1.00	0.28 (<.0001)
Uric acid												1.00

P-values are shown in parentheses.
1,5-AG, 1,5-anhydro-D-glucitol.

on the subjects whose periods of time from blood sample collection to diagnosis of pancreatic cancer were from 0 to 6 years. We considered that pancreatic cancer may exist in the periods of time from 0 to 6 years. As shown in Table 4, higher levels of 1,5-AG, asparagine, tyrosine and uric acid showed a decreasing trend in the risk of pancreatic cancer (*P* for trend = .04, .04, .04 and .02, respectively), even after adjustment for smoking, BMI, and past history of DM. When excluding those with renal diseases (4 case-control trios and 9 controls), which could have affected their plasma 1,5-AG levels, we observed a slightly evident decrease in trend with increasing levels of 1,5-AG (*P* for trend = .03).

4 | DISCUSSION

In this prospective case-control study, we measured 12 metabolites for their association with pancreatic cancer risk. However, we did not observe an apparent association. In the subjects whose periods of time from blood sample collection to diagnosis of pancreatic cancer were from 0 to 6 years, we found that 1,5-AG, asparagine, tyrosine and uric acid showed a similar tendency to our previous reports of metabolic changes in pancreatic cancer patients.[13] These findings may suggest that changes in our diagnostic metabolites are mainly as a result of the existence of pancreatic cancer.

Researchers examined the risk of prostate and colon cancer in a prospective metabolite profiling study.[17,18] Cross et al reported that no overall associations were observed between serum metabolites and colorectal cancer, but serum glycochenodeoxycholate, which is a bile acid metabolite, was positively associated with colorectal cancer among women.[18] A study by Mondul et al[17] also indicated that several metabolomic profiles may have pathophysiological relevance to prostate carcinogenesis. There is also a report about pancreatic cancer in a prospective metabolic profiling study. Mayers et al[10] reported that plasma branched-chain amino acids were elevated among subjects with sample collected from 2 to 5 years before pancreatic cancer diagnosis, when occult disease is probably present. However, it was not sufficient to evaluate the risk factors of pancreatic cancer before the onset, because this period from sample collection to pancreatic cancer diagnosis was short. In the present study, we investigated whether the levels of 12 metabolites, the levels of which differ significantly between pancreatic cancer patients and healthy volunteers,[13] are changed among subjects with sample collected from 0 to 19 years before pancreatic cancer diagnosis in order to evaluate their association with pancreatic cancer risk, and the possibility of these metabolites as environmental risk factors. Increasing levels of methionine, and decreasing levels of 1,5-AG were associated with increased risk of pancreatic cancer. However, these metabolites did not show a statistically significant difference after adjustment for potential confounding factors (ie, smoking, BMI, and past history of DM). This result suggested that prediction of pancreatic cancer risk was difficult using these metabolites.

In the subjects whose periods of time from blood sample collection to diagnosis of pancreatic cancer were from 0 to 6 years,

TABLE 3 Odds ratios (OR) and 95% CI for the associations between plasma metabolite levels and pancreatic cancer risk

