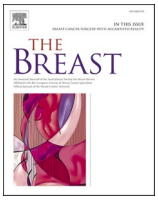
The Breast 73 (2024) 103620

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast



Male breast cancer: No evidence for mosaic *BRCA1* promoter methylation involvement

Mathias Schwartz [a,b,c,*], Sabrina Ibadioune [a,c], Sophie Vacher [a,c], Marie-Charlotte Villy [a,d], Olfa Trabelsi-Grati [a,c], Jessica Le Gall [a,c], Sandrine M. Caputo [a,c], Hélène Delhomelle [a,c], Mathilde Warcoin [a,c], Virginie Moncoutier [a,c], Christine Bourneix [a,c], Nadia Boutry-Kryza [e], Antoine De Pauw [a,c], Marc-Henri Stern [a,c,f], Bruno Buecher [a,c], Emmanuelle Mouret-Fourme [a,c], Chrystelle Colas [a,c], Dominique Stoppa-Lyonnet [a,d,f], Julien Masliah-Planchon [a,c], Lisa Golmard [a,c], Ivan Bieche [a,d]

[a] *Department of genetics, Curie Institute, Paris, France*
[b] *UMR3244, Curie Institute, Paris, France*
[c] *Paris Sciences & Lettres Research University, Paris, France*
[d] *Université Paris Cité, Paris, France*
[e] *Service de génétique, Hospices Civils de Lyon, Lyon, France*
[f] *DNA Repair and Uveal Melanoma (D.R.U.M.), Inserm U830, Paris, France*

ARTICLE INFO

Keywords:
HBOC syndrome
Breast neoplasm
Genetic testing
Homologous recombination
DNA methylation
Epigenomics

ABSTRACT

Breast cancers (BC) are rare in men and are often caused by constitutional predisposing factors. In women, mosaic *BRCA1* promoter methylations (MBPM) are frequent events, detected in 4–8% of healthy subjects. This constitutional epimutation increases risk of early-onset and triple-negative BC. However, the role of MBPM in male BC predisposition has never been assessed. We screened 40 blood samples from men affected by BC, and performed extensive tumour analysis on MBPM-positive patients. We detected two patients carrying MBPM. Surprisingly, tumour analysis revealed that neither of these two male BCs were caused by the constitutional *BRCA1* epimutations carried by the patients.

1. Introduction

Breast cancer (BC) is very rare in men, with less than five cases per million per year [1]. Genetic testing in affected males is recommended as approximately 10 % of cases are caused by germline pathogenic variants (gPV) in *BRCA2,* and another 2 % by gPV in *BRCA1* [2]. In most cases however, no genetic cause is identified.

Constitutional methylation, where methylation of specific genes is detectable in normal tissues, is increasingly being considered as a mechanism for cancer predisposition. For instance, up to 1 % of Lynch syndrome cases could be due to constitutional *MLH1* promoter methylation [3]. For now, constitutional epimutations cannot be used to adapt cancer prevention strategies, but they may help to guide BC screening in the future [4]. Indeed, mosaic *BRCA1* promoter methylations (MBPM) are much more frequent than *BRCA1* gPV in healthy women population (4–8% *versus* around 0.5 %), and several studies have shown that MBPM increase the risk of triple-negative or early-onset BC [5–8,15]. However, there is no consensus about the use of MBPM in clinical practice. Indeed, MBPM occur *in utero* in a subset of cells [9,15]. Thus, the proportion of cells carrying *BRCA1* methylation varies among tissues making follow-up guidelines difficult to establish. This variability has also led to heterogenous results in studies using different techniques to detect MBPM [4]. High-sensitivity techniques targeting core *BRCA1* promoter, such as Methylation-Sensitive High-Resolution Melting (MS-HRM), are recommended [10,11]. Among remaining questions, involvement of MBPM in male BC has never been assessed. We describe here the first male BC patients carrying MBPM.

2. Patients and methods

We selected 40 men affected by invasive BC of no special type between 32 and 85 years old (Table 1). They did not carry germline

* Corresponding author. Department of Genetics, Institut Curie 26 rue d'Ulm, 75005, Paris, France.
E-mail address: mathias.schwartz@curie.fr (M. Schwartz).

https://doi.org/10.1016/j.breast.2023.103620
Received 17 November 2023; Received in revised form 5 December 2023; Accepted 7 December 2023
Available online 10 December 2023

Table 1
Cohort of 40 men affected by breast cancer of no special type. ER: Estrogen Receptors; PR: Progesterone Receptors.

		Number (%)
Age at onset (years)	<40	1 (2.5)
	40–49	2 (5)
	50–59	7 (17.5)
	60–69	13 (32.5)
	70–79	12 (30)
	>80	5 (12.5)
	Total	40 (100)
Immunochemistry	ER +	39 (97.5)
	PR +	33 (82.5)
	Her2 amplification	1 (2.5)
Other cancers	Prostate	6 (15)
	Bladder	1 (2.5)
	Seminoma	1 (2.5)

pathogenic single nucleotide or copy number variant in 37 previously tested cancer-predisposing genes (Supplementary Table 1). All patients were born XY males and self-identified as men. According to French regulation, they had given informed written consent for genetic analysis for research purposes. We performed MS-HRM with EpiMelt *BRCA1* kit (Methyldetect) on DNA extracted from blood samples of each patient.

