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Validation of plasma metabolites associated with breast cancer risk among Mexican Americans

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Abstract

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Author contribution HZ, JS, YY, XW, FJE, DT, and WC participated in study design. ZH, JS, and YY performed the data analysis. JS, HZ, and WC drafted the manuscript.

Credit Author Statement

Hua Zhao: Conceptualization, Methodology, Writing- Reviewing and Editing.
Jie Shen: Investigation, Data curation, Writing- Original draft preparation.
Yuanqing Ye: Data analysis.
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Conflict of Interest Statement

The study protocol was approved by MD Anderson's Institutional Review Board.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

All procedures performed in this study were approved by the Institutional Review Board at M D Anderson Cancer Center and in accordance with the ethical standards of 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Written informed consent was obtained from all participants.

Data sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

In our previous breast cancer case control study in Hispanics, we found 14 metabolites whose levels differed between cases and controls. To validate the results, we carried out a nested case control study of 100 incident breast cancer and 100 matched healthy women identified from the Mano-A-Mano Mexican American Cohort study. With the adjustment of parity, education, birth place, language acculturation, BMI category, smoking, drinking, physical activity, and sitting time, 4 metabolites were associated with breast cancer risk: 3-hydroxyoctanoate (Odds ratio (OR)=1.51, 95% confidence interval (CI): 1.10, 3.47), 3-hydroxybutyrate (BHBA) (OR=1.42, 95%CI: 1.01, 3.72), linoleate (18:2n6) (OR =1.39, 95% CI: 1.07, 4.04), and bilirubin (OR=0.54, 95%CI: 0.42, 0.95). Then, we used 3 non-redundant metabolites, namely 3-hydroxyoctanoate, linoleate (18:2n6), and bilirubin, to generate a metabolic risk score. Increased metabolites risk score was associated with a 1.67-fold increased risk of breast cancer (OR =1.67, 95%CI: 1.32, 3.94). And the significant association was more evident among those who were diagnosed with cancer earlier during the follow-up (≤ 5 years) than their counterparts. In conclusion, we identified four significant metabolites which may help elucidate metabolic pathways that contribute to breast carcinogenesis. Our findings warrant further replication efforts.

Keywords

metabolomics; breast cancer; Mexican Americans

Introduction

Currently, breast cancer is the most frequently diagnosed cancer among Hispanic women (1). Known risk factors account for only about 30% of breast cancers (2, 3). Therefore, a better understanding of the biological etiology and mechanisms is warranted. The metabolome reveals endogenous activities as well as ecological and lifestyle factors (4, 5). Metabolomics can identify delicate signals in metabolism and is therefore a promising means to pinpoint new etiological pathways.

In our prior two-stage analysis, we identified 14 candidate metabolites that significantly differed between breast cancer cases and healthy controls in Hispanic women (6). However, due to case-control study design, the results cannot be properly interpreted. In the current study, we attempted to validate the 14 previously identified metabolites using pre-diagnostic plasma samples obtained from 100 incident breast cancer and 100 age and gender matched healthy Mexican American (MA) women identified from the Mano-A-Mano Mexican American Cohort study (MACS).

Materials and Methods

Study population

Our study utilized self-identified Mexican or MA participants from the ongoing MACS, a large population-based prospective cohort study of MA households. Participants were individuals of Mexican descent who lived in the Houston area for at least one year. Details of the recruitment strategy and data collection procedures have been previously described (7). Our study included female participants from MACS who were followed for a median of

8.2 years until December 1, 2017. Breast cancer cases were identified during follow-up and were further verified through the Texas Cancer Registry. Of the validated cases, we randomly selected 100 index cases. We chose 1 matched control for each index case using an incidence density sampling protocol from appropriate risk sets of cohort members who were both alive and free of cancer at the time of diagnosis of the index case. Our matching criteria included age at recruitment (±2 years), date of biospecimen collection (±1 year), and gender. The study protocol was approved by MD Anderson's Institutional Review Board.

