Can chimerism explain breast/ovarian cancers in \textit{BRCA} non-carriers from \textit{BRCA}-positive families?

Rachel Mitchell\textsuperscript{1*, Lela Buckingham\textsuperscript{2*, Melody Cobleigh\textsuperscript{1}, Jacob Rotmensch\textsuperscript{3}, Kelly Burgess\textsuperscript{1}, Lydia Usha\textsuperscript{1*}}

\textsuperscript{1}Department of Internal Medicine, Division of Hematology, Oncology and Cell Therapy, Rush University Medical Center, Chicago, Illinois, United States of America, \textsuperscript{2}Department of Pathology, Division of Medical Laboratory Science, Rush University Medical Center, Chicago, Illinois, United States of America, \textsuperscript{3}Department of Obstetrics and Gynecology, Division of Gynecologic, Rush University Medical Center, Chicago, Illinois, United States of America

\textsuperscript{*}These authors contributed equally to this work.

\textsuperscript{*}Lydia_usha@rush.edu

Abstract

Hereditary breast and ovarian cancer syndrome (HBOC) is most frequently caused by mutations in \textit{BRCA1} or \textit{BRCA2} (in short, \textit{BRCA}) genes. The incidence of hereditary breast and ovarian cancer in relatives of \textit{BRCA} mutation carriers who test negative for the familial mutation (non-carriers) may be increased. However, the data is controversial, and at this time, these individuals are recommended the same cancer surveillance as general population.

One possible explanation for \textit{BRCA} phenocopies (close relatives of \textit{BRCA} carriers who have developed cancer consistent with HBOC but tested negative for a familial mutation) is natural chimerism where lack of detectable mutation in blood may not rule out the presence of the mutation in the other tissues. To test this hypothesis, archival tumor tissue from eleven \textit{BRCA} phenocopies was investigated. DNA from the tumor tissue was analyzed using sequence-specific PCR, capillary electrophoresis, and pyrosequencing. The familial mutations were originally detected in the patients’ first-degree relatives by commercial testing. The same testing detected no mutations in the blood of the patients under study. The test methods targeted only the known familial mutation in the tumor tissue. Tumor diagnoses included breast, ovarian, endometrial and primary peritoneal carcinoma. None of the familial mutations were found in the tumor samples tested. These results do not support, but do not completely exclude, the possibility of chimerism in these patients. Further studies with comprehensive sequence analysis in a larger patient group are warranted as a chimeric state would further refine the predictive value of genetic testing to include \textit{BRCA} phenocopies.

Introduction

Germline mutations in \textit{BRCA1} or \textit{BRCA2} genes cause over 90\% of hereditary breast and ovarian cancer (HBOC) syndrome [1–3]. \textit{BRCA1} and \textit{BRCA2} proteins play a critical role in repair
of double-stranded DNA breaks and the maintenance of the genome integrity [3, 4]. Gene mutations result primarily in female cancers and an estrogen effect on tissue susceptibility has been postulated [5–7]. Truncating germline \textit{BRCA1} and \textit{BRCA2} (\textit{gBRCA}) mutations are called deleterious or pathogenic and confer an extremely high risk of cancer. The lifetime risk of breast cancer in female \textit{gBRCA} mutation carriers is up to 85%; the lifetime risk of ovarian cancer is up to 50% [8–16]. Some data suggest that the risk of endometrial cancer is also increased in \textit{gBRCA} mutation carriers [17], especially, the risk of uterine papillary-serous carcinoma. If a woman previously diagnosed with breast cancer has a \textit{gBRCA} mutation, she has up to a 65% risk of another breast cancer and a 16% lifetime risk of ovarian cancer [18, 19]. Female \textit{gBRCA} mutation carriers typically present with breast cancer under age 50, but may also present with ovarian, fallopian tube, primary peritoneal, and pancreatic cancer occurring at any age. Because the risk of cancer is so high, it is recommended that these women undergo prophylactic surgery (removal of the breasts, fallopian tubes, and ovaries). If a woman declines surgery, it is recommended to undergo increased surveillance for breast and ovarian cancers which is, however, not as effective as prophylactic surgery at reducing cancer risk and may be not effective at all for ovarian cancer [20–25].

HBOC is inherited in an autosomal dominant pattern, meaning that affected individuals are heterozygous for \textit{gBRCA} mutations. Patients suspected of carrying \textit{gBRCA} mutations based on personal and family histories are recommended to undergo a test involving isolation of DNA from their white blood cells (or saliva) and sequencing of their \textit{BRCA1} and \textit{BRCA2} genes with deletion/duplication analyses.

If a relative of a \textit{gBRCA} carrier has a blood test that is negative for the known familial \textit{gBRCA} mutation, that individual is deemed to have a normal (wild-type) germline \textit{BRCA1} and \textit{BRCA2} genes and is often referred to as "\textit{BRCA} non-carrier" [26]. Generally, there is no recommendation to undergo prophylactic surgery or increased surveillance for cancer. However, some \textit{BRCA} non-carriers do develop breast or ovarian cancers. These individuals are referred to as "\textit{BRCA} phenocopies," meaning that they have the same phenotype (affected by an HBOC-associated cancer) as their relative, but do not have the same genotype (the known \textit{gBRCA} mutation as shown by blood testing). Studies report conflicting results on the relative risk ratio (RR) of breast and ovarian cancers in \textit{BRCA} non-carriers with the breast cancer RR up to 5.1 [27, 28]. Some authors argue that their cancer risk is the same as in the general population [29, 30], some conclude that their risk is the same as in high risk families without identified \textit{gBRCA} mutations [26], but overall most authors agree that their risk is increased [31, 32]. The studies are difficult to compare because they use different methods and are applied to different populations [33]. Currently, the only explanations offered to \textit{BRCA} phenocopies on the cause of their cancers are: 1) their cancers are sporadic; 2) they may have germline mutations in other genes that cause HBOC which have not yet been identified; 3) there are familial environmental factors that lead to their cancer. All of these explanations assume that cancers in \textit{BRCA} non-carriers are not related to the familial \textit{gBRCA} mutation. The risk of \textit{BRCA} non-carriers developing an HBOC is clinically important because it determines their cancer surveillance and prevention recommendations [34].

An alternate explanation for \textit{BRCA} phenocopies that we further explored was natural chimerism. We hypothesized that in at least some \textit{BRCA} phenocopies, breast and ovarian cancer are still caused by familial \textit{BRCA} mutations, but the mutant genes are transmitted through an alternative non-mendelian inheritance, via chimeric cells harboring the mutation rather than through the germline [35, 36]. We further hypothesized that it is these \textit{BRCA}-mutant cells that give rise to breast and ovarian cancers in chimeric individuals as these cells are known to be susceptible to malignant transformation. See Fig 1A. Therefore, the progeny of these cells (the majority of cells in the tumor) would be \textit{BRCA}-mutant as well. This hypothesis gave us a
potential explanation for the existence of BRCA phenocopies but also, the method to test the hypothesis by analyzing the patient's tumor tissue for the known familial mutation. Finding the familial mutation in the patient's tumor would have no other explanation except for chimerism. Even though somatic BRCA mutations are common in breast and especially, in ovarian
cancer, it would be highly improbable to find the same somatic mutation in the patient’s tumor as the germline mutation present in her first-degree relative.

