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Circulating Plasma Metabolites and Breast Cancer Risk

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Background

Metabolite profiles reflect the integrated impact of the genome and exogenous exposures on the metabolic state. As cancer is defined by altered cellular processes and metabolic state, examination of metabolite profiles can provide insight into biologic mechanisms contributing to cancer development. Studies have uncovered associations between plasma metabolites and risk of several cancers, including hormonal cancers: ovarian cancer,[1] prostate cancer,[2] and breast cancer.[3] However, the biologic implication of these associations remains unknown. Small sample sizes of previous studies necessitate the further study of such associations. Moreover, metabolite changes may be matched to specific periods in cancer development, though most studies are limited by a single blood collection. This study explores metabolite profiles associated with breast cancer among postmenopausal women and explores the changes over time.

Identification of novel metabolites associated with breast cancer risk can provide insights into the biochemical mechanisms underlying breast cancer development.

Objective

Discover metabolite and metabolite pathway associations with breast cancer risk. Further evaluate how these associations differ with time of blood collection.

Methods

Cohort and Inclusion. Participants included cases and controls from the Nurses' Health Study (est. 1976) that contributed a blood sample ≥10 years prior to breast cancer diagnosis (collected 1989-1990) (N=939). 592 participants also contributed a blood sample <10 years prior to breast cancer diagnosis (collected 2000-2002).

Exposure. Prediagnostic plasma metabolites were profiled for cases and controls via liquid chromatography tandem mass spectrometry (LC-MS) at the Broad Institute via two platforms. A total of 307 known metabolites were measured; those with <10% missingness were imputed with ½ the minimum value. Odds ratios were assessed comparing the 90th-10th percentile of metabolite level.

Outcome. Case status for overall breast cancer, as well as ER+ and ER- breast cancers.

Statistical Methods. Individual metabolites were assessed via multivariable conditional logistic regression to determine metabolite associations with overall BC at distant and proximate blood measures. Unconditional logistic regressions were used for ER+ and ER- BC. We further investigated the association with breast cancer for the difference between proximate and distant metabolite measures, controlling for distant metabolite measure, and for the average of distant and proximate metabolites. Multiple testing was accounted for using the number of effective tests (NEF). Metabolite set enrichment analysis (MSEA) was used to analyze the association between metabolite groups (defined by subclasses) and breast cancer risk. Weighted metabolite co-expression network analysis (WMCNA) identified data-driven metabolite modules based on their interconnectedness; associations between resulting module scores and breast cancer risk were examined. Multiple testing in GSEA and WGCNA was accounted for using the false discovery rate.

Covariates: Matched factors: age at blood draw, month of blood draw, fasting status at blood draw, menopausal status at blood draw, hormone use at blood draw. Other factors: BMI at age 18, weight change from age 18 to blood draw, age at menarche, combined age at first birth and parity, breastfeeding history, history of benign breast disease, family history of breast cancer, alcohol use (g/day), activity level (met hrs/week).

Results

Table 1. Descriptive characteristics of NHS participants who provided blood samples at distant (N=939) and proximate (N=592) timepoints.

Characteristic	Distant Blood (>10y before dx)		Proximate Blood (≤10y before dx)	
	Case (N=939)	*Control (N=939)*	*Case (N=592)*	*Control (N=592)*
Age*, mean (SD)	55.5 (6.9)	55.6 (6.9)	66.4 (6.9)	66.5 (6.8)
Fasting*(%)	67	73	87	92
Postmenopausal* (%)	62	62	98	98
Hormone use (any)* (%)	68	68	81	81
Age at menarche, mean (SD)	12.5 (1.4)	12.6 (1.4)	12.5 (1.4)	12.6 (1.4)
Breastfeeding history* (%)	604 (64.3)	583 (62.1)	399 (67.4)	381 (64.4)
History of BBD* (%)	53	46	53	47
Family history of BC* (%)	15	11	23	15
Weight change from age 18*, mean (SD)	12.3 (10.9)	10.6 (11.2)	15.1 (12.8)	13.53 (12.8)
BMI*, mean (SD)	25.7 (4.3)	25.2 (4.7)	26.7 (5.0)	26.4 (5.2)
Average alcohol consumption in g/day*, mean (SD)	7.0 (9.9)	5.9 (8.2)	6.7 (9.2)	5.8 (7.7)
Activity level in met hours/week*, mean (SD)	15.4 (18.8)	15.9 (17.6)	25.7 (42.0)	23.4 (31.7)