Metabolomics Analysis

Plasma samples underwent metabolomics profiling at Metabolon Inc (Durham, NC) via ultra-high-performance liquid chromatography/mass spectroscopy and gas chromatography/mass spectroscopy. The protocol has been previously described in detail (8). After disregarding compounds that had ≥30% missing values, 526 identified metabolites were left for analysis. The missing values were deemed as the outcome of low signal strength and were substituted by half of the minimum positive values revealed in the data. The median proportion of below-limit-of-detection values was 0%. Metabolite peak intensities were run-day-normalized and log-transformed for analysis. Metabolite measurements were highly reliable in masked replicates. Over the 526 metabolites, the median intraclass correlation coefficient (ICC) was 0.93 (interquartile range (IQR)=0.86–0.97), similar to previous reports analyzed by Metabolon (9).

Statistical Analysis

Any missing values were supposed to be under the limits of detection, and these values were imputed with the metabolite minimum (minimum value imputation). Non-parametric Wilcoxon signed-rank tests were applied to assess levels of 14 candidate metabolites between breast cancer cases and healthy controls. To evaluate the risk of breast cancer, we utilized conditional logistic regression analysis. The matching variables were age at recruitment and date of biospecimen collection. The covariates included parity, education, birth place, language acculturation, and BMI category in both models 1 and 2, and smoking, drinking, physical activity, and sitting time in model 2. Bonferroni criterion was used to correct the multiple comparison. We further performed stratified analysis to assess the difference of association by social-demographics, healthy behaviors, and time duration between blood collection and cancer diagnosis. To identify the possible redundancy, we evaluated the pairwise correlations between all 14 metabolites among the controls. Next, using 3 non-redundant significant metabolites, we created a metabolic risk score. For each metabolite, we classified the study participants into high and low groups by means of the median level in the controls as the cutoff point. Next, based on the association between metabolite levels with risk of breast cancer, we counted the study subjects as either high or low risk (0 or 1) and added scores across 3 metabolites to create a risk score (range: 0–3). Conditional logistic regression analysis was used to evaluate the association between the risk score with the risk of breast cancer. Co-variates were adjusted as appropriate. In addition, stratified analysis was applied to assess the impact of time duration between blood collection and cancer diagnosis. STATA software version 14.1 (STATA, College Station, TX) were used for all analyses.

Results

Table 1 illustrated the basic sociodemographic characteristics and lifestyle behaviors of the 100 breast cancer cases and 100 healthy controls. Overall, the cases and controls were matched very well. No significant difference was observed for parity, education level, place of birth, language acculturation, BMI category, smoking status, alcohol drinking status, physical activity, and sitting time. Forty-two of cases were diagnosed within 5 years after the blood was collected.

In the first model with the adjustment of parity, education, birth place, language acculturation, and BMI category, we observed 5 metabolites whose levels in plasma significantly differed between breast cancer cases and controls (Table 2). Higher levels of 3-hydroxyoctanoate, 3-hydroxybutyrate (BHBA), linoleate (18:2n6), and 10-nonadecenoate (19: ln9) were associated with 1.55, 1.47, 1.38, and 1.33-fold elevated risk of breast cancer. Meanwhile, higher levels of bilirubin were associated with 50% decreased risk of breast cancer. In the second model with further adjustment of smoking, drinking, physical activity, and sitting time, 4 of the 5 metabolites remained significant: 3-hydroxyoctanoate (OR=1.51, 95%CI: 1.10, 3.47), 3-hydroxybutyrate (BHBA) (OR=1.42, 95%CI: 1.01, 3.72), linoleate (18:2n6) (OR=1.39, 95%CI: 1.07, 4.04), and bilirubin (OR=0.54, 95%CI: 0.42, 0.95). However, none of the associations remained significant after Bonferroni multiple comparison adjustment.

To account for potential collinearity between metabolites, we evaluated the pairwise correlations between all 14 metabolites among the controls (Figure 1). Metabolites whose pairwise correlations greater than 0.5 were considered highly correlated and to have possible redundancy. For four significant metabolites, the only significant correlation was between 3-hydroxyoctanoate and 3-hydroxybutyrate (BHBA) (Rho=0.629, P<0.001). Thus, 3-hydroxybutyrate (BHBA) was not included in further analysis.