Chimeric or mosaic individuals harbor two different cell populations with different genetic compositions, arising from two different zygotes. For our purposes, “chimeric” will be preferred to “mosaic” as “mosaic” usually refers to cells arising from the same zygote with an acquired mutation in a daughter population.

Chimerism in animals was first reported in 1917 [37]. Human chimerism has been observed in naturally-occurring instances as well as iatrogenic cases (such as transplant recipients and rarely, following blood transfusions) [38]. Here, we focus on naturally-occurring chimerism which is a result of pregnancy.

Several types of naturally-occurring chimerism have been described in humans including tetragametic chimerism (TGC), fetal-maternal microchimerism (FMMc) and maternal-fetal microchimerism (MFMc) [39] (Fig 1). TGC has been observed in twin pregnancies. Occasionally, a twin may die early in pregnancy (“vanishing twin”) resulting in the singleton birth; however, his/her cells may be admixed to the body of the surviving twin [40]. The rate of twinning and chimerism was reported to increase with the wide use of assisted reproduction techniques [41–46]. In contrast to TGC, FMMc and MFMc are thought to be quite common if not ubiquitous. These events could lead to a chimeric individual with tissues of different genotype. In the current study, such chimerism is proposed to explain the lack of predisposing mutations by blood tests, whereas the mutations might be present in tumor tissue.

**Methods**

**Patients and clinical assessment**

Patients for this study were selected based on the absence of a known familial BRCA mutation found in a first-degree relative. Approval for this study was obtained by the Rush University Medical Center Institutional Review Board. After subjects signed an informed consent form, tumor specimens were obtained from the Department of Pathology, Rush University Medical Center (Chicago, IL) and other respective Pathology Departments of institutions where participants had their cancer surgery. Diagnosis of breast, ovarian or endometrial cancer was obtained from pathology reports and histologic evaluation. Clinical data were established from chart review. Patients were eligible if they were affected by HBOC-associated cancer and had previously tested negative for a known familial mutation. Breast cancer patients under 45 or women with ovarian, fallopian tube, primary peritoneal cancer at any age, any male with breast cancer, and any patient with pancreatic cancer were all considered eligible for this study.

**Isolation of DNA**

Hematoxylin and eosin-stained tissue sections cut adjacent to unstained 4 μm were examined by a pathologist. Using the stained slide as a guide, approximately 2mm² of tumor tissue was manually scraped from the slides. The tissue was digested in a solution of 1.0 mg/mL proteinase K (Sigma) in 10mM Tris, pH 8.3, 50 mM KCl. Digestions proceeded overnight at 56 °C. The lysate was used directly for analysis.

**Tumor mutation detection**

Familial mutations included BRCA1 187delAG (a founder mutation in Ashkenazi Jewish population; 2 patients), 1793delA, IVS17+3A>G, 2841G>T, 3109insAA, 5215G>A, 8107G>A, and BRCA2 6794 insA, 5645C>A and 6174delT (another founder mutation in Ashkenazi Jewish population). Tumor tissues were tested using sensitive PCR methods to optimize detection.
Tissue from families with the BRCA1 187delAG mutations were analyzed by amplicon size [47] and pyrosequencing [48]. Amplicon size was also applied to detection of the BRCA1 3109 insAA, and BRCA2 6794 insA mutations using PCR with fluorescently labeled primers and capillary electrophoresis (Table 1). Fluorescent PCR products were resolved by capillary electrophoresis. Fragment size analysis was performed using GeneMapper Software. Synthetic oligomers (Integrated DNA Technologies, Des Moines, IA) were used as controls for all mutations.

BRCA1 187delAG, BRCA1 3109 insAA and BRCA2 6794 insT and 6174delT were tested by PCR with fluorescently labeled primers followed by capillary electrophoresis. The 5’ end of one primer for amplification of each gene mutation in the reaction mix described above was covalently bound to fluorescein. Following PCR, amplicons were diluted 1/400 in formamide and separated by capillary electrophoresis. Peak migration compared to controls was used to determine the presence of deletion or insertion mutations.

Detection of the BRCA1 187delAG mutation was also performed by pyrosequencing. A 110 bp region covering the BRCA1 2 bp deletion was amplified on an ABI9700 Thermal Cycler (ThermoFisher, Waltham, MA). Single strands from the PCR products were isolated and subjected to pyrosequencing on a Q24 Pyrosequencer (Qiagen, Inc, Valencia, CA) using primers and sequence to analyze as previously reported [49].

| Mutation   | Primer Sequences | Product Size (bp) |
|------------|------------------|-------------------|
| BRCA1 IVS17+3A>G | ACTACTCATGTGGTTAGAATGAAAAGCAGTGTG (Forward inner G allele) | 163, 137 |
|            | GCCAGTCTCTCTCTCTCTCTCAGG (Reverse inner A allele) | |
|            | TCTTTTAAATGGATGCTGTTG (Forward outer primer) | |
|            | GCCTGATATTGTCTCTCTCTTCACT (Reverse outer primer) | |
| BRCA2 8107 A>T | GAATGGTGGTTTATAAAATCATAGATCGA (Forward inner A allele) | 92, 80 |
|            | CAGGAGCTGGCCAGTCTATCCATACAA (Reverse inner T allele) | |
|            | GGCTGTAGAATCAAGGCTCTAGTG (Forward outer primer) | |
|            | AGGATCTTATTGAAATCTCTTACC (Reverse outer primer) | |
| BRCA1 5215G>A | AAAACAGATGTGAGTTGGTGTTGACCA (Forward inner A allele) | 227, 155 |
|            | CGCAATTCTAGAAATAATTTTGATGTC (Reverse inner G allele) | |
|            | TGACCCCAGAAGATATTTATGCTGTGTA (Forward outer primer) | |
|            | TCAAGCCCCTGAGACAGTTTCCTGTCTC (Reverse outer primer) | |

https://doi.org/10.1371/journal.pone.0195497.t001

Tissue from families with the BRCA1 187delAG mutations were analyzed by amplicon size [47] and pyrosequencing [48]. Amplicon size was also applied to detection of the BRCA1 3109 insAA, and BRCA2 6794 insA mutations using PCR with fluorescently labeled primers and capillary electrophoresis (Table 1). Fluorescent PCR products were resolved by capillary electrophoresis. Fragment size analysis was performed using GeneMapper Software. Synthetic oligomers (Integrated DNA Technologies, Des Moines, IA) were used as controls for all mutations.

