

Identification of Breast Cancer-Associated Lipids in Scalp Hair

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Abstract: A correlation between the presence of breast cancer and a change in the synchrotron-generated X-ray diffraction (XRD) pattern of hair has been reported in several publications by different groups, and on average XRD-based assays detect around 75% of breast cancer patients in blinded studies. To date, the molecular mechanisms leading to this alteration are largely unknown. We have determined that the alteration is likely to be due to the presence of one or more breast cancer-associated phospholipids. Further characterization of these lipids could be used to develop a novel, sensitive and specific screening test for breast cancer, based on hair initially, and potentially extendable to other biological samples.

Keywords: hair, breast cancer, lipids, phospholipids, hair

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Introduction

Breast cancer is the most common cancer among women and is also the second leading cause of cancer deaths in women.² Over 1.3 million women worldwide are diagnosed annually with breast cancer and approximately 458,000 will die from the disease.⁴ Currently, mammography (X-ray of the breast) and ultrasound are used to screen for breast cancer and tissue biopsies are used to confirm a diagnosis. Mammography can be unreliable in dense breasts and the accuracy of the test relies on the skill of the analyst⁵ while ultrasound also relies heavily on the operator's ability to detect the tumour.⁶ The accuracy of mammography varies significantly, in a 2005 US study of 49,528 symptomatic women, the sensitivity of both film and digital mammography was shown to be 41% {Pisano, 2005 #52}. Therefore, breast cancer may require multiple screenings before a confident diagnosis can be confirmed.⁷⁻⁹ However, regular exposure to X-rays can increase the chance of breast cancer development.¹⁰ It is generally agreed therefore that a more reliable and less invasive method for screening and diagnosing breast cancer would significantly reduce mortality of this disease.

Whilst many biomarkers have been tried as candidates without success, a promising novel technology was reported in 1999. The authors showed a correlation between the presence of breast cancer and an observed change in the X-ray diffraction (XRD) pattern of hair from afflicted individuals.¹¹ Using synchrotron-generated X-rays, the diffraction patterns of hair samples collected from women diagnosed with breast cancer all exhibited a circular feature specific to the pathological state. This observed change presented as a new circular feature (in a specific region of 4.6 Angstroms) superimposed on the normal X-ray diffraction pattern of hair (Fig. 1). Of the samples taken from patients not suspected of having breast cancer, 86% of the diffraction patterns were normal. Further studies by James et al in 2005, using a xenograft model of breast cancer in nude mice, indicated that the diffraction change specific to breast cancer was observed in hair or whiskers in early stages of tumour development. The data illustrated that the change occurred within days of cell implantation and provided support for the potential that this technique could be used as an early diagnostic test for the presence of breast cancer.¹² Other groups, independent of the original inventor,

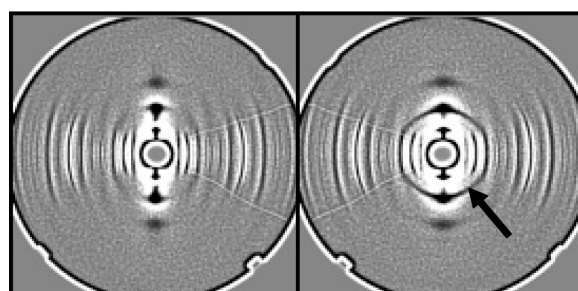


Figure 1. XRD patterns of hair from a healthy woman (left) and from a woman with breast cancer (right).

Notes: The arrow indicates the altered feature in the breast cancer patient's hair, referred to as the "ring". These patterns were taken using the Small Angle X-ray (SAXS) at the Australian Synchrotron. This pattern is consistent with the patterns reported by Corino et al¹ in 2009, which reported the feature present in greater than 60% of patients with breast cancer.

reported their inability to reproduce similar results to those obtained in the initial study.^{13,14} Some subsequent studies by other groups however, confirmed an association between the XRD hair pattern alteration and the presence of breast cancer, although the initial reported accuracy was not attained.^{1,15} The authors agreed that that the analysis and interpretation of the data is complex and requires strict adherence to the methodology to ensure accurate reproduction.¹⁶⁻¹⁹

The biological mechanisms leading to the ring phenomenon have not been identified. Speculations on the origin of the ring have included that it is derived from calcium-associated soap residues,³ or from the interaction of hair tissue and calcium with proteoglycans, which cause a structural transformation of the extracellular matrix.²⁰