In further stratified analysis by sociodemographic characteristics and lifestyle behaviors, we evaluated the association between 3-hydroxyoctanoate, linoleate (18:2n6), and bilirubin with breast cancer risk in each stratified category (Table 3). In general, the association between the metabolite and breast cancer risk didn't differ between the categories. The only exception was linoleate (18:2n6) by BMI category. The association between linoleate (18:2n6) and breast cancer risk was more evident in study participants who were obese (OR=1.48, 95%CI: 1.01, 5.14) than those who were non-obese (OR=1.32, 95%CI: 0.76, 5.48). We also assessed whether the association differed by the time duration between blood collection and cancer diagnosis. The association apparently was stronger among those who had cancer diagnosis earlier (≤ 5 years after blood collection) than those who had cancer diagnose later (> 5 years after blood collection). For example, higher 3-hydroxyoctanoate was associated with 1.65-fold increased risk of breast cancer among those who had cancer diagnosis earlier (≤ 5 years after blood collection) and 1.33-fold increased risk of breast cancer among those who had cancer diagnose later (> 5 years after blood collection) (OR=1.65, 95%CI; 1.07, 4.38; OR=1.33, 95%CI: 1.01, 5.06, respectively).

Then, we generated a risk score using those three non-redundant metabolites, namely 3-hydroxyoctanoate, linoleate (18:2n6), and bilirubin. Using the risk score as a continuous variable, we noted that increased risk score was associated with a 1.72-fold increased risk of breast cancer in model 1 and a 1.67-fold increased risk in model 2, (OR=1.72, 95%CI=1.46, 3.70; OR=1.67, 95%CI=1.32, 3.94) (Table 4). When stratified by the time duration between blood collection and cancer diagnosis (≤ 5 years vs >5 years), the association remained significant in both categories. However, it was stronger among those who had cancer diagnosis earlier (≤ 5 years after blood collection) (model 1: OR=1.90, 95%CI=1.27, 4.89; model 2: OR=1.84, 95%CI=1.15, 4.78) than those who had cancer diagnosis later (> 5 years after blood collection) (model 1: OR=1.49, 95%CI=1.04, 5.02; model 2: OR=1.43, 95%CI=1.02, 5.16). After multiple comparison adjustment, the significant association remained only for those had cancer diagnosis earlier, but not for those who had cancer diagnosis later.

Next, we classified the study subjects into two strata based on the risk score among the control subjects: low (0–1) and high (2–3). When compared with study subjects with low risk scores, those with high risk scores exhibited a significantly increased risk of breast cancer in both model 1 and model 2 (OR=3.91, 95%CI=1.87, 10.32); and OR=3.82, 95%CI=1.78, 11.56). Similar stratified analysis was further applied to assess the impact of time duration between blood collection and cancer diagnosis. We found that the association was stronger among those who had cancer diagnosis earlier (model 1: OR=5.55, 95%CI=1.65, 13.58; model 2: OR=5.38, 95%CI=1.62, 13.57) than those who had cancer diagnosis later (model 1: OR=2.47, 95%CI=1.08, 15.06; model 2: OR=2.36, 95%CI=1.07, 14.85). When adjusted by Bonferroni multiple comparison, the association remained significant for those who had cancer diagnosis earlier.

Discussion

To our best knowledge, this is the first prospective study to assess the role of plasma metabolites in the development of breast cancer among MAs. In our study, we validated four metabolites, namely 3-hydroxyoctanoate, 3-hydroxybutyrate (BHBA), linoleate (18:2n6), and bilirubin, which were identified in our previous breast cancer case control study. As the associations we observe are more pronounced among cases occurring earlier during follow-up, our results suggest altered levels of circulating metabolites may be an early biomarker of subclinical cancer in Mexican Americans.

