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BRIEF REPORT

Null effect of circulating sphingomyelins on risk for breast cancer: a Mendelian randomization report using Breast Cancer Association Consortium (BCAC) data. [version 1; peer review: 2 approved with reservations]

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Abstract

Background: Changes in cellular metabolism are a hallmark of cancer and are linked with sphingolipid synthesis. Due to immense interest in how sphingolipids influence chemoresistance, more is known about the impact of sphingolipids during cancer treatment and progression than about the potential role of sphingolipids in the induction of tumors in humans.

Methods: Because estrogen triggers sphingolipid signaling cascades, the causal role of circulating levels of sphingomyelin (a type of sphingolipid) on breast cancer was investigated with a well-powered Mendelian randomization design.

Results: The results reveal a null effect (OR = 0.94; 95% CI = 0.85, 1.05; $P = 0.30$).

Conclusion: Despite the role sphingomyelins play during chemoresistance and cancer progression, circulating sphingomyelins do not appear to initiate or protect from breast cancer. This finding comprises the first causal report in humans that sphingomyelins on breast cancer initiation is null. Future investigations of risk in other cancer types are needed to further explore the potential role of sphingolipid biology in cancer etiology.

Keywords

Mendelian randomization, breast cancer, sphingomyelins, lipids, metabolism

Open Peer Review

Approval Status

	1	2
version 1
18 Dec 2019	view	view
1. Jorge Simon , Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Bizkaia, Spain		
2. Rezvan Esmaeili , Motamed Cancer Institute, Tehran, Iran		

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Adams CD: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Introduction

Changes in cellular metabolism are a hallmark of cancer¹. Sphingolipids can control the rate of cell proliferation during malignant transformation and affect chemoresistance². Sphingomyelin is a type of sphingolipid, a class of lipids containing sphingoid bases (Figure 1). As a response to cellular stress, sphingolipids mediate apoptosis and autophagy, through the synthesis and/or accumulation of ceramide. Ceramide can be hydrolyzed from sphingomyelin³. Due to immense interest in how sphingolipids influence chemoresistance, much is known about the impact of sphingolipids on cancer treatment and little is known about role sphingolipids in the induction of tumors in humans.

Estrogen triggers sphingolipid signaling cascades². Due to this, it was hypothesized that circulating sphingomyelins might be involved in acquisition of breast cancer. The causal impact of circulating levels of sphingomyelins on risk for breast cancer was appraised with Mendelian randomization (MR).

Methods

Conceptual framework

MR is an instrumental variables technique; i.e., genetic variants (typically single-nucleotide polymorphisms, SNPs) strongly associated with traits are used in statistical models instead of the traits themselves. This avoids most environmental sources of confounding and averts reverse causation, which preclude causal inference in observational studies. Two-sample MR is an adaptation of the procedure that uses summary statistics from two genome-wide association (GWA) studies⁴.

MR assumptions

MR depends on the validity of three assumptions: (i) the SNPs acting as the instrumental variables must be strongly associated with the exposure; (ii) the instrumental variables must be independent of confounders of the exposure and the outcome; and (iii) the instrumental variables must be associated with the outcome only through the exposure.

Data sources

Step 1. Kettunen *et al.* (2016) performed a genome-wide association (GWA) study of 123 circulating metabolites—including sphingomyelins—in European participants (n=13,476 for sphingomyelins)⁵. From this, independent (those not in linkage disequilibrium; $R^2 < 0.01$) SNPs associated at genome-wide significance ($P < 5 \times 10^{-8}$) with a standard-deviation (SD) increase in circulating sphingomyelins were identified. The Kettunen GWA is available through MR-Base⁵.

Figure 1. Cartoon of a sphingomyelin. The bolder print indicates the lipidic sphingoid base that is carrying a saturated fatty acid amine bonded to an amino group at the C2 position. Attached to the polar head is a phosphocholine. The cartoon has been reproduced with permission from Holthuis *et al.* (2001)⁶.

Step 2. A publicly available GWA study of breast cancer performed by the Breast Cancer Association Consortium (BCAC) on 122,977 breast cancer cases and 105,974 controls of European ancestry was chosen as the outcome GWA for breast cancer⁷.

Statistical approach

A seven-SNP instrument for circulating sphingomyelins was constructed from the SNPs strongly associated with circulating sphingomyelin levels. Estimates of the proportion of variance in circulating sphingomyelins explained by the genetic instrument (R^2) and the strength of the association between the genetic instrument and sphingomyelins (F -statistic) were generated (conventionally F -statistics < 10 are weak). The instrument for sphingomyelins has an $R^2 = 0.032$ and the F -statistic = 1089. The study was powered using the mRnd MR power calculator (available at <http://cnsgenomics.com/shiny/mRnd/>). It had $> 90\%$ power to detect an OR of 0.90.

The log-odds for breast cancer per SD increase in circulating sphingomyelins was calculated, using the inverse-variance weighted (IVW) MR method. The “TwoSampleMR” package⁴ was used for the MR analysis.

All described analyses were performed in R version 3.5.2.