BRCA1 187delAG, BRCA1 3109 insAA and BRCA2 6794 insT and 6174delT were tested by PCR with fluorescently labeled primers followed by capillary electrophoresis. The 5’ end of one primer for amplification of each gene mutation in the reaction mix described above was covalently bound to fluorescein. Following PCR, amplicons were diluted 1/400 in formamide and separated by capillary electrophoresis. Peak migration compared to controls was used to determine the presence of deletion or insertion mutations.

Detection of the BRCA1 187delAG mutation was also performed by pyrosequencing. A 110 bp region covering the BRCA1 2 bp deletion was amplified on an ABI9700 Thermal Cycler (ThermoFisher, Waltham, MA). Single strands from the PCR products were isolated and subjected to pyrosequencing on a Q24 Pyrosequencer (Qiagen, Inc, Valencia, CA) using primers and sequence to analyze as previously reported [49].

BRCA1 IVS17+3A>G, and 5215 G>A and BRCA2 8107A>T were detected using sequence-specific PCR. Four primers were designed by public access software (http://cedar.genetics.soton.ac.uk/public_html/primer1.html). The four primers (Table 1) were used to amplify the isolated DNA in the following amplification reaction mix: 400 nM inner and outer forward and reverse primers, 1X Taq Gold Buffer (Applied Biosystems), 2.5 mM MgCl₂, 320 μM dNTP’s, 0.25 unit AmpliTaq Gold polymerase (Applied Biosystems) and 5μl template DNA in a total volume of 25 μL. For some assays, primer pairs recognizing the mutant and normal sequences were used in separate reactions. The two inner primers from each group of four are designed to end on either the normal or mutated base. If the normal base is present, the inner primer ending with the complement to the normal base will be extended with one of the outer primers to yield a product of the indicated size. For example detection of the BRCA1 IVS17+3A>G, the normal sequence will yield a product of 137 bp. The other inner primer will yield a product of 163 bp if the mutant base is present in the template (Table 1).

BRCA1 2841G>T and BRCA2 5645C>A were analyzed by dye terminator sequencing (Illumina). 50 ng of tumor DNA was subjected to a first round of target selection by PCR, followed by a second round of indexing. Pooled samples were cleaned with magnetic beads, and 12
pmol was loaded into the Illumina MiSeq. Sequence results were screened for coverage (500X) and variant frequency.

**Results.** The eleven patients studied carried diagnoses of infiltrating ductal carcinoma, ductal carcinoma in situ, invasive lobular carcinoma, ovarian adenocarcinoma as well as endometrial and primary peritoneal carcinoma. Patient ages at diagnosis ranged from 26 to 66 years. None of the eleven patient tumors tested displayed the familial BRCA mutation alleles (Table 2). In addition to testing for gBRCA mutations, four patients underwent gene panel testing and were negative for mutations in other cancer-associated genes. Patients 2 and 9 underwent gene panel testing that included 23 genes: ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, and TP53. Patient 3 underwent a more comprehensive gene panel test due to her significant personal and family history of cancer that included 49 genes: APC, ATM, BAP1, BARD1, BRCA1, BRCA2, BRIP1, BMPR1A, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, FH, FLCN, GREM1, MAX, MEN1, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TME127, TP53, TSC1, TSC2, and VHL. Patient 7 underwent a gene panel specific to breast cancer risk that included 14 genes: ATM, BARD1, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, PALB2, PTEN, RAD50, RAD51C, STK11, and TP53. Patient 10 underwent Lynch syndrome testing (MLH1, MSH2, and MSH6) in addition to BRCA1/2 due to a personal history of early onset endometrial cancer. None of the patients had a pathogenic mutation in the other genes tested.

Patient 3 underwent genetic counseling following her sister was found to have a deleterious BRCA1 mutation and was found to be a non-carrier. Despite these findings, she underwent prophylactic bilateral salpingo-oophorectomy with hysterectomy and pathology was negative for occult malignancy. She was diagnosed with primary peritoneal carcinoma 12 months after her prophylactic surgery. See Fig 2 for this patient’s pedigree. Families of patients 1 and 5 carried the BRCA1 187 delAG mutation. Assessment of the tumor tissue from the two phenocopy patients by PCR/capillary electrophoresis and by pyrosequencing revealed no evidence of the mutation (Fig 3A).

Testing for the BRCA1 IVS17+3A>G, 5215 G>A and 8107A>T mutations (tumors from patients 6, 7, 8) was performed by sequence-specific PCR. Peak height patterns of the resulting pyrograms were analyzed to detect the 187 delAG mutations (Fig 3B). In all three cases, the mutation would be detected as the intermediate sized band of three bands on the gel. The BRCA2 8107 A>T mutation (tumor from patient 7) was tested using sequence-specific PCR (Fig 3C). This method was originally designed as a multiplex PCR, however, due to primer competition, individual primer sets for mutant and wild type alleles were used in separate reaction mixes. The amplicons of the two separate reactions for each sample were then mixed and loaded into a single well for electrophoresis. While the product of the A allele was present in the patient sample, the 92 bp T allele seen in the positive control was not. The results in Fig 3B and 3C show no detection of mutations in the phenocopy tumor tissue.

BRCA1 3109 insAA and BRCA2 6794 insA (tumors from patients 10, 11) were assayed using PCR with fluorescently labeled primers and capillary electrophoresis (Fig 3D). These analyses yielded some equivocal results. As shown in Fig 3D, PCR products from the patient DNA migrated in a pattern different from the positive controls (shifted 1–2 bp to the right from the negative control from 74 to 75 or 82 to 84 bp). Migration was partially consistent with the negative control, however, an additional (n-1) product was apparent in both specimens. Subsequent reversible dye terminator sequencing (Illumina) did not detect the respective mutations in either sample.
Table 2. Patient clinical characteristics and family history.