Among 4 validated metabolites mentioned above, 3 were lipids, including 1 monohydroxy fatty acid (3-hydroxyoctanoate), 1 ketone body (3-hydroxybutyrate (BHBA), and 1 polyunsaturated fatty acid (linoleate (18:2n6)). Higher levels of monohydroxy fatty acids may signify altered fatty acid β-oxidation in patients with breast cancer (8). Fatty acid β-oxidation has been shown to support functional mitochondria and is vital for the rapid growth of cancer cells (10, 11). In a recent study, fatty Acid β-Oxidation was found to be implicated in breast cancer stem cell self-renewal and chemoresistance (12). Therefore, altered fatty acids β-oxidation may promote breast carcinogenesis and foster the aggressive tumorigenic phenotypes (13). Similar observations were also reported in several other breast cancer case control analyses (14, 15). Ketone body 3-hydroxybutyrate (BHBA), the end

product of ketogenesis and downstream of fatty acid β-oxidation, was noted associated with breast cancer risk in our study. The elevated 3-hydroxybutyrate (BHBA) level in the cases is another signal of altered fatty acid β-oxidation in breast cancer subjects. Linolenic acid has been suggested to support the tumorigenic phenotype of breast cancer (16). In our previous breast cancer study, we found that levels of linoleate (18:2n6) were significantly higher in the cases than controls (8). Interestingly, in a genome-wide association study, genetic variants in genes involved in the regulation of linolenic acid metabolism were associated with breast cancer risk (17).

We also found that increased levels of bilirubin were associated with decreased risk of breast cancer. Our results are in consistent with Bilirubin's anti-carcinogenic property (18, 19). Endogenous antioxidant bilirubin has shown to inhibit cancer development (20). In a previous study using pre-diagnostic serum samples, bilirubin was not associated with breast cancer (20). However, serum bilirubin has been reported to associated with reduced lung cancer risk in two prospective studies (21, 22) and reduced cancer mortality in a population-based set (23). Clearly, more research is needed to clarify the role of bilirubin in breast carcinogenesis.

Interestingly, none of our validated metabolites were overlapped with significant metabolites identified in previous studies in Whites (24, 25). Metabolites are the end products of intercellular pathways and are susceptible to host and ecological stimuli. Thus, metabolite profiles can be influenced significantly by factors ranging from genetics, demographic factors, and co-morbidities to environmental exposure (26). We examined 4 breast cancer related metabolites reported in the study by Moore et al (25), namely 16a-hydroxy-DHEA-3-sulfate, 3-methylglutarylcarnitine, allo-isoleucine, and 2-methylbutyrylcarnitine. Though we did not observe any significant association for those metabolites (16a-hydroxy-DHEA-3-sulfate: OR=1.24, 95%CI=0.65–4.58; allo-isoleucine: OR=1.40, 95%CI=0.77–4.93; and 2-methylbutyrylcarnitine: OR=1.28, 95%CI=0.67–4.59), borderline association was observed for 3-methylglutarylcarnitine (OR=1.71, 95%CI=0.96–4.72).

Although our study is the largest study in MAs, relatively small sample size is a major limitation. Another limitation of our study is that we were unable to obtain repeated measures of circulating metabolites; a single measurement may not reflect circulating metabolites over a lifetime. In addition, we did not have matched tumor and normal tissues, which would have enabled us to compare metabolites in target and surrogate tissues. Nevertheless, our study is the first prospectively designed study to show the significant relationship between plasma metabolites and breast cancer risk in MAs. Larger future studies should be conducted in order to further validate our observed associations.

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Highlights

- We have validated 4 plasma metabolites associated with breast cancer risk in Mexican Americans. They are 3-hydroxyoctanoate, 3-hydroxybutyrate (BHBA), linoleate (18:2n6), and bilirubin.
- We have constructed a metabolic risk score. Higher risk score was associated with increased breast cancer risk.