Sensitivity analyses

Several sensitivity estimators can be used appraise pleiotropic bias. Three were chosen to complement the primary IVW causal tests: MR Egger regression, weighted median, and weighted mode estimations. In addition to these sensitivity estimators, a test for heterogeneity was performed, since variability in the causal estimates between SNPs can indicate pleiotropy. The test for heterogeneity was performed using Cochrane’s Q -statistic.

Results

There was a null effect for circulating sphingomyelins on breast cancer (OR = 0.94; 95% CI = 0.85, 1.05; $P = 0.30$). The sensitivity estimators had effect estimates in the same direction and were of comparable magnitude to the IVW estimate, indicating no evidence for substantial bias due to unwanted pleiotropy. There was no evidence for heterogeneity in the estimates (Table 1). The MR-Egger intercept test, which provides an assessment of potential directional pleiotropy in the IVW was null. A null effect indicates a lack of evidence for pleiotropy (Estimate = 1.01; 95% CI = 0.97, 1.04; $P = 0.55$).

Discussion

This is the first causal report in humans that sphingomyelins on breast cancer initiation is null. The null effect might reflect the complex interplay of pro-apoptotic and pro-growth ceramides⁸, perhaps with greater upregulation of the pro-apoptotic pathways, which may be different for different tissues. Future investigations of risk in other cancer types are needed to further explore the potential role of sphingolipid biology in cancer etiology.

One potential limitation of this analysis is that unwanted pleiotropy cannot be entirely ruled out in MR studies. However, the sensitivity estimators provide evidence against this. Given the many

Table 1. Causal estimates for the association of circulating sphingomyelin levels on risk breast cancer.

Method	OR	Lower 95% CI	Upper 95% CI	P value	Q- statistic	Q-diff	Q P-value
IVW	0.94	0.85	1.05	0.30	8	6	0.27
MR Egger*	0.88	0.68	1.13	0.36	7	5	0.22
Weighted median*	0.92	0.81	1.04	0.19	NA	NA	NA
Weighted mode*	0.91	0.78	1.06	0.26	NA	NA	NA

IVW, inverse-weighted variance. *Denotes a sensitivity estimator.

ways in which a finding could be a false-positive, null findings from well-powered MR studies are, in some ways, more believable than reports of causal associations⁹. A major strength of this two-sample MR analysis is that it capitalized on the power of very large GWA studies. If sphingomyelins were causal for breast cancer initiation, it is highly unlikely that the effect would go undetected with more than 100,000 cases and 100,000 controls in BCAC.

Data availability

The sphingomyelin data are publicly available through the MR-Base repository (<http://www.mrbase.org/>) under a GNU General Public License v3.

The breast cancer outcome data are freely available on the BCAC website (<http://bcac.ccge.medschl.cam.ac.uk/>).

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Version 1

Reviewer Report 27 July 2020

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Rezvan Esmaeili

Breast Cancer Research Center, Motamed Cancer Institute, Tehran, Iran

In this manuscripts, authors used Mendelian randomization to determine the role of circulating sphingomyelin in breast cancer. Although the manuscript is worth indexing, the following issues should be addressed.

1. The introduction needs more explanation about breast cancer, the possible role of sphingomyelin, and MR for the general readers of the journal.
2. How the second and third MR assumptions (which they mentioned in the manuscript) were met?
3. As the authors said, the sphingomyelin is affected by estrogen signaling. Why did authors not do the subgroup analysis and not investigated the MR assumption in the ER-positive vs. negative patients? It seems the result may be significant in the ER-positive group. This analysis is necessary.
4. A similar publication in the same data set ([10.2217/bmt-2020-0002¹](#)) is available. Please explain the novelty or differences of the recent manuscript with the mentioned paper? Why is it not in the references?
5. The authors should provide a table containing a summary of the statistical data. Mentioned the selected SNP, allele frequency, hazard ratio, p values, etc.
6. Survival analysis should be included.
7. A table of demographic data and clinicopathologic characteristics of patients should be included.
8. It seems more data should be included in regards to sensitivity analysis and power estimate. I am not an expert in this field, and another person should revise this part.

9. The authors should mention the previous studies related to sphingomyelin and breast cancer in the discussion.
10. The body of the manuscript needs some English polishing. The text should be more fluent to read.

References

1. D Adams C: Circulating sphingomyelins on estrogen receptor-positive and estrogen receptor-negative breast cancer-specific survival. *Breast Cancer Management*. 2020. [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: cancer, biomarkers

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 23 March 2020

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? Jorge Simon

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I consider this manuscript suitable for indexing with your journal with some changes recommended:

- Make a more user-friendly introduction and be sure about some concepts (e.g sphingomyelin is a type of sphingolipid containing sphingomyelin, where every sphingolipid contains it).
- Introduce some main concepts about breast concept to contextualize your research.
- In the part of results, include what does each variable mean. I have found difficult to understand all the data from the table and the reader could have the same "problem".
- In the part of discussion you should focus on the possible explanation about the crosstalk between proliferative and pro-apoptotic SPHINGOLIPIDS (not ceramides, please confirm every concept (e.g. sphingomyelin is proliferative while ceramide apoptotic, and C1P pro-apoptotic). I would like to read a little bit more about your hypothesis instead of the limitations of your study.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hepatology, metabolomics, lipidomics, sphingolipids

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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