Two groups have proposed that the ring shown by the James group was lipid in nature. In 2003, Bertrand et al simply referred to the breast cancer-associated feature shown by James and colleagues as "hair lipid diffraction rings".³ A study of hairs from cancer and normal subjects by Fourier transform infrared attenuated total reflection provided independent validation of the underlying hypothesis that hair from individuals with breast cancer exhibits a structural abnormality. Interpretation of the spectra of these regions led the researchers to conclude that there was a modification of the hair fiber growth as a result of the presence of a developing cancer. The changes in one of the regions of interest were suggestive of an increase in lipid content.²¹ The fact that the XRD feature associated with breast cancer is a ring gives some clues as to its chemical nature. A circular feature (a "broad halo")

in an XRD pattern can be produced by an amorphous material such as oils.²² Thus, a possible explanation for the presence of the ring in the XRD patterns seen in the hair from breast cancer patients is an incorporation of lipid into the fibre. In this study we conducted a range of experiments to determine if the feature was related to lipids and if so, what is the nature of the lipids. We conclude that the breast cancer-associated feature, seen in the XRD pattern of hair, is phospholipid in nature.

Methods

X-ray diffraction of hair

Samples

Donated human hair samples were cut close to the scalp from a total of 18 individuals whose breast cancer status was determined by mammography. Women were excluded if their scalp hair had been dyed or chemically treated. Samples were collected in accordance with the ethical standards of the Fermiscan Human Research Ethics Committee, an HREC constituted under the NHMRC Guidelines.

Synchrotron X-ray diffraction

X-ray diffraction assessment required the analysis of individual hair fibers from each subject, which were loaded onto specially, designed sample holders. Synchrotron X-ray diffraction experiments were carried out as described in previous publications¹⁵ on the Small Angle X-ray Scattering—Wide Angle X-ray Scattering (SAXS-WAXS) beam line at the Australian Synchrotron, Melbourne. A MAR165 detector was used online to aid in the alignment of the sample and also for data collection.

Image analysis

Image analysis was performed according to that described previously.¹⁵

Diffraction patterns were analysed using FIT2D (<http://www.esrf.fr/computing/scientific/FIT2D>) and Saxes15ID.³⁰ Both programs offer the data processing and smoothing routines that are required to perform the data reduction and subsequent analysis. Background correction was carried out without compromising any of the features present in the original pattern using FIT2D. One-dimensional data was extracted from each X-ray diffraction pattern and presented in the form of a graph alongside the corrected image. Samples were determined as either “positive for breast cancer” or

“negative for breast cancer” by examination of the resulting X-ray diffraction patterns by two trained analysts who were not provided with the clinical status of the patient. A negative call was one where the circular feature was not present in the pattern but the underlying keratin reflections were. A positive call was one where the circular feature was present at a q space of 1.32 ± 0.02 angstroms along with the underlying keratin reflections. A standard set of hairs was used (hairs from patients confirmed to have breast cancer and confirmed to be free of breast cancer) to calibrate the assay. The pathology results (following biopsy) were forwarded to an auditor. The diffraction analyses were separately forwarded to the independent auditor, who de-coded the results, matched the imaging and pathology results with the X-ray diffraction data, and communicated the results to the investigators.

Addition of fatty acids to hair

In order to attempt to recreate the feature seen in the XRD pattern of hair using lipids, hair fibers from subjects confirmed to be free of breast cancer were initially checked by SAXS to confirm the absence of the circular feature associated with breast cancer. Each fiber was removed from the holder and soaked in olive oil for 10 minutes, then patted dry with tissue paper and re-exposed to the SAXS beam for another 20 seconds. The resulting image was analyzed for the presence/absence of a breast cancer-associated feature.

Chemical enhancement of lipids in hairs and solvent extraction of hairs

Two hair fibers from each patient with breast cancer were soaked in 5 mL of 100% acetone for 5 minutes to remove external lipids. They were then washed in 5 mL of water for 5 minutes. This was repeated three more times. They were patted dry with tissue paper, mounted in the sample holder and exposed to the SAXS beam for 20 seconds. The resulting image was analyzed. For each sample with the circular breast cancer-associated feature, the two fibers were treated differently:

- One hair fiber from each patient was soaked in 4 mL chloroform: methanol (1:2) for 2 hours.
- The other hair fiber from each patient was soaked in 4 mL acetone for 5 minutes, wiped dry and exposed to the SAXS beam for 20 seconds, and



then soaked in 4 mL of unbuffered 0.1 M/L of lead nitrate for 3.5 hours.