| Patient | Age | Cancer                        | Stage | Ethnicity     | Family History                                                                 | Family Mutation | Patient Mutation identified on additional blood testing | Panel Testing |
|---------|-----|-------------------------------|-------|---------------|--------------------------------------------------------------------------------|-----------------|----------------------------------------------------------|---------------|
| 1       | 47  | Infiltrating ductal carcinoma, ER/PR-, HER2+ | 2     | Ashkenazi     | • Mother, breast (40s), ovarian (70s): BRCA+                                                                                            | BRCA1 187delAG  | No                                                      |               |
| 2       | 58  | Ovarian cancer                | 1     | Mexican       | • Sister, breast (55), ovarian (56): BRCA+                                                                                            | BRCA1 1793delA  | No                                                      |               |
| 3       | 57  | Primary peritoneal cancer     | 4     | German, Irish | • Mother, breast (42)                                                                                                                  | BRCA1 2841G>T   | VUS (MSH2- p.S87C)                                       | Yes           |
| 4       | 57  | Ductal carcinoma in situ      | 0     | Ashkenazi     | • Mother, colon (61) Father, colon (73)                                                                                                | BRCA2 6174delT  | No                                                      |               |
| 5       | 66  | Ductal carcinoma in situ      | 0     | Ashkenazi     | • Mother, breast (70)                                                                                                                  | BRCA1 187delAG  | No                                                      |               |
| 6       | 61  | ILC, ER/PR+, HER2-            | 1     | Danish, Polish, English, Welsh, German                                               | BRCA1 5215G>A   | No                                                      |               |
| 7       | 51  | Infiltrating ductal carcinoma, ER/PR+, HER2- | 2     | Unknown       | • Father, prostate: BRCA+                                                                                                             | BRCA1 8107G>A   | VUS (ATM- c.496+4T>C)                                   | Yes           |
| 8       | 51  | Infiltrating ductal carcinoma | U     | German, Polish | • Mother, ovarian (64) Unaffected sister: BRCA+                                                                                         | BRCA1 IVS17+3A>G | No                                                      |               |

(Continued)
Discussion

The current study addresses the presence of the disease phenocopy in the absence of a familial gene mutation. Genetic testing is most often performed on blood which is an easily accessible, abundant source of high quality DNA. Blood is thought to provide a genotype representative to all other tissues. Rare genetic events early in embryonic development could theoretically result in an individual with different genotypes in different tissues (chimera). In this case, mutations in tissue may not be present in blood. If these mutations are cancer-associated, risk of malignancies in the tissue would be increased, without the presence of the genetic mutation in the blood.

Our study included patients with ovarian, primary peritoneal and endometrial cancers which are uncommon in the general population and less likely to develop sporadically, especially, in families with known HBOC syndrome suggesting an underlying cause for these phenocopies. We tested DNA from eleven tumors from women who come from families carrying BRCA1 and BRCA2 mutations, but who do not carry the familial mutation themselves (phenocopies) as defined by blood testing. Phenocopies are rare but their exact incidence is unknown. Their personal and family history of cancer may suggest a genetic cause for the phenotype and we postulated that they may represent a genetically chimeric state. To investigate this, we looked for the familial mutation for each patient in the tumor tissue. Tumor tissue is not commonly tested for familial mutations. We used highly sensitive PCR methods to determine if the familial mutations might be present in the affected tissues. None of the eleven samples displayed these mutations. This observation does not support that chimerism is responsible for the phenocopies.

This result is concordant with the result of a recent study by Azzollini et al. However, there are significant differences between the studies. Specifically, in their study [49], the authors test the hypothesis of spontaneous reversal of germline BRCA mutations to the wild-type in blood
Maternal Ancestry: German
Paternal Ancestry: Irish
No Ashkenazi Jewish ancestry or consanguinity

This pedigree represents the family history of cancer in patient number 3. The proband, or original patient, is denoted by the arrow. The type of cancer each relative has a history of is denoted below their pedigree symbol. If a relative had genetic testing and was found to carry the known familial BRCA1 mutation, this is also denoted below their pedigree symbol. If a relative tested negative for the known familial BRCA1 mutation, this is also denoted below their pedigree symbol. The proband had full gene sequencing and deletion/duplication analysis of the BRCA1/2 genes; therefore, ‘BRCA1/2-‘ is listed below their pedigree symbol. The proband was also found to carry a variant of uncertain significance in MSH2.

Key
Proband/patient
Ovarian/Peritoneal cancer
Breast cancer
Skin cancer
Leukemia
Deceased
Male
Female
Gender not specified

Fig 2. Patient 3 pedigree. This pedigree represents the family history of cancer in patient number 3. The proband, or original patient, is denoted by the arrow. The type of cancer each relative has a history of is denoted below their pedigree symbol. If a relative had genetic testing and was found to carry the known familial BRCA1 mutation, this is also denoted below their pedigree symbol. If a relative tested negative for the known familial BRCA1 mutation, this is also denoted below their pedigree symbol. The proband had full gene sequencing and deletion/duplication analysis of the BRCA1/2 genes; therefore, ‘BRCA1/2-‘ is listed below their pedigree symbol. The proband was also found to carry a variant of uncertain significance in MSH2.
cells which may account for the negative result of genetic testing performed on blood. To our knowledge, this phenomenon was observed in malignant tissue from germline BRCA carriers not randomly, but rather due to their exposure to cytotoxic chemotherapy (most commonly, platinum agents) [50–52]. This reversal is believed to account for developing platinum resistance in some gBRCA carriers with cancer treated with Carboplatin. In addition, spontaneous reversal of the germline mutations is unlikely to lead to the wild-type gene sequence in the majority of white blood cells. Since genetic testing has a sensitivity of 99% [53] for detection of a mutation, the germline mutation present even in 1% of blood cells should have been still detected. In contrast to the study Azzollini et al, our study analyzed only tumor samples from BRCA phenocopies but not other tissues. Also, for the purpose of our study, BRCA phenocopies were defined as the first-degree relatives of the known BRCA carriers as opposed to less closely related family members.

Finally, our hypothesis is in fact quite different from the hypothesis of Azzollini et al as it is based on the suggestion that an individual can develop a tumor originating from the cells of

Fig 3. Molecular testing in tumor tissue. A. The BRCA1 187del AG deletion heterozygote (families 1 and 5) is detected as an n-2 product by capillary electrophoresis (left) and by an indicative peak pattern by pyrosequencing (top right). Neither the deletion product nor the mutant peak pattern was detected in the patient tumors (bottom panels). B. The heterozygote detected as a 163 bp product by gel electrophoresis. The synthetic oligomer carrying the mutation confirmed the detection of the mutation bymutation sequence specific primers. This band is not present in the negative control nor family 8 DNA (left four lanes). The 137 bp band specific to the normal A allele was detected by primers specific to that allele in the patient sample. Tumor DNA tested for the familial mutation 5215G>A gave similar results (not shown). C. The BRCA2 8107 A—T mutation was tested by sequence-specific PCR. The 92 bp product (T allele, positive) is not present in the patient’s tissue where only the A allele (80bp) is observed. Reagent blanks for the A and T allele primer sets (BI A, BI T) are shown. D. BRCA1 3109 ins AA (left), and BRCA2 6794 insA (patients 10 and 11, respectively; right) mutation analysis by PCR-capillary electrophoresis. Amplified products from DNA without (negative control, top) and with (positive control, middle panels) demonstrate the expected right shift in migration for the BRCA1 3109 insAA n+2 product (76 bp) and the BRCA2 6794 insA n+1 product (82 bp)(bottom panels). Patient samples show an unexpected left shift (n-1) product.
another individual. Cancers, allogeneic in origin, have been observed in other species such as an aggressive devil facial tumor disease in Tasmanian devils and an indolent transmissible venereal tumor in dogs [54]. In humans, rare cases of allogeneic tumors were observed in infants born to mothers with a metastatic malignancy (maternal tumors) [55] and in transplant recipients with suppressed immunity (tumors of donor origin) [56–62]. Even more intriguing is the recent research on the role of fetal-maternal and maternal-fetal microchimerism in human cancers [63–66]. According to these studies, fetal cells are found in maternal tumors many years after pregnancy as low-abundance cells. Their role in carcinogenesis remains unknown. Some studies suggest their protective role in breast [64, 67] and other maternal cancers, while others point towards their unfavorable role in promoting tumorigenesis [66]. However, so far no studies showed that fetal cells can directly give rise to cancer by forming a malignant clone in a mother, although some research suggests that it is possible [68]. Given these considerations, we were planning to test our study participants for chimerism using STR-based (Short tandem repeat genotyping) methods had we demonstrated the presence of the mutation from the family member in their tumor.