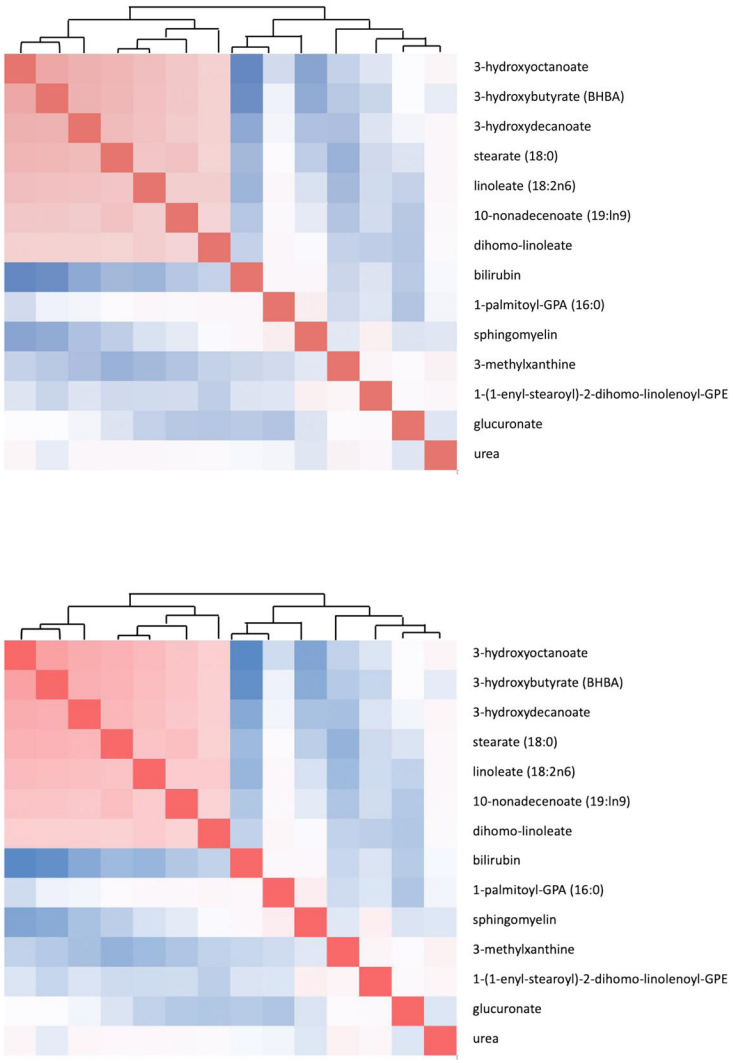

Fig. 1.

Pair-wise correlation analysis to assess the redundancy among significant metabolites.

Table 1

Distribution of characteristics among participants by case control status

Variable	Controls, n (%)	Cases, n (%)	*P* value
Overall	100 (100)	10 (100)	
Age at enrollment, years			
<51 years	50 (50.00)	46 (46.00)	
≥51 years	50 (50.00)	54 (54.00)	0.730
Parity			
Nulliparous	9 (9.00)	8 (8.00)	
1 or 2 children	43 (43.00)	42 (42.00)	
>2 children	48 (48.00)	50 (50.00)	0.964
Education level			
<High school	68 (68.00)	70 (70.00)	
High school	21 (21.00)	20 (20.00)	
>High school	11 (11.00)	10 (10.00)	0.967
Place of birth			
Mexico	70 (70.00)	70 (70.00)	
United States	30 (30.00)	30 (30.00)	1.000
Language acculturation			
Low	69 (69.00)	60 (60.00)	
High	31 (31.00)	40 (40.00)	0.279
BMI category			
Underweight/normal weight	15 (15.00)	12 (12.00)	
Overweight	36 (36.00)	38 (38.00)	
Obese	49 (49.00)	50 (50.00)	0.879
Smoking status			
Never	73 (73.00)	64 (64.00)	
Former	19 (19.00)	28 (28.00)	
Current	8 (8.00)	8 (8.00)	0.446
Alcohol drinking			
Never	67 (67.00)	66 (66.00)	
Former	21 (21.00)	26 (26.00)	
Current	12 (12.00)	8 (8.00)	0.646
Physical activity			
Low	72 (72.00)	80 (80.00)	
Medium or high	28 (28.00)	20 (20.00)	0.325
Sitting hours per day			
<2	27 (27.00)	24 (24.00)	
2–4	28 (28.00)	36 (36.00)	
4–6	23 (23.00)	24 (24.00)	
>6	22 (22.00)	16 (16.00)	0.700
Time between blood collection and cancer diagnosis			

Variable	Controls, n (%)	Cases, n (%)	*P* value
≤5 years		42 (42.00)	
>5 years		48 (48.00)	

Table 2.