All fibers were then washed in 10 mL of water for 5 minutes. This was repeated three times. Hairs were wiped dry and exposed to the SAXS beam for 20 seconds. The resulting image was analyzed for the presence/absence of a breast cancer-associated feature.

Lipid profiling of hair

Samples

Seven hair samples from breast cancer patients were pooled according to their cancer stage (P1-P3) and five healthy un-pooled controls (H1-H5) were chosen for lipid extraction. Replicates were taken from each sample.

Extraction of lipid from hair

Hair samples were cut very finely using surgical grade scissors and placed into a glass vial. Samples were then washed in acetone 5 minutes to remove exterior fatty acids. 30 mg of air-dried sample were weighed into cryo-mill tubes and 1000 μ L of extraction solution (methanol, chloroform, water (2:1:0.6)) was added into each tube. Samples were spiked with 10 μ L of 100 μ M internal standard (Cholesterol ester (18:0) d) to 10 μ M final concentration. Hair was ground using a cryo-mill (Precellys) with 1.4 mm ceramic beads at 6800 rpm for three times 30 second with 45 second between intervals and the samples were centrifuged for 10 minutes at 10000 rpm. Supernatant (500 μ L) was transferred into Eppendorf tubes and dried in a speed-vac. Samples were resuspended in 100 μ L of n-butanol and methanol (v/v 1:1), centrifuged for 10 min at 10000 rpm and 50 μ L of supernatant transferred to high pressure liquid chromatography (HPLC) vials for further analysis.

LCMS analysis

Lipids were separated by injecting 5 μ L aliquots onto a 50 mm \times 2.1 mm \times 2.7 μ m Ascentis Express RP Amide column (Supelco) using an Agilent LC 1200. Briefly, sample elution was performed at 0.2 mL/min over a gradient of 50% water (v/v)/methanol 20% (v/v)/tetrahydrofuran 20% (v/v) to water/methanol/tetrahydrofuran (5:20:75, v/v/v). Lipids were analysed by electrospray ionisation-mass spectrometry (ESI-MS) using an Agilent Triple Quad 6460. The

mass spectrometer was set up to specifically identify phosphatidylcholines. The capillary voltage used was 4000 V, fragment or voltage 140–380 V, and collision energy 15–60 V. The collision gas throughout was nitrogen at a rate of 7 L/min. LCMS data was processed using an Agilent MassHunter quantitative software.

Data processing for all LCMS/GCMS data

1. Data: Mass spectra data collected from QTOF mass spectrometer in positive mode
2. Pre-processing: The masses in the mass spectra were presented as a matrix table using XCMS software
3. Filtration: all the masses that were present in blanks were deleted
4. Normalization: sample weight + median
5. Multivariate Statistical Analysis: Principal Component Analysis (unsupervised analysis) and Principle Least Squares—Discriminant Analysis (supervised analysis).

Results

Addition of fatty acids to hair

XRD of hair before and after soaking in olive oil showed that a circular feature similar to the previously reported breast cancer-associated feature could be induced and suggested that the feature was lipid in nature (Fig. 2). There was some variability in the strength of the olive oil-induced feature. This variability could be attributed to the differential uptake of oil into the hair fibre due to the individual variability in structural features such as in the arrangement of the cuticle and intermediate filaments of the fibres. Cuticles that are more tightly packed are less likely to absorb as much external lipid as those cuticles that are more loosely packed.

Chemical enhancement of lipids in hairs and solvent extraction of hairs

Chloroform/methanol solutions are used routinely to extract lipids from biological samples.²³ When hair fibres, which initially exhibited the feature, were soaked in chloroform: methanol (1:2), the intensity of the feature diminished in XRD analysis compared to pre-exposure to the solvent solution. No other component of the pattern was similarly diminished, indicating that the molecular nature of the feature was susceptible to solvent