Potentially, germline mutations in other cancer-associated genes may account for cancers in BRCA non-carriers. However, in our study, some of the participants underwent multi-gene panel testing looking for mutations in other genes commonly associated with predisposition to breast and ovarian cancers. We did not find any pathogenic germline mutations to explain the HBOC phenotype in BRCA phenocopies. Even though we found two variants of unknown significance (VUS) in the ATM and MSH2 genes, they are likely to be eventually re-classified into benign polymorphisms like most of other VUSes. Pathogenic mutations in the MSH2 gene are associated with Lynch syndrome. Lynch syndrome is known to cause colorectal, endometrial, gastric, ovarian, and urinary tract cancers. Lynch syndrome does not currently have a definitive link to increased risk for breast cancer [69]. Case 3 who had a MSH2 VUS does not have a family history consistent with Lynch syndrome which makes the VUS finding less concerning. Mutations in the ATM gene are associated with an increased risk for many of the same cancers associated with BRCA mutations, such as breast, pancreatic, and prostate. While case 7 has a personal and family history of cancers that could be associated with a germline ATM mutation, most affected relatives tested positive for the familial BRCA mutation, explaining the cancer history in these individuals.

It has been observed that individuals who test negative for a known familial mutation in other cancer-associated genes may still be at an increased risk to develop certain types of cancer. One example is the CHEK2 gene. It has been well-established in multiple studies that germline CHEK2 mutations are associated with an increased risk for breast, colon and other cancers. Current research has shown discrepancies in the level of breast cancer risk in families with CHEK2 mutations suggesting that there are additional factors that influence the risk of breast cancer in these families in addition to the CHEK2 mutation. Therefore, even when a person tests negative for a known familial CHEK2 mutation previously identified in a blood relative, they are still considered to be at an increased risk to develop breast cancer. On the other hand, in Lynch syndrome, the study of non-carrier relatives did not demonstrate an increased cancer risk [70].

Aside from other pathogenic gene mutations in other high or moderate penetrance genes, single nucleotide polymorphisms (SNPs) could be an additional explanation for BRCA phenocopies. A SNP occurs when a single nucleotide differs from the nucleotide seen at this locus in the genome of the general population [71]. SNPs are normal and occur approximately once in every 300 nucleotides. They are population-specific. Typically they are thought to have no effect on a person’s health. However, more recent studies have shown a potential link between certain SNPs and an increased risk of breast cancer. Combinations of SNPs have been
proposed to assess a woman’s risk of breast cancer if she has a family history of breast cancer but no identifiable pathogenic mutation in a cancer-associated gene [72–75]. Penetrance of gBRCA mutations vary in families and populations. Some SNPs have been reported to modify cancer risk in gBRCA mutation carriers [76–77]. It is possible that the same SNPs that increase cancer risk in gBRCA carriers can increase the cancer risk in their non-carrier relatives and thus, account for the phenomenon of BRCA phenocopies.

The BRCA phenocopy phenomenon undermines the value of genetic testing for HBOC for some health care providers and patients alike. Accordingly, some women from the BRCA positive families affected by HBOC make the decision for prophylactic surgery even in the absence of the known familial mutation. Our case 3 is one of the most striking examples. This patient decided to undergo risk-reducing bilateral oophorectomy and hysterectomy despite of the negative blood test for the familial mutation. Nonetheless, she was diagnosed with primary peritoneal carcinoma within a year after the prophylactic surgery.

Conclusion
Hereditary breast and ovarian cancer syndrome most frequently occur through inheritance of mutations in the BRCA1 and BRCA2 genes. Non-carriers in a family with a known mutation in either gene could be at higher risk for cancer but the current recommendations are for that of the general population. Our hypothesis and the hypothesis of Azzollini et al, presumed a link between a familial BRCA mutation and HBOC in BRCA phenocopies, albeit through different mechanisms (chimerism and mosaicism, respectively). Although both studies were negative, which strengthens the conclusion that there is no association between the familial mutation and cancer development in BRCA phenocopies, full tumor sequencing is now possible on tumor cells which may provide further insight into the pathogenesis of the tumors. Currently, the ultimate cause of this phenomenon of phenocopies remains unknown. Further investigation on these tumor specimens with comprehensive sequence analysis of additional five hundred cancer-associated genes by next-generation sequencing is underway. We believe that different approaches to study phenocopies are warranted including investigation of genome-wide associations (GWAS) and SNPs.

Acknowledgments
Heidi Schlehlein (www.scisculpt.com) for images in Fig 1. Peggah Ghoreishi, Rush McCormick Educational Center for images in Fig 3. We are grateful to Mr. and Mrs. Mark Ditsch, Mr. George Ruwe, Douglas and Sarah Criner for their generous contributions that made this research possible.

Author Contributions
Conceptualization: Lela Buckingham, Lydia Usha.
Data curation: Rachel Mitchell, Lela Buckingham, Lydia Usha.
Formal analysis: Lela Buckingham.
Funding acquisition: Lydia Usha.
Methodology: Lydia Usha.
Project administration: Lydia Usha.
Resources: Rachel Mitchell, Melody Cobleigh, Jacob Rotmensch, Lydia Usha.
Software: Kelly Burgess.
Supervision: Lydia Usha.
Visualization: Lydia Usha.
Writing – original draft: Rachel Mitchell, Lela Buckingham, Lydia Usha.
Writing – review & editing: Rachel Mitchell, Lela Buckingham, Kelly Burgess, Lydia Usha.