Metabolite	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Xylitol					
Median, μg/mL	0.06	0.14	0.27	0.34	
Cases (n)/controls (n)	34/85	50/85	47/85	39/85	
OR (95% CI)[a]	1.00 (reference)	1.80 (0.94-3.44)	1.88 (0.81-4.34)	1.52 (0.64-3.60)	.57
OR (95% CI)[b]	1.00 (reference)	1.88 (0.95-3.72)	1.77 (0.73-4.28)	1.39 (0.56-3.46)	.78
1,5-AG					
Median, μg/mL	14.7	22.2	29.3	39.9	
Cases (n)/controls (n)	53/85	49/85	35/85	33/85	
OR (95% CI)[a]	1.00 (reference)	0.93 (0.58-1.51)	0.59 (0.34-1.02)	0.50 (0.27-0.93)	.02
OR (95% CI)[b]	1.00 (reference)	1.03 (0.62-1.71)	0.66 (0.37-1.17)	0.56 (0.29-1.09)	.06
Histidine					
Median, nmol/mL	67.6	80.2	89.2	100.9	
Cases (n)/controls (n)	38/85	41/85	43/85	48/85	
OR (95% CI)[a]	1.00 (reference)	1.23 (0.63-2.39)	1.35 (0.66-2.77)	1.53 (0.75-3.12)	.24
OR (95% CI)[b]	1.00 (reference)	1.16 (0.58-2.30)	1.29 (0.61-2.72)	1.47 (0.70-3.09)	.28
Inositol					
Median, μg/mL	2.74	3.55	4.38	5.55	
Cases (n)/controls (n)	37/85	48/85	27/85	58/85	
OR (95% CI)[a]	1.00 (reference)	1.34 (0.77-2.36)	0.76 (0.39-1.48)	1.75 (0.94-3.24)	.17
OR (95% CI)[b]	1.00 (reference)	1.52 (0.85-2.71)	0.85 (0.42-1.68)	1.85 (0.98-3.49)	.17
Threonine					
Median, nmol/mL	167.4	212.6	245.3	310.3	
Cases (n)/controls (n)	36/85	45/85	48/85	41/85	
OR (95% CI)[a]	1.00 (reference)	1.30 (0.74-2.30)	1.39 (0.79-2.45)	1.19 (0.65-2.20)	.57
OR (95% CI)[b]	1.00 (reference)	1.39 (0.77-2.51)	1.38 (0.77-2.46)	1.19 (0.63-2.25)	.64
Methionine					
Median, nmol/mL	19.6	24.6	27.9	34.0	
Cases (n)/controls (n)	34/85	34/85	53/85	49/85	
OR (95% CI)[a]	1.00 (reference)	1.09 (0.61-1.95)	1.91 (1.03-3.53)	1.79 (0.94-3.40)	.03
OR (95% CI)[b]	1.00 (reference)	1.06 (0.59-1.91)	1.82 (0.97-3.43)	1.64 (0.85-3.17)	.07
Arabinose					
Median, μg/mL	0.15	0.23	0.31	0.49	
Cases (n)/controls (n)	41/85	45/85	41/85	43/85	
OR (95% CI)[a]	1.00 (reference)	1.10 (0.64-1.88)	1.01 (0.54-1.87)	1.05 (0.55-2.01)	.94
OR (95% CI)[b]	1.00 (reference)	1.04 (0.59-1.83)	0.98 (0.52-1.85)	1.05 (0.53-2.09)	.93
Asparagine					
Median, nmol/mL	46.2	52.6	61.2	73.3	
Cases (n)/controls (n)	50/85	48/85	29/85	43/85	
OR (95% CI)[a]	1.00 (reference)	0.88 (0.51-1.52)	0.51 (0.28-0.95)	0.74 (0.40-1.36)	.18
OR (95% CI)[b]	1.00 (reference)	0.93 (0.53-1.63)	0.54 (0.29-1.01)	0.82 (0.44-1.55)	.32
Glutamine					
Median, nmol/mL	515.8	604.7	676.8	766.0	
Cases (n)/controls (n)	44/85	42/85	39/85	45/85	
OR (95% CI)[a]	1.00 (reference)	0.93 (0.50-1.71)	0.86 (0.45-1.65)	1.00 (0.51-1.99)	1.00
OR (95% CI)[b]	1.00 (reference)	0.96 (0.51-1.80)	0.88 (0.45-1.73)	0.98 (0.48-1.98)	.92

(Continues)

TABLE 3 (Continued)

Metabolite	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Lysine					
Median, nmol/mL	164.1	198.2	226.5	269.9	
Cases (n)/controls (n)	35/85	41/85	44/85	50/85	
OR (95% CI)[a]	1.00 (reference)	1.39 (0.75-2.56)	1.58 (0.81-3.10)	1.82 (0.92-3.58)	.09
OR (95% CI)[b]	1.00 (reference)	1.53 (0.81-2.89)	1.67 (0.82-3.38)	1.86 (0.92-3.77)	.12
Tyrosine					
Median, nmol/mL	93.1	116.0	134.1	158.4	
Cases (n)/controls (n)	36/85	46/85	39/85	49/85	
OR (95% CI)[a]	1.00 (reference)	1.32 (0.75-2.33)	1.15 (0.63-2.09)	1.45 (0.80-2.63)	.30
OR (95% CI)[b]	1.00 (reference)	1.26 (0.71-2.26)	1.00 (0.54-1.84)	1.15 (0.62-2.14)	.85
Uric acid					
Median, mg/dL	3.48	4.83	5.93	7.38	
Cases (n)/controls (n)	47/85	50/85	26/85	47/85	
OR (95% CI)[a]	1.00 (reference)	1.01 (0.60-1.68)	0.52 (0.28-0.95)	0.94 (0.53-1.66)	.45
OR (95% CI)[b]	1.00 (reference)	0.98 (0.58-1.65)	0.48 (0.25-0.90)	0.82 (0.45-1.50)	.27

Based on conditional logistic regression analysis.

[a]OR was adjusted for age (5-y age group), gender, public health center (PHC) area, and duration of the fasting period prior to blood sampling (less than 7 hours, 7 hours or more, or unknown).