Conditional logistic regression analysis to identify metabolites significantly associated with breast cancer

			Model 1*		Model 2#	
Metabolites	**Pathways**	**Sub-pathways**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
3-hydroxyoctanoate	Lipid	Fatty Acid, Monohydroxy	**1.55 (1.13, 3.32)**	**0.016**	**1.51 (1.10, 3.47)**	**0.022**
3-hydroxybutyrate (BHBA)	Lipid	Ketone Bodies	**1.47 (1.11, 3.72)**	**0.020**	**1.42 (1.01, 3.72)**	**0.049**
stearate (18:0)	Lipid	Long Chain Fatty Acid	1.22 (0.83, 4.01)	0.235	1.26 (0.79, 4.24)	0.332
3-hydroxydecanoate	Lipid	Fatty Acid, Monohydroxy	1.29 (0.87, 4.09)	0.203	1.35 (0.86, 4.27)	0.194
linoleate (18:2n6)	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	**1.38 (1.06, 3.93)**	**0.043**	**1.39 (1.07, 4.04)**	**0.041**
10-nonadecenoate (19:1n9)	Lipid	Long Chain Fatty Acid	**1.33 (1.05, 4.04)**	**0.044**	1.30 (0.96, 4.14)	0.060
dihomo-linoleate	Lipid	Polyunsaturated Fatty Acid (n3 and n6)	1.20 (0.73, 3.99)	0.589	1.15 (0.63, 4.02)	0.764
3-methylxanthine	Xenobiotics	Xanthine Metabolism Hemoglobin and Porphyrin	0.80 (0.32, 2.80)	0.504	0.85 (0.33, 3.51)	0.619
bilirubin	Cofactors/Vitamins	Metabolism	**0.50 (0.47, 0.90)**	**0.034**	**0.54 (0.42, 0.95)**	**0.041**
1-palmitoyl-GPA (16:0) glucuronate	Lipid	Lysolipid	0.89 (0.45, 3.16)	0.744	0.90 (0.40, 3.81)	0.778
	Carbohydrate	Aminosugar Metabolism	0.90 (0.32, 4.53)	0.796	0.95 (0.33, 4.72)	0.904
1-(1-enyl-stearoyl)-2-dihomo-linolenoyl-GPE	Lipid	Plasmalogen Urea cycle; Arginine and Proline	0.62 (0.33, 1.25)	0.146	0.70 (0.35, 1.30)	0.166
urea	Amino Acid	Metabolism	1.11 (0.59, 4.08)	0.646	1.12 (0.58, 4.16)	0.639
sphingomyelin	Lipid	Sphingolipid Metabolism	0.90 (0.41, 4.28)	0.826	0.92 (0.34, 4.51)	0.854

*.Adjusted by parity, education, birth place, language acculturation, and BMI category.

#.Adjusted by parity, education, birth place, language acculturation, and BMI category, smoking status, alcohol status, sitting time, and physical activity.

Table 3.

Stratified analysis to assess the association between candidate metabolites and breast cancer