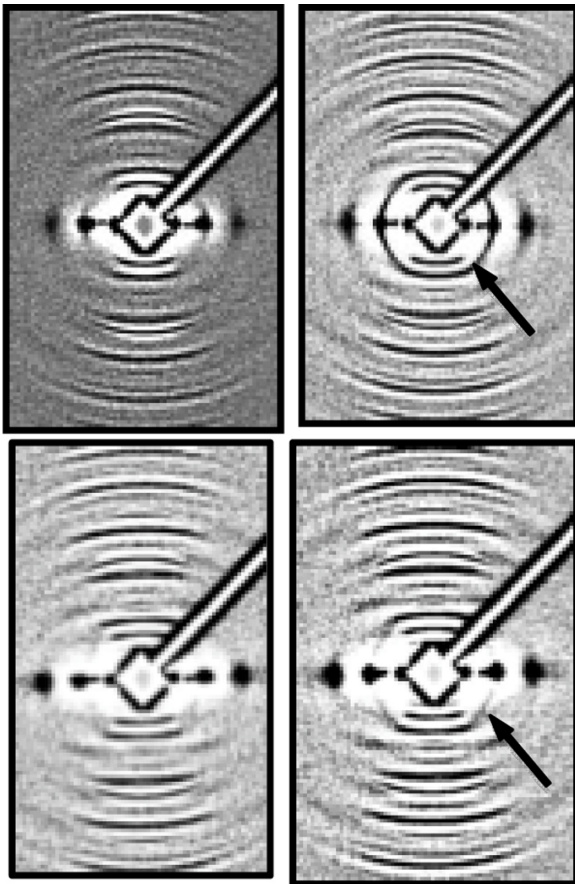


Figure 2. Addition of fatty acids to hair ($n = 6$).
Notes: In the first experiment, hair that did not have a ring (confirmed by XRD) (left) was soaked in olive oil for 10 minutes, wiped dry, and then re-exposed to X-rays. A clear “breast cancer-like” ring was seen post-soaking (right, arrow indicates breast cancer-associated feature).

extraction (Fig. 3). Out of the 15 samples (and replicates) this “extraction” occurred in 9 of the samples.

Lead has been shown to enhance the intensity of lipid-derived features (corresponding to the “ring” that we see in our data) in the XRD pattern of hair.³ This is thought to be due to the binding of lead ions to free fatty acids. Lead nitrate-treated hairs show an enhancement of the intensity of the ring and an additional reflection of the ring in 5 out of the 15 samples (Fig. 4). Again, in both experiments, the variation in chemical absorption could be attributed to the structural differences between hairs from different individuals.

Extraction of lipid from hair

When samples of hair from breast cancer patients and controls were extracted and then analysed for phosphatidylcholine lipid species, using liquid chromatography mass spectrometry (LCMS), the profiles

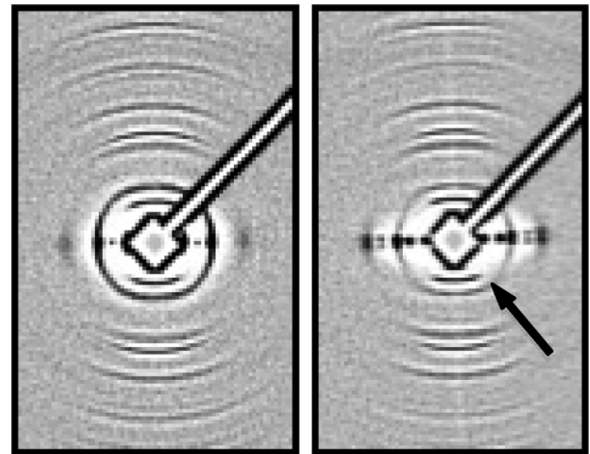


Figure 3. Solvent extraction of hairs.
Notes: Hairs from breast cancer patients, with XRD alterations (left), were treated with chloroform/methanol, a solvent solution known to extract lipids. The intensity of the ring was diminished (right) and the other (keratin-associated) features in the patterns were not affected. Arrow indicates location of the breast cancer-associated feature.

showed that overall, the level of particular species of phosphatidylcholine were higher in the hair of breast cancer patients than in controls (Fig. 5). The sensitivity of the preliminary analysis was not high enough to detect different amounts of the lipids. Some of the lipids found to be significantly different were PC18:6 ($P = 0.0023$), PC30:0 ($P = 0.0314$), PC32:0 ($P = 0.0417$), PC 32:6 ($P = 0.0064$) and PC23:4 ($P = 0.0333$), which showed the most, marked change in breast cancer compared to hair from healthy controls.

Discussion

We believe that we are the first to demonstrate that the previously reported breast cancer-associated change seen in XRD analysis of hair samples is of lipid origin, in particular phospholipids.