[b]OR was adjusted for age (5-y age group), gender, PHC area, duration of the fasting period prior to blood sampling (less than 7 hours, 7 hours or more, or unknown), smoking, body mass index, and past history of diabetes mellitus.

CI, confidence interval; 1,5-AG, 1,5-anhydro-D-glucitol.

decreasing levels of 1,5-AG, asparagine, tyrosine and uric acid were related to an increased risk of pancreatic cancer (Table 4). These metabolic changes were consistent with our previous report for pancreatic cancer patients.[13] In addition, we verified the usefulness of these metabolites for detection of early-stage pancreatic cancer patients (stages I and II),[14] and these metabolites had a high sensitivity for early-stage pancreatic cancer. Therefore, these previous results support the high reliability of our present findings. In the study by Mayers et al[10], elevated plasma levels of branched-chain amino acids were associated with a greater than twofold increased risk of near future pancreatic cancer diagnosis in profiling of prediagnostic plasma metabolites. These elevations may result from breakdown of peripheral protein stores by the influence of early pancreatic cancer. Early pancreatic cancer may induce metabolic change. Therefore, in our study, the cause of metabolic changes may be occult pancreatic cancer.

1,5-AG is a metabolically stable small molecule that is well absorbed in the intestinal tract and mainly originates from the diet. Blood level of 1,5-AG is constant as a result of equilibrium between 1,5-AG absorption, urinary reabsorption, and secretion by the intestinal tract. Inhibition of reabsorption by glucose can occur in hyperglycemia. Therefore, 1,5-AG has been used as a short-term biomarker of DM,[19-21] which is a well-known risk factor for pancreatic cancer.[22-24] 1,5-AG, which is a biomarker of DM, showed a statistically significant difference between cases and controls in the present study (Table 1), but did not show an apparent association with the risk of pancreatic cancer (Table 3). In the subjects who were diagnosed in the first 0-6 years, however, low 1,5-AG level showed a statistically significantly higher risk (Table 4). Some studies have indicated that a greater risk of pancreatic cancer was seen in the 2-4 years after subjects were diagnosed with DM,[25-27] and that the risk was decreased at 4 years after DM diagnosis.[28] However, an increased risk of pancreatic cancer, while not statistically significant, was also observed among the subjects who had DM for over 10 years.[28] It is unclear whether DM induces pancreatic cancer, or whether pancreatic cancer induces DM, or both.

Asparagine, uric acid, and tyrosine were associated with pancreatic cancer in subjects who were diagnosed in the first 0-6 years (Table 4). L-Asparaginase catalyzes the extracellular conversion of asparagine into aspartic acid and ammonia.[29] It is important for solid tumor progression to maintain asparagine production, because solid tumor is nutritionally dependent on an external supply of asparagine.[30] Therefore, L-asparaginase is an important chemotherapeutic agent, especially in acute lymphoblastic leukemia.[29] Some reports indicated that it may be effective for diagnosis and therapy of pancreatic cancer.[31,32] Purine nucleic acids are catabolized to hypoxanthine, then xanthine and, finally, to uric acid by xanthine oxidase. Uric acid is an indicator of high turnover of nucleic acids.[33] Stotz et al[34] identified that uric acid level at the time of diagnosis is an independent prognostic factor in pancreatic cancer patients. The high level of uric acid may depend on metabolic changes as a result of pancreatic cancer. Tyrosine is also reported to likely be useful for the diagnosis of pancreatic cancer.[31] Catecholamines, which are metabolically produced from tyrosine, increased proliferation of pancreatic cancer cells.[35] In addition, Miyajima et al[36] pointed out the possibility that tyrosine is incorporated into activated T cells in

TABLE 4 Odds ratios (OR) and 95% CI for the associations between plasma metabolite levels and pancreatic cancer during the first 6 y of follow up