References
1. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet. 1998; 62(3):676–89. PMID: 9497246
2. Daly MB, Axilbund JE, Buys S, Crawford B, Farrell CD, Friedman S, et al. Genetic/familial high-risk assessment: breast and ovarian. J Natl Compr Canc Netw. 2010 May; 8(5):562–94. PMID: 20495085
3. O’Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis. 2010 Jun; 31(6):961–7. https://doi.org/10.1093/carcin/bgq069 PMID: 20400477
4. Huen MS, Sy SM, Chen J. BRCA1 and its toolbox for the maintenance of genome integrity. Nat Rev Mol Cell Biol. 2010 Feb; 11(2):138–48. https://doi.org/10.1038/nrm2831 PMID: 20029420
5. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell. 2002 Jan 25; 108(2):171–82. PMID: 11832208
6. Zheng L, Annab LA, Afshari CA, Lee WH, Boyer TG. BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. Proc Natl Acad Sci USA. 2001 Aug 14; 98(17):9587–92. https://doi.org/10.1073/pnas.171174298 PMID: 11493692
7. Nosey AM, Gorski JJ, Murray MM, Quinn JE, Chung WY, Stewart GE, et al. Molecular basis for estrogen receptor alpha deficiency in BRCA1-linked breast cancer. J Natl Cancer Inst. 2007 Nov 21; 100(2):1683–94. https://doi.org/10.1093/jnci/djm207 PMID: 1800219
8. Schorge JO, Mosdesit SC, Coleman RL, Cohn DE, Kauff ND, Duska LR, et al. SGO White Paper on ovarian cancer: etiology, screening and surveillance. Gynecol Oncol. 2010 May; 119(2):562–94. PMID: 20400477
9. Satagopan JM, Offit K, Foulkes W, Robson ME, Wacholder S, Eng CM, et al. The lifetime risks of breast cancer in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. Cancer Epidemiol Biomarkers Prev. 2001 May; 10(5):467–73. PMID: 11352856
10. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in BRCA1 mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet 1995 Jan; 56(1):265–71. PMID: 7825587
11. Brose MS, Rebbeck TR, Calzone KA, Stopfer JE, Nathanson KL, Weber BL. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst. 2002 Sep 18; 94(18):1365–72. PMID: 12237282
12. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet 1995 Jan; 56(1):265–71. PMID: 7825587
13. Brose MS, Rebbeck TR, Calzone KA, Stopfer JE, Nathanson KL, Weber BL. Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program. J Natl Cancer Inst. 2002 Sep 18; 94(18):1365–72. PMID: 12237282
14. Thorlacius S, Struwing JP, Hartge P, Olafsdottir GH, Sigvaldason H, Tryggvadottir L, et al. Population-based study of risk of breast cancer in carriers of BRCA2 mutation. Lancet. 1998 Oct 24; 352(9137):1337–9. PMID: 9802270
15. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet. 2003 May; 73(5):1117–30. https://doi.org/10.1086/375033 PMID: 12677558
16. Chen S, Iversen ES, Friel T, Finkelstein D, Weber BL, Eisen A, et al. Characterization of BRCA1 and BRCA2 mutations in a large United States sample. J Clin Oncol. 2006 Feb 20; 24(6):863–71. https://doi.org/10.1200/JCO.2005.03.6772 PMID: 16484695
17. Thompson D, Easton DF, Breast Cancer Linkage Consortium. Cancer Incidence in BRCA1 mutation carriers. J Natl Cancer Inst. 2002; 94(18):1365. PMID: 12237282
18. Malone KE, Begg CB, Haile RW, Borg A, Concannon P, Tellhed L, et al. Population-based study of the risk of second primary contralateral breast cancer associated with carrying a mutation in BRCA1 or...
19. Marcus JN, Watson P, Page DL, Narod SA, Lenoir GM, Tonin P, et al. Hereditary breast cancer: pathology, prognosis, and BRCA1 and BRCA2 gene linkage. Cancer 1996 Feb 15; 77(4):697–709. PMID: 8616762

20. Metcalfe K, Lynch HT, Ghadirian P, Tung N, Olivotto I, Warner E, et al. Contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. J Clin Oncol. 2004 Jun 15; 22(12):2328–35. https://doi.org/10.1200/JCO.2004.04.033 PMID: 15197194

21. Rebbeck TR, Friebele T, Lynch HT, Neuhausen SL, van ’t Veer L, Garber JE, et al. Bilateral prophylactic mastectomy reduces breast cancer risk in BRCA1 and BRCA2 mutation carriers: the PROSE Study Group. J Clin Oncol. 2004 Mar 15; 22(6):1055–62. https://doi.org/10.1200/JCO.2004.04.188 PMID: 14981104

22. Hartmann LC, Sellers TA, Schaid DJ, Frank TS, Soderberg CL, Sitta DL, et al. Efficacy of bilateral prophylactic mastectomy in BRCA1 and BRCA2 gene mutation carriers. J Natl Cancer Inst. 2001 Nov 7; 93(21):1633–7. PMID: 11698567

23. Hartmann LC, Schaid DJ, Woods JE, Crotty TP, Myers JL, Arnold PG, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med. 1999 Jan 14; 340(2):77–84. https://doi.org/10.1056/NEJM199901143400201 PMID: 9867158

24. Heemskerk-Gerritsen BA, Brekelmans CT, Menke-Pluymers MB, van Geel AN, Tilanus-Linthorst MM, Bartels CC, et al. Prophylactic mastectomy in BRCA1/2 mutation carriers and women at risk of hereditary breast cancer: long-term experiences at the Rotterdam Family Cancer Clinic. Ann Surg Oncol. 2007 Dec; 14(12):3335–44. https://doi.org/10.1245/s10434-007-9449-x PMID: 17541692

25. Geiger AM, Yu O, Herrinton LJ, Barlow WE, Harris EL, Rolnick S, et al. A population-based study of bilateral prophylactic mastectomy efficacy in women at elevated risk for breast cancer in community practices. Arch Intern Med. 2005 Mar 14; 165(5):516–20. https://doi.org/10.1001/archinte.165.5.516 PMID: 15767526

26. Domcek SM, Friebele TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010 Sep 1; 304(9):967–75. https://doi.org/10.1001/jama.2010.1237 PMID: 20810374

27. Kurian AW, Gong GD, John EM, Johnston DA, Felberg A, West DW, et al. Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: findings from the Breast Cancer Family Registry. J Clin Oncol. 2011 Dec 1; 29(34):4505–9. https://doi.org/10.1200/JCO.2010.34.4440 PMID: 22042950

28. Smith A, Moran A, Boyd MC, Bulman M, Shenton A, Smith L, et al. Phenocopies in BRCA1 and BRCA2 families: evidence for modifier genes and implications for screening. J Med Genet. 2007 Jan; 44(1):10–5. https://doi.org/10.1136/jmg.2006.043091 PMID: 17079251

29. Metcalfe KA, Finch A, Poll A, Horsman D, Kim-Sing C, Scott J, et al. Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation. Br J Cancer. 2009 Jan 27; 100(2):421–5. https://doi.org/10.1038/sj.bjc.6604830 PMID: 1908722