* Signifies measure was taken at blood draw

Individual Metabolite Associations

No metabolites were significantly associated with breast cancer risk after adjustment by NEF (adjusted p-value threshold=0.0003) though several patterns emerged by class of metabolite and a few metabolites stood out as prominent (**Table 2 & 2b**). For example, phenylalanine was strongly associated with increased risk at both distant and proximate bloods. Steroid esters were inversely associated with risk at distant blood. Triacylglycerols (TAGs) with ≥3 double bonds were inversely associated with risk at proximate blood. TAGs with <3 double bonds appeared positively associated with risk, especially at the distant time point. Based on these findings, TAGs were further explored by number of Carbon atoms and number of double bonds (**Figure 1**).

Table 2. Odds ratios for breast cancer risk comparing 90th to 10th percentiles of selected metabolite levels, fully adjusted CLR, measured at distant blood.

Metabolite	Subclass	OR (95% CI)	p value
phenylalanine	Amino acids, peptides	1.41 (1.08-1.85)	0.012
proline	Amino acids, peptides	1.33 (1.03-1.72)	0.032
homoarginine	Amino acids, peptides	1.3 (1.01-1.68)	0.039
lysine	Amino acids, peptides	1.31 (1.01-1.69)	0.04
acetyl-galactosamine	Carbohydrates	1.35 (1.02-1.77)	0.035
C5:1 carnitine	Fatty acid esters	0.73 (0.57-0.93)	0.01
C5-DC carnitine	Fatty acid esters	0.73 (0.57-0.93)	0.012
C22:0 LPE	Glycerophosphoethanolamines	0.75 (0.58-0.98)	0.035
C38:6 PE plasmalogen	Glycerophosphoethanolamines	0.78 (0.61-0.99)	0.039
thyroxine	NA	1.56 (1.19-2.05)	0.001
2-methylguanosine	NA	1.32 (1.01-1.72)	0.039
guanosine	NA	0.78 (0.61-0.99)	0.041
C22:5 CE	Steroid esters	0.67 (0.52-0.86)	0.002
C18:3 CE	Steroid esters	0.69 (0.54-0.89)	0.004
C20:5 CE	Steroid esters	0.74 (0.58-0.95)	0.017

Table 2b. Odds ratios for breast cancer risk comparing 90th to 10th percentiles of selected metabolite levels, fully adjusted CLR, measured at proximate blood.

Metabolite	Sub Class	OR (95% CI)	p value
phenylalanine	Amino acids, peptides	1.76 (1.25-2.48)	0.001
proline	Amino acids, peptides	1.59 (1.13-2.22)	0.007
isoleucine	Amino acids, peptides	1.56 (1.12-2.17)	0.009
C16:0 Ceramide (d18:1)	Ceramides	1.72 (1.23-2.4)	0.002
myristoleic acid	Fatty acids and conjugates	1.58 (1.11-2.24)	0.012
C58:7 TAG	Triacylglycerols	0.59 (0.42-0.82)	0.002
C56:9 TAG	Triacylglycerols	0.64 (0.46-0.87)	0.004
C56:10 TAG	Triacylglycerols	0.63 (0.46-0.86)	0.004
C54:9 TAG	Triacylglycerols	0.64 (0.47-0.87)	0.005
C54:8 TAG	Triacylglycerols	0.65 (0.47-0.88)	0.006
C58:11 TAG	Triacylglycerols	0.64 (0.47-0.88)	0.006
C56:8 TAG	Triacylglycerols	0.66 (0.48-0.9)	0.008
C58:9 TAG	Triacylglycerols	0.66 (0.47-0.9)	0.01
C58:10 TAG	Triacylglycerols	0.66 (0.48-0.9)	0.01
C56:7 TAG	Triacylglycerols	0.68 (0.5-0.94)	0.017