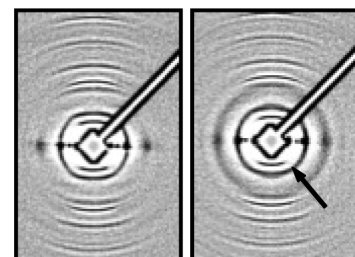


Figure 4. Lead nitrate treatment of hair.
Notes: When hairs, which produce the ring in XRD, were soaked in lead nitrate solution, the intensity of the rings was reflected in another circular feature around the original one. Lead nitrate has been shown to bind to lipids in hair and reflect the diffraction pattern.³ Arrow indicates location of the breast cancer-associated feature.

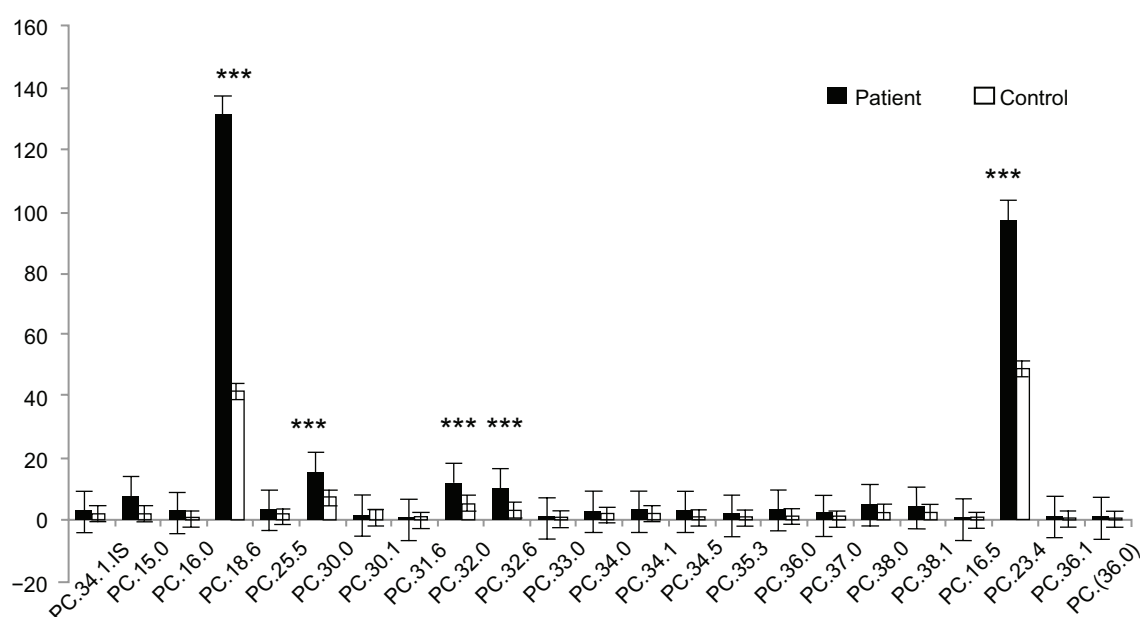


Figure 5. Average concentration of specific phosphatidylcholine (PC) species in hair samples collected from patients with breast cancer and controls. **Note:** ***Significantly different phospholipids (n = 8).

To demonstrate that a similar feature to that reported for XRD analysis of hair from breast cancer patients could be added to hair samples *ex vivo*, we applied olive oil to hair samples that did not demonstrate the breast cancer ring, and reported the presence of a ring in those samples subsequently. We used olive oil because it is the vegetable oil that is most similar to human fatty acids.²⁴ Interestingly, olive oil contains significant amounts of phospholipids, including phosphatidylinositol.²⁵ We were able to remove the ring from a majority of samples using a range of organic solvents known to solubilise lipids. We were also able to enhance the XRD intensity of the ring in several of the breast cancer positive samples using a chemical known to bind to lipids. Whilst not every sample reacted the same to the treatments, we speculate that this was due to the variation in the ability of the solvents and chemicals to penetrate the hair fibre, which could be attributed to the individual differences in the cuticle density packing.

In attempting to characterise the lipid(s) associated with breast cancer, we used mass spectrometry on hair samples that had been extensively extracted using a cryo-mill, to overcome the individual extractability variation. This showed that there is significantly more phosphatidylcholine present in the hairs of breast cancer patients when compared to controls.