30. Domcek SM, Gaudet MM, Stopefer JE, Fleischauh MH, Powers J, Kauff N, et al. Breast cancer risks in individuals testing negative for a known family mutation in BRCA1 or BRCA2. Breast Cancer Res Treat. 2010 Jan; 119(2):409–14. https://doi.org/10.1007/s10549-009-0611-y PMID: 19885732

31. Korde LA, Mueller CM, Loud JT, Struwing JP, Nichols K, Greene MH, et al. No evidence of excess breast cancer risk among mutation-negative women from BRCA mutation-positive families. Breast Cancer Res Treat. 2011 Jan; 125(1):169–73. https://doi.org/10.1007/s10549-010-0923-y PMID: 20458532

32. Robson M. Do women remain at risk even if they do not inherit a familial BRCA1/2 mutation? J Clin Oncol. 2011 Dec 1; 29(34):4477–8. https://doi.org/10.1200/JCO.2011.37.6483 PMID: 22042956

33. Fischer C, Engel C, Sutter C, Zachariae S, Schmutzler R, Meindl A, et al. BRCA2 testing: uptake, phenocopies, and strategies to improve detection rates in initially negative families. Clin Genet. 2012 Nov; 82(5):478–83. https://doi.org/10.1111/j.1399-0004.2011.01788.x PMID: 21919902

34. Gronwald J, Cybulski C, Lubinski J, Narod SA. Phenocopies in breast cancer 1 (BRCA1) families: implications for genetic counselling. J Med Genet. 2007 Apr; 44(4):e76. https://doi.org/10.1136/jmg.2006.048462 PMID: 17400795

35. Goldgar D, Venne V, Conner T, Buys S. BRCA phenocopies or ascertainment bias? J Med Genet. 2007 Aug; 44(8):e68; author reply e68. PMID: 17673440

36. Maurel MC and Kanellopoulos-Langevin C. Heredity–venturing beyond genetics. Biol Reprod. 2008 Jul; 79(1):2–8. https://doi.org/10.1095/biolreprod.107.065607 PMID: 18401011

37. Ye Y, Berendine VZ, Hellmich C, Gillespie K. Microchimerism: convert genetics? Int J Mol Epidemiol Genet. 2010; 1(4):350–7. PMID: 21532844
38. Abuelo D. Clinical significance of chimerism. Am J Med Genet C Semin Med Genet. 2009 May 15; 151C(2):148–51. https://doi.org/10.1002/ajmg.c.30213 PMID: 19378333
39. Adams KM, Nelson JL. Microchimerism: an investigative frontier in autoimmunity and transplantation. JAMA. 2004 Mar 3; 291(9):1127–31. https://doi.org/10.1001/jama.291.9.1127 PMID: 14996783
40. Yunis EJ, Zuniga J, Romero V, Yunis EJ. Chimerism and tetragametic chimerism in humans: implications in autoimmunity, alloreognition and tolerance. Immunol Res. 2007; 38(1–3):213–36. PMID: 17917028
41. Lipsker D, Flory E, Wiesel ML, Hanau D, de la Salle H. Between light and dark, the chimera comes out. Arch Dermatol. 2008 Mar; 144(3):327–30. https://doi.org/10.1001/archderm.144.3.327 PMID: 18347288
42. Miura K, Niikawa N. Do monochorionic dizygotic twins increase after pregnancy by assisted reproductive technology? J Hum Genet. 2005; 50(1):1–6. https://doi.org/10.1007/s10038-004-0216-6 PMID: 15599781
43. Williams CA, Wallace MR, Drury KC, Kipersztok S, Edwards RK, Williams RS, et al. Blood lymphocyte chimerism associated with IVF and monochorionic dizygous twinning: Case report. Hum Reprod. 2004 Dec; 19(12):2816–21. https://doi.org/10.1093/humrep/deh533 PMID: 15375077
44. Pinborg A, Lidegaard O, Andersen AN. The vanishing twin: A major determinant of infant outcome in IVF singleton births. Br J Hosp Med (Lond). 2006 Aug; 67(8):417–20.
45. Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. Hum Reprod. 2005 Oct; 20(10):2821–9. https://doi.org/10.1093/humrep/dei142 PMID: 15979998
46. Peters HE, Konig TE, Verhoeven MO, Schats R, Mijatovic V, Ket J, et al. Unusual twinning resulting in chimerism: a systematic review on monochorionic dizygotic twins. Twin Res Hum Genet. 2017 Apr; 20(2):161–8. https://doi.org/10.1017/thg.2017.4 PMID: 28236812
47. Uysal NS, Gulmese C, Celik ZY, Zeyneloglu HB, Yanik FFB. Fetal sex discordance in a monochorionic twin pregnancy following intracytoplasmic sperm injection: A case report of chimerism and review of the literature. J Obstet Gynaecol Res. 2017 Dec 3. https://doi.org/10.1111/jog.13514 [Epub ahead of print] PMID: 29205694
48. Mangold KA, Wang V, Weissman SM, Rubenstein WS, Kaul KL. Detection of BRCA1 and BRCA2 Ashkenazi Jewish Founder Mutations in Formalin-Fixed Paraffin-Embedded Tissues Using Conventional PCR and Heteroduplex/Amplicon Size Differences. J Mol Diagn. 2010; 12:20–6. https://doi.org/10.2353/jmoldx.2010.090023 PMID: 19959799
49. Zhang L, Kirchhoff T, Yee CJ and Offit K. A Rapid and Reliable Test for BRCA1 and BRCA2 Founder Mutation Analysis in Paraffin Tissue Using Pyrosequencing. J Mol Diagn. 2009; 11:176–81. https://doi.org/10.1016/j.jmoldx.2009.080137 PMID: 19324993
50. Azzollini J, Presenti C, Ferrari L, Fontana L, Calvello M, Peissel B, et al. Revertant mosaicism for family mutations is not observed in BRCA1/2 phenocopies. PLoS ONE. 2017; 12(2):e0171663. https://doi.org/10.1371/journal.pone.0171663 PMID: 28199346
51. Hilton JL, Geisler JP, Rathe JA, Hattermann-Zogg MA, DeYoung B, Buller FE. Inactivation of BRCA1 and BRCA2 in ovarian cancer. J Natl Cancer Inst. 2002; 94:1396–406. PMID: 12237285
52. Dhillon KK, Swisher EM, Taniguchi T. Secondary mutations of BRCA1/2 and drug resistance. Cancer Sci. 2011 Apr; 102(4):663–9. https://doi.org/10.1111/j.1349-7006.2010.01840.x PMID: 21205087
53. Sakai W, Swisher EM, Karlson BY, Agarwal MK, Higgins J, Friedman C, et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. Nature. 2008 Feb; 451(7182):1116–20. https://doi.org/10.1038/nature06633 PMID: 18264087
54. The myriad advantage. Myriad Health. 2017. Available from: https://myriad.com/patients-families/genetic-testing-101/the-myriad-advantage/
55. Rinkevich B. Quo vadis chimerism? Chimerism. 2011 Jan-Mar; 2(1):1–5. https://doi.org/10.4161/chim.2.1.14725 PMID: 21547028
56. Jackisch C, Louwen F, Schwenkhagen A, Karbowski B, Schmid KW, Schneider HP, et al. Lung cancer during pregnancy involving the products of conception and a review of the literature. Arch Gynecol Obstet. 2003 Jun; 268(2):69–77. https://doi.org/10.1007/s00404-002-0356-x PMID: 12768292
57. Broestl L, Rubin JB, Dahiya S. Fetal microchimerism in human brain tumors. Brain Pathol. 2017 Sept. https://doi.org/10.1111/bpa.12557 PMID: 28921714
58. von Boehmer H, Draenert A, Jungraithmayr W, Inci I, Niklaus S, Boehler A, et al. Immunosuppression and lung cancer of donor origin after bilateral lung transplantation. Lung Cancer. 2012 Apr; 76(1):118–22. https://doi.org/10.1016/j.lungcan.2011.10.001 PMID: 22088939
59. Fatt MA, Horton KM, Fishman EK. Transmission of metastatic glioblastoma multiforme from donor to lung transplant recipient. J Comput Assist Tomogr. 2008 May-Jun; 32(3):407–9. https://doi.org/10.1097/RICT.0b013e31807674f2 PMID: 18520546