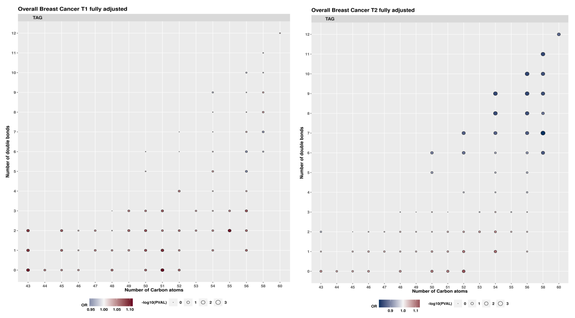

Figure 1. Odds ratios for breast cancer risk comparing 90th to 10th percentile of TAGs, by number of Carbon atoms and double bonds at T1 (distant blood) and T2 (proximate blood). Models are CLR equations, fully adjusted. Protective associations are shown in blue, harmful associations are shown in red.

Metabolite Set Enrichment Analysis

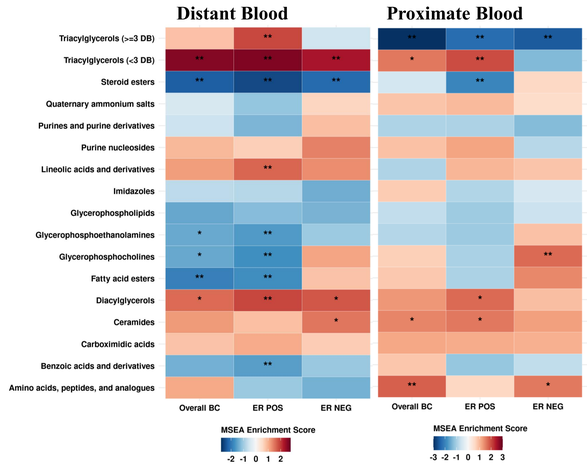

Figure 2b. MSEA by subclass of metabolites for overall, ER+, and ER- breast cancer, proximate blood. Overall breast cancer results use conditional logistic regression; ER status specific models use unconditional logistic regression models adjusted for matched factors. Models are fully adjusted for all covariates. Stars denote p-values adjusted by FDR: * (padj <0.2); ** (padj<0.05). Darker blue is a more negative enrichment score; darker red is a more positive enrichment score.

TAGS with ≥3 DB are strongly protective measured closer to diagnosis, though are null (overall & ER-) or harmful (ER+) when measured at the distant time point. The harmful effect of TAGs with <3 DB is more prominent at the distant blood, though it remains consistent for overall and ER+ breast cancer at the proximate blood. Protective association of steroid esters is stronger when measured at the distant blood.

In line with differences seen in GSEA by blood draw, a higher protective association for breast cancer was observed when TAGs with ≥3 DB increased from distant to proximate blood (e.g.: C58:9 TAG OR for 2.5 SD change from distant to proximate blood, adjusted for distant blood=0.60 (0.26-0.85), p=0.004.

WGCNA Analysis

No metabolite modules were significantly associated with breast cancer risk in WGCNA analysis, though modules characterized by steroid esters and TAGs were identified at both time points.

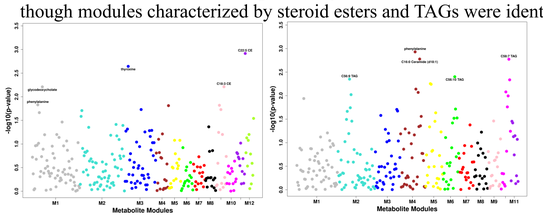

Figure 3. Metabolite associations with overall breast cancer risk (by Module grouping) at distant blood (left) and proximate blood (right). WGCNA module scores derived from fully adjusted conditional logistic results. The top 5 metabolites are highlighted.

Conclusions

While individual metabolite associations were not significantly associated with breast cancer risk, several stood out as noteworthy for future investigation. MSEA analysis revealed subclasses associated with breast cancer risk at both time points, and highlighted differences between the two time points, which were clarified in difference analyses. In particular, TAGs with ≥3 double bonds appear very protective against breast cancer at the proximate time point, whereas the protective effect of steroid esters is more readily seen at the distant blood draw. Some associations are in opposition given ER type of breast cancer. Further exploration of these metabolite pathways and their action throughout the development of breast cancer should be explored.

Acknowledgements

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