Variable	3-hydroxyoctanoate*	linoleate (18:2n6)*	bilirubin*
	OR (95%CI)	OR (95%CI)	OR (95%CI)
Age at enrollment, years			
<51 years	**1.53 (1.03, 4.46)**	1.41 (0.89, 5.98)	0.57 (0.35, 1.06)
≥51 years	**1.47 (1.05, 4.37)**	1.37 (0.82, 5.71)	0.50 (0.31, 1.01)
Parity			
0–2 children	**1.49 (1.06, 4.40)**	1.44 (0.85, 5.83)	0.51 (0.36, 1.04)
>2 children	**1.54 (1.05, 4.33)**	1.35 (0.80, 5.64)	0.58 (0.39, 1.07)
Education level			
<High school	**1.54 (1.07, 3.92)**	1.40 (0.86, 5.77)	0.55 (0.35, 1.05)
≥High school	1.44 (0.90, 4.67)	1.37 (0.81, 5.58)	0.53 (0.34, 1.03)
Place of birth			
Mexico	**1.49 (1.04, 4.04)**	**1.37 (1.00, 5.36)**	0.51 (0.32, 0.99)
United States	1.61 (0.72, 5.73)	1.47 (0.65, 6.34)	0.63 (0.26, 1.63)
Language acculturation			
Low	**1.50 (1.03, 4.12)**	**1.36 (1.01, 5.29)**	0.52 (0.34, 0.98)
High	1.55 (0.82, 5.69)	1.48 (0.62, 6.55)	0.64 (0.24, 1.71)
BMI category			
Non-obese	**1.46 (1.04, 4.12)**	1.32 (0.76, 5.48)	**0.53 (0.33, 0.99)**
Obese	**1.58 (1.06, 4.30)**	**1.48 (1.01, 5.14)**	**0.54 (0.34, 0.98)**
Smoking status			
Never	**1.54 (1.07, 3.99)**	**1.38 (1.02, 4.56)**	**0.54 (0.35, 0.98)**
Ever	1.40 (0.76, 5.68)	1.45 (0.65, 6.39)	0.57 (0.21, 1.89)
Alcohol drinking			
Never	**1.53 (1.06, 4.01)**	**1.40 (1.04, 4.69)**	**0.55 (0.34, 0.97)**
Ever	1.46 (0.86, 5.09)	1.34 (0.60, 6.12)	0.51 (0.22, 1.84)
Physical activity			
Low	**1.52 (1.07, 3.86)**	**1.41 (1.05, 4.71)**	**0.53 (0.35, 0.97)**
Medium or high	1.39 (0.70, 5.38)	1.32 (0.59, 6.03)	0.59 (0.24, 1.91)
Sitting hours per day			
0–4	**1.47 (1.03, 4.39)**	**1.38 (1.02, 4.49)**	0.52 (0.29, 1.06)
>4	**1.55 (1.06, 4.48)**	**1.41 (1.03, 4.51)**	0.56 (0.33, 1.09)
Time between blood collection and cancer diagnosis			
≤5 years	**1.65 (1.07, 4.38)**	**1.57 (1.06, 4.78)**	**0.51 (0.30, 0.96)**
>5 years	**1.33 (1.01, 5.06)**	1.30 (0.85, 5.43)	**0.55 (0.25, 0.99)**

*Adjusted by parity, education, birth place, language acculturation, and BMI category, smoking status, alcohol status, sitting time, and physical activity, as appropriate.

Table 4.

Conditional logistic regression to assess the relationship between metabolite risk score associated with breast cancer risk

	Model 1*		Model 2#	
	OR (95%CI)	**P value**	**OR (95%CI)**	**P value**
Continuous variable	1.72 (1.46–3.70)	<0.001	1.67 (1.32–3.94)	<0.001
Stratified by time between blood collection and cancer diagnosis				
≤ 5 years	1.90 (1.27, 4.89)	0.003	1.84 (1.15, 4.78)	0.011
> 5 years	1.49 (1.04, 5.02)	0.044	1.43 (1.02, 5.16)	0.047
Categorical variable				
Low (0–1)	Reference		Reference	
High (2–3)	3.91 (1.87, 10.32)	<0.001	3.82 (1.78–11.56)	<0.001
Stratified by time between blood collection and cancer diagnosis				
≤ 5 years	5.55 (1.65, 13.58)	<0.001	5.38 (1.62, 13.57)	<0.001
≤ 5 years	2.47 (1.08, 15.06)	0.028	2.36 (1.07, 14.85)	0.030

*. Adjusted by parity, education, birth place, language acculturation, and BMI category.

#. Adjusted by parity, education, birth place, language acculturation, and BMI category, smoking status, alcohol status, sitting time, and physical activity.