From this data we propose that the tumour in the breast produces and releases phospholipids, probably from the tumour cell membrane, which enter the circulation and are incorporated into the matrix of the hair fibre resulting in an alteration in the pattern represented by a circular feature when the hair is subjected to XRD.

There are several lines of evidence which support this proposal and suggest that lipogenesis is closely linked to tumorigenesis. BRCA1 (breast cancer susceptibility gene 1) was the first susceptibility gene linked to breast and ovarian cancer.²⁶ It is frequently lost in breast cancer. In 1999, James also reported that women who tested positive for a mutation of the BRCA1 gene also showed the alteration in their hair XRD pattern.¹¹ A study by Moreau et al demonstrated that BRCA1 negatively regulates lipogenesis through binding to phosphorylated and inactive form of acetyl coenzyme A carboxylase, P-ACCA.²⁷ Thus there is a link between loss of BRCA1 and increased lipogenesis.

A number of other groups have also suggested that lipogenesis is closely linked to tumorigenesis in breast cancer. Chajes et al examined lipids in breast cancer tissues in comparison to normal tissue from the same patients and found that mechanisms specifically related to malignant transformation and tumour



progression influence the membrane fatty-acid profile of breast carcinoma.²⁸ A mouse study showed similar effects. Only mammary tumour tissues showed a drastic increase in the total phospholipid content ($P < 0.0001$) associated with a significant upregulation of phosphatidylethanolamine, phosphatidylcholine (PC), and sphingomyelin ($P < 0.05$).²⁹ Breast cancer has been associated with increased levels of PC in several studies which have suggested it can serve as a biomarker of breast cancer reflecting upregulation of specific choline transporters and choline kinase genes.³⁰ The level of PC in human breast cancer cells has been reported to be 10-fold higher than in normal mammary epithelial cells.³¹

In addition to being upregulated in breast cancer tissues and in cultured breast cancer cell membranes, it has been reported that lipids are also elevated in serum. In 1971 Feldman and Carter reported the association between serum lipids and breast cancer.³² More recently, Alexopoulos et al found that there was a significant difference in serum phospholipid content between stage-IV breast cancer patients and disease-free individuals. The most significant differences in lipid profiles among disease-free and cancer subjects were attributed to three phosphocholine species and to three unidentified fatty acid species.³³ Serum lipids elevated in breast cancer decrease significantly after treatment,³⁴ in a parallel fashion to the reported disappearance of the ring following mastectomy and chemotherapy.¹⁵ There are no reports which have looked at whether elevated levels of phosphocholine in serum are also seen in hair from the same patient.

The results presented in this study are from a small sample size designed as a pilot study to establish the nature of the circular breast cancer-associated XRD feature and formulate an idea of the likely lipids involved. We can conclude that phosphatidylcholine, and in particular sub-species thereof, is the most likely candidate, however, the possible association of other phospholipid molecules needs to be investigated and an understanding of the relationship between lipid shedding and the cancer process needs to be elucidated in a larger sample size. Furthermore, it is possible that hair from patients with other cancers may also exhibit incorporation of cancer-associated phospholipids. Therefore in future studies on breast

cancer it would be necessary to rule out the presence of other cancers in the patients from which the hair samples have been collected.

Further characterization of the phospholipids associated with breast cancer could be used to develop a novel sensitive and specific diagnostic screening test for breast cancer, based on hair initially, and potentially extendable to other biological samples.

Author Contributions

Conceived and designed the experiments: DM, PF, JH. Analysed the data: DM. Wrote the first draft of the manuscript: DM. Contributed to the writing of the manuscript: DM, PF. Agree with manuscript results and conclusions: DM, PF, JH. Jointly developed the structure and arguments for the paper: DM, PF. Made critical revisions and approved final version: PF. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

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Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is