60. Bajaj NS, Watt C, Hajjialidadi D, Gillespie C, Haas AR, Pochettino A, et al. Donor transmission of malignant melanoma in a lung transplant recipient 32 years after curative resection. Transpl Int. 2010 Jul; 23(7):e26–31. https://doi.org/10.1111/j.1432-2277.2010.01090.x PMID: 20444242

61. Kim JK, Carmody IC, Cohen AJ, Loss GE. Donor transmission of malignant melanoma to a liver graft recipient: case report and literature review. Clin Transplant. 2009 Aug-Sep; 23(4):571–4. https://doi.org/10.1111/j.1399-0012.2008.00928.x PMID: 19681978

62. Jonas S, Bechstein WO, Lemmens HP, Neuhaus R, Thalmann U, Nehuhaus P. Liver graft-transmitted glioblastoma multiforme. A case report and experience with 13 multiorgan donors suffering from primary cerebral neoplasia. Transpl Int. 1996; 9(4):426–9. PMID: 8819282

63. Val-Bernal F, Ruiz JC, Cotorruelo JG, Arias M. Glioblastoma multiforme of donor origin after renal transplant: case report and literature review. Clin Transplant. 2009 Aug-Sep; 23(4):571–4. https://doi.org/10.1111/j.1399-0012.2008.00928.x PMID: 19681978

64. Nemescu D, Ursu RG, Nemescu ER, Negura L. Heterogeneous distribution of fetal microchimerism in local breast cancer environment. PLoS One. 2016 Jan; 11(1):e0147675. https://doi.org/10.1371/journal.pone.0147675 PMID: 26808509

65. Cirello V, Fugazzola L. Novel insights into the link between fetal cell microchimerism and maternal cancers. J Cancer Res Clin Oncol. 2016 Aug; 142(8):1697–704. https://doi.org/10.1007/s00432-015-2110-3 PMID: 26746656

66. Gadi VK, Nelson JL. Fetal microchimerism in breast cancer. Cancer Res. 2007 Oct; 67(19):9305–8. https://doi.org/10.1158/0008-5472.CAN-06-4209 PMID: 17900006

67. Kallenbach LR, Johnson KL, Bianchi DW. Fetal cell microchimerism and cancer: a nexus of reproduction, immunology, and tumor biology. Cancer Res. 2011 Jan; 71(1):8–12. https://doi.org/10.1158/0008-5472.CAN-10-0618 PMID: 21199793

68. Gadi VK. Fetal microchimerism in breast from women with and without breast cancer. Breast Cancer Res Treat. 2010 May; 121(1):241–4. https://doi.org/10.1007/s10549-009-0548-1 PMID: 19768535

69. Sawicki JA. Fetal microchimerism and cancer. Cancer Res. 2008 Dec; 68(23):9567–9. https://doi.org/10.1158/0008-5472.CAN-08-4209 PMID: 19074129

70. Genetic/Familial High-Risk Assessment: Breast and Ovarian. National Comprehensive Cancer Network Guidelines Version 1.2018. 3 October 2017. Available from: https://www.nccn.org/professionals/physician_gls/default.aspx#genetics_screening

71. Win AK, Young JP, Lindor NM, Tucker KM, Ahnen DJ, Young GP, et al. Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. J Clin Oncol. 2012 Mar; 30(9):958–64. https://doi.org/10.1200/JCO.2011.39.5590 PMID: 22331944

72. What are single nucleotide polymorphisms (SNPs)? Help me understand genetics-genomic research. Genetics Home Reference. Available from: https://ghr.nlm.nih.gov/primer/genomicresearch.snp

73. Evans DG, Brentnall A, Byers H, Harkness E, Stavrino P, Howell A, et al. The impact of a panel of 18 SNPs on breast cancer risk in women attending a UK familial screening clinic: a case-control study. J Med Genet. 2017 Feb; 54(2):111–3. https://doi.org/10.1136/jmedgenet-2016-104125 PMID: 27794048

74. Cuzick J, Brentnall AR, Segal C, Byers H, Reuter C, Datre S, et al. Impact of a panel of 88 single nucleotide polymorphisms on the risk of breast cancer in high-risk women: results from two randomized tamoxifen prevention trials. J Clin Oncol. 2017 Mar; 35(7):743–50. https://doi.org/10.1200/JCO.2016.69.8944 PMID: 28029312

75. Mavaddat N, Pharoah PD, Michaailidou K, Tyer J, Brook MN, Bolla MK, et al. Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst. 2015 May; 107(5): .

76. Hamdy F, Soucy P, Kuchenbaeck KB, Pastinen T, Droit A, Lemacon A, et al. Association of breast cancer risk in BRCA1 and BRCA2 mutation carriers with genetic variants showing differential allelic expression: identification of a modifier of breast cancer risk at locus 11q22.3. Breast Cancer Res Treat. 2017 Jan; 161(1):117–34. https://doi.org/10.1007/s10549-016-4018-2 PMID: 27796716

77. Kuchenbaeck KB, McGuffog L, Barrowdale D, Lee A, Soucy P, Dennis J, et al. Evaluation of polygenic risk scores for breast and ovarian cancer risk prediction in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst. 2017 Jul; 109(7): .