	Pancreatic cancer diagnosed in the first 6 y of follow up (n = 48)				
Metabolite	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	***P* for trend**
Xylitol					
Median, μg/mL	0.06	0.12	0.29	0.49	
Cases (n)/controls (n)	12/27	23/42	8/11	5/16	
OR (95% CI)[a]	1.00 (reference)	0.91 (0.26-3.20)	1.00 (0.12-8.58)	0.39 (0.05-3.09)	.29
1,5-AG					
Median, μg/mL	15.5	22.2	30.3	41.7	
Cases (n)/controls (n)	16/22	16/16	10/33	6/25	
OR (95% CI)[a]	1.00 (reference)	1.44 (0.47-4.37)	0.29 (0.08-1.00)	0.37 (0.08-1.66)	.04
Histidine					
Median, nmol/mL	66.1	81.1	89.3	100.8	
Cases (n)/controls (n)	6/11	13/20	11/22	18/43	
OR (95% CI)[a]	1.00 (reference)	1.15 (0.20-6.43)	0.68 (0.12-3.72)	0.66 (0.14-3.17)	.31
Inositol					
Median, μg/mL	2.79	3.67	4.40	5.81	
Cases (n)/controls (n)	8/20	9/22	8/28	23/26	
OR (95% CI)[a]	1.00 (reference)	1.30 (0.37-4.51)	1.14 (0.28-4.58)	3.23 (0.90-11.63)	.054
Threonine					
Median, nmol/mL	174.5	213.0	249.0	316.7	
Cases (n)/controls (n)	7/11	14/24	13/30	14/31	
OR (95% CI)[a]	1.00 (reference)	0.77 (0.18-3.26)	0.52 (0.15-1.81)	0.51 (0.13-1.96)	.22
Methionine					
Median, nmol/mL	20.9	24.6	27.5	36.7	
Cases (n)/controls (n)	5/13	10/20	19/30	14/33	
OR (95% CI)[a]	1.00 (reference)	1.30 (0.34-4.95)	1.70 (0.46-6.31)	0.77 (0.17-3.44)	.79
Arabinose					
Median, μg/mL	0.18	0.23	0.31	0.58	
Cases (n) / controls (n)	9/14	16/21	9/32	14/29	
OR (95% CI)[a]	1.00 (reference)	0.91 (0.26-3.16)	0.21 (0.04-1.04)	0.49 (0.09-2.57)	.26
Asparagine					
Median, nmol/mL	45.9	53.2	61.3	74.8	
Cases (n)/controls (n)	13/16	15/23	8/25	12/32	
OR (95% CI)[a]	1.00 (reference)	0.97 (0.34-2.74)	0.30 (0.09-1.02)	0.34 (0.09-1.28)	.04
Glutamine					
Median, nmol/mL	513.4	613.6	686.2	800.8	
Cases (n)/controls (n)	6/15	11/18	9/24	22/39	
OR (95% CI)[a]	1.00 (reference)	1.14 (0.22-5.87)	0.62 (0.12-3.22)	0.93 (0.20-4.28)	.94
Lysine					
Median, nmol/mL	167.4	195.6	225.7	276.5	
Cases (n)/controls (n)	6/13	11/19	10/27	21/37	
OR (95% CI)[a]	1.00 (reference)	1.45 (0.34-6.23)	0.78 (0.15-4.05)	1.37 (0.29-6.53)	.80
Tyrosine					
Median, nmol/mL	95.0	116.2	133.9	156.8	
Cases (n)/controls (n)	7/11	17/19	8/30	16/36	
OR (95% CI)[a]	1.00 (reference)	1.82 (0.45-7.41)	0.35 (0.08-1.56)	0.42 (0.10-1.76)	.04

(Continues)

TABLE 4 (Continued)

Metabolite	Pancreatic cancer diagnosed in the first 6 y of follow up (n = 48)				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Uric acid					
Median, mg/dL	3.16	4.77	5.87	7.95	
Cases (n)/controls (n)	11/10	17/29	10/30	10/27	
OR (95% CI)[a]	1.00 (reference)	0.37 (0.13-1.08)	0.23 (0.07-0.82)	0.17 (0.04-0.78)	.02

Based on conditional logistic regression analysis.
[a]OR was adjusted for age (5-y age group), gender, PHC area, and duration of the fasting period prior to blood sampling (less than 7 hours, 7 hours or more, or unknown), smoking, body mass index, and past history of diabetes mellitus.
CI, confidence interval; 1,5-AG, 1,5-anhydro-D-glucitol.

lymph nodes, which are observed in patients with pancreatic cancer. These facts indicate that tyrosine metabolism may be related to pancreatic cancer. In subjects who were diagnosed in the first 0-6 years, asparagine, uric acid, and tyrosine showed a similar tendency (Table 4), and these results support our previous report.[13,14]

The present study has several limitations. First, the fasting time of all plasma samples was not constant. Although we matched samples for fasting time at more than 7 hours or under 7 hours, some metabolites may be affected by fasting status.[37] Second, we could not compare with cancer stage, because the number of early-stage pancreatic cancers was low because of its low detection ratio. Third, we could not assess the association with conventional markers of DM, such as HbA1c, insulin, and glucose tolerance test.

In conclusion, we prospectively collected and analyzed metabolic changes in the plasma of a large number of prediagnostic pancreatic cancer patients. We found that 12 target metabolites were not associated with pancreatic cancer risk. However, metabolic changes in subjects diagnosed during the first 0-6 years showed a similar tendency to our previous reports. These results might support that at least 1,5-AG, asparagine, tyrosine and uric acid are useful for early detection of pancreatic cancer.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest for this article.

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