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References

- Corino GL, French PW, Lee M, et al. Characterization of a test for invasive breast cancer using X-ray diffraction of hair—results of a clinical trial. *Breast Cancer (Auckl)*. 2009;3:83–90.
- DeSantis C, Siegel R, Bandi P, Jemal A. Breast Cancer Statistics, 2011: American Cancer Society. *CA Cancer J Clin*. 2011.
- Bertrand L, Doucet J, Simionovici A, et al. Lead-revealed lipid organization in human hair. *Biochim Biophys Acta*. 2003;1620:218–24.
- Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global Cancer Statistics. *CA Cancer J Clin*. 2011;61:69–90.
- Yankaskas BC, Haneuse S, Kapp JM, et al. Performance of first mammography examination in women younger than 40 years. *Journal of the National Cancer Institute*. 2010;102:692.
- Sohn C, Blohmer JU. Breast ultrasound: a systematic approach to technique and image interpretation Thieme, 1999.
- Kuhl CK, Schrading S, Leutner CC, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. *J Clin Oncol*. 2005;23:8469–76.
- Chen TH, Yen AM, Wu GH, et al. Multiple detection modalities and disease natural history of breast cancer. *Stud Health Technol Inform*. 2007;129:78–81.
- Lee JM, Kopans DB, McMahon PM, et al. Breast cancer screening in brca1 mutation carriers: Effectiveness of MR imaging—markov monte carlo decision analysis. *Radiology*. 2008;246:763–71.
- Jansen-van der Weide MC. *Abstract—Mammography Screening and Radiation-induced Breast Cancer among Women with a Familial or Genetic Predisposition: A Meta-analysis*. Presented at the RSNA, 2009.
- James V, Kearsley J, Irving T, et al. Using hair to screen for breast cancer. *Nature*. 1999;398:33–4.
- James V, Corino G, Robertson T, et al. Early diagnosis of breast cancer by hair diffraction. *Int J Cancer*. 2005;114:969–72.
- Laaziri K, Sutton M, Ghadirian P, et al. Is there a correlation between the structure of hair and breast cancer or BRCA1/2 mutations? *Phys Med Biol*. 2002;47:1623–32.
- Briki F, Busson B, Salicru B, et al. Breast-cancer diagnosis using hair. *Nature*. 1999;400:226.
- Corino GL, French PW. Diagnosis of breast cancer by X-ray diffraction of hair. *Int J Cancer*. 2008;122:847–56.
- James VJ. Changes in the diffraction pattern of hair resulting from mechanical damage can occlude the changes that relate to breast cancer. *Phys Med Biol*. 2003;48:37–41.
- James VJ. The traps and pitfalls inherent in the correlation of changes in the fibre diffraction pattern of hair with breast cancer. *Phys Med Biol*. 2003;48:5–9.
- James VJ. The importance of good images in using hair to screen for breast cancer. *J Med Genet*. 2001;38:16.
- James VJ. False-positive results in studies of changes in fiber diffraction of hair from patients with breast cancer may not be false. *J Natl Cancer Inst*. 2003;95:170–1.
- Vazina AA, Brasb W, Dolbnyab IP, et al. Peculiarities of human hair structural dynamics. *Nuclear Instruments and Methods in Physics Research*. 2005;543:153–7.
- Lyman DJ, Murray-Wijelath J. Fourier transform infrared attenuated total reflection analysis of human hair: comparison of hair from breast cancer patients with hair from healthy subjects. *Appl Spectrosc*. 2005;59:26–32.
- Clark GL, Aborn RH, Brugmann EW, et al. On X-ray diffraction patterns from liquids and colloidal gels. *Proc Natl Acad Sci U S A*. 1927;13:549–52.
- Seppanen-Laakso T, Oresic, M. How to study lipidomes. *J Mol Endocrinol*. 2009;42:185–90.
- Robbins C. *Chemical and Physical Behavior of Human Hair*. ed 4. 2002:213.
- Hatzakis E, Koidis A, Boskou D, et al. Determination of Phospholipids in Olive Oil by 31P NMR Spectroscopy. *Agric Food Chem*. 2008;56:6232–40.
- Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*. 1994;266:66–71.
- Moreau K, Dizin E, Ray H, et al. BRCA1 affects lipid synthesis through its interaction with acetyl-CoA carboxylase. *J Biol Chem*. 2006;281:3172–81.
- Chajes V, Lanson M, Fetissof F, et al. Membrane fatty acids of breast carcinoma: contribution of host fatty acids and tumor properties. *Int J Cancer*. 1995;63:169–75.
- Monteggia E, Colombo I, Guerra A, et al. Phospholipid distribution in murine mammary adenocarcinomas induced by activated neu oncogene. *Cancer Detect Prev*. 2000;24:207–11.
- Eliyahu G, Kreizman T, Degani H. Phosphocholine as a biomarker of breast cancer: molecular and biochemical studies. *Int J Cancer*. 2007;120:1721–30.
- Katz-Brull R, Seger D, Rivenson-Segal D, et al. Metabolic markers of breast cancer: enhanced choline metabolism and reduced choline-ether-phospholipid synthesis. *Cancer Res*. 2002;62:1966–70.
- Feldman EB, Carter AC. Circulating lipids and lipoproteins in women with metastatic breast carcinoma. *J Clin Endocrinol Metab*. 1971;33:8–13.
- Alexopoulos CG, Blatsios B, Avgerinos A. Serum lipids and lipoprotein disorders in cancer patients. *Cancer*. 1987;60:3065–70.
- Ray A, Jain D, Yadav R, et al. Effect of cancer treatment modalities on serum lipids and lipoproteins among women with carcinoma of the breast. *Indian J Physiol Pharmacol*. 2001;45:337–44.
- Ryu HK, Jung BH, Kim KM, et al. Determination of cholesterol in human hair using gas chromatography-mass spectrometry. *Biomed Chromatogr*. 2006;20:999–1003.

Supplementary Tables

Table S1A. All images showing solvent extraction.

Sample	Before	Solvent Extraction
1		
2		
3		
4		
5		
6		
7		
8		

Notes: n = 15. Arrow indicates approximate region where breast cancer-associated feature is typically located.

Table S1B. All images showing solvent extraction.

9		
10		
11		
12		
13		
14		
15		

Notes: n = 15. Arrow indicates approximate region where breast cancer-associated feature is typically located.

Table S2A. All images showing lead nitrate treatment.

Sample	Before	LeadNitrateTreatment
1		
2		
3		
4		
5		
6		
7		
8		

Notes: n = 15. Arrow indicates approximate region where breast cancer-associated feature is typically located.

Table S2B. All images showing lead nitrate treatment.

9		
10		
11		
12		
13		
14		
15		

Notes: n = 15. Arrow indicates approximate region where breast cancer-associated feature is typically located.



Table S3. Concentration (nM/mg) of Phosphatidylcholine lipids species extracted from hair (from Fig. 5).

Sample	D-PC 34.1	PC.15.0	PC.16.0	PC.18.6	PC.25.5	PC.30.0	PC.30.1	PC.31.6	PC.32.0	PC.32.6	PC.33.0	PC.34.0
Concentration (nM/mg)												
S2	3.42	6.46	2.38	160.00	4.54	14.44	1.32	0.30	9.01	11.80	1.06	2.45
S2B	2.55	5.57	2.12	122.27	3.00	12.87	1.85	0.21	9.85	7.24	0.62	1.60
SP	2.95	10.14	2.94	109.62	2.71	18.77	1.62	0.58	16.51	11.23	0.85	4.08
C1	1.95	3.36	0.25	63.17	1.42	12.45	1.41	0.27	6.06	5.23	0.33	1.92
C2	4.89	4.99	1.51	62.17	2.01	10.80	1.27	0.29	7.11	5.93	0.95	2.75
C3	2.22	2.03	0.71	47.29	1.46	3.10	0.08	0.00	2.41	1.60	0.20	0.67
C4	0.94	0.86	0.21	23.06	0.64	7.48	0.80	0.11	9.08	2.48	0.41	2.65
C5	0.19	0.57	0.05	13.02	0.41	3.41	0.19	0.03	2.34	1.14	0.09	0.74
PC.34.1												
S2	3.42	4.55	2.03	3.54	1.80	6.70	5.45	0.35	130.49	1.12	1.00	
S2B	2.55	1.90	1.25	1.89	1.30	3.49	3.76	0.29	89.97	0.77	0.77	
SP	2.95	2.23	1.67	3.82	1.75	4.35	3.11	0.00	70.29	0.83	0.84	
C1	1.95	1.03	1.35	1.43	0.26	3.14	3.30	0.15	74.19	0.38	0.34	
C2	4.89	1.22	0.86	2.87	1.66	4.50	4.67	0.25	64.31	0.66	0.73	
C3	2.22	0.78	0.64	1.34	0.73	2.22	1.61	0.02	44.01	0.26	0.35	
C4	0.94	0.76	0.55	1.06	0.30	1.55	2.18	0.07	34.36	0.43	0.28	
C5	0.19	0.50	0.23	0.35	0.01	0.97	0.55	0.00	29.16	0.08	0.00	
PC.35.3												
PC.36.0												
PC.37.0												
PC.38.0												
PC.38.1												
PC.16.5												
PC.23.4												
PC.36.1												
PC.36.0												