In MBPM-positive patients, we performed MS-HRM on DNA extracted from buccal swabs and fresh frozen tumour samples, as well as in available blood samples from affected relatives. Genomic tumour analysis consisted in targeted sequencing of more than 500 genes involved in cancer, detection of deletions causing loss of heterozygosity (LOH) at the *BRCA1* locus, and a Shallow Whole Genome Sequencing to count "large-scale genomic alterations" (LGA), *i.e.* copy number variations larger than 10 Mega-bases. Counting LGA is an effective method for detecting homologous recombination deficiency (HRD) [8,12,13]. A tumour is considered HRD with a high level of certainty when it carries more than 20 LGAs.

3. Results

Two patients (5 %) carried MBPM (Fig. 1), a similar proportion as in the non-cancer female population, and much less than in a previous cohort of female family breast/ovarian cancer (44 %) [5,6,8].

Clinically, Patient 1 was diagnosed at age 83 years with a tumour expressing estrogen receptors (ER) and progesterone receptors (PR), without Her2 overexpression. Treatments included mastectomy with lymphadenectomy (4 out of 19 lymph nodes invaded) followed by adjuvant chemotherapy and radiotherapy. He had no clinical recurrence after six years of hormonotherapy. No other personal or family history were noted.

The medical history of Patient 2 included excess weight, diabetes, and arterial hypertension. His BC was diagnosed at age 69 years and expressed ER but not PR, without Her2 overexpression. He was treated by mastectomy with lymphadenectomy (0 of 6 lymph nodes invaded). He could not receive chemotherapy because of his comorbidities, but he underwent adjuvant radiotherapy and maintenance hormonotherapy. No clinical recurrence was noted four years after surgery. Several BCs were reported in his family (Fig. 2).

In Patient 1, MBPM was detected in <1 % of alleles in blood. As expected for a constitutional mosaic event, the proportion of methylated alleles was different in another non-cancerous tissue: ~10 % in buccal mucosal smears. In Patient 2, ~50 % of alleles were methylated in blood, and between 10 % and 50 % in buccal mucosal smears. His female cousin, affected with a bilateral BC at age 45 years and a third BC at age 63 years, was also analysed (Fig. 2). She had no gPV in 7 BC predisposing genes (*BRCA1*, *BRCA2*, *PALB2*, *RAD51C*, *RAD51D*, *CDH1*, *TP53*) and no MBPM detected in blood.

We then explored tumours from Patients 1 and 2. *BRCA1* methylation could be detected in both patients, but at lower levels than in non-cancerous tissues: ~1 % in Patient 1 and ~10 % in Patient 2. These results suggested that tumours did not arise from cells carrying *BRCA1* methylation, and that *BRCA1* methylation was detected from adjacent non-cancerous tissue in those tumour samples. Tumour DNA sequencing showed no genetic variants of interest, and no LOH at the *BRCA1* locus. Contrary to BC from female MBPM carriers, none of the two tumours presented an HRD signature, with only 3 and 9 LGAs in Patient 1 and Patient 2, respectively [8].

The reduced level of *BRCA1* methylation in tumour samples compared to non-cancerous samples, the absence of *BRCA1* LOH, and the absence of HRD signature were consistent with the absence of causality between MBPM and BC in these two patients.

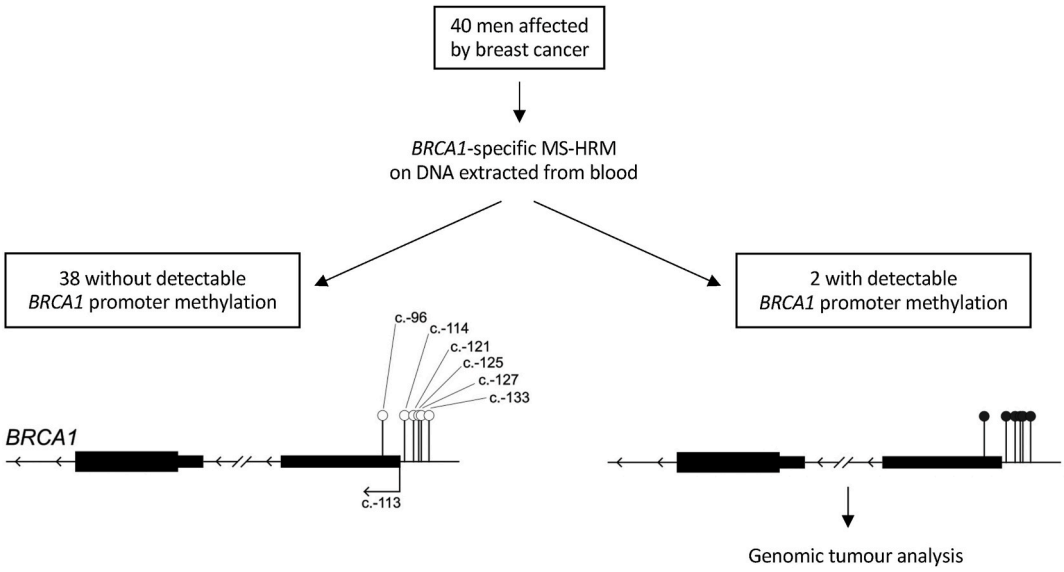


Fig. 1. Study design and graphic representation of *BRCA1* (NM_007294) promoter analysis. *BRCA1*-specific Methylation-Sensitive High-Resolution Melting (MS-HRM) analyse 6 CpGs located in core *BRCA1* promoter, nearby transcription start site. Unmethylated CpGs are represented by empty circles, methylated CpGs are represented by black-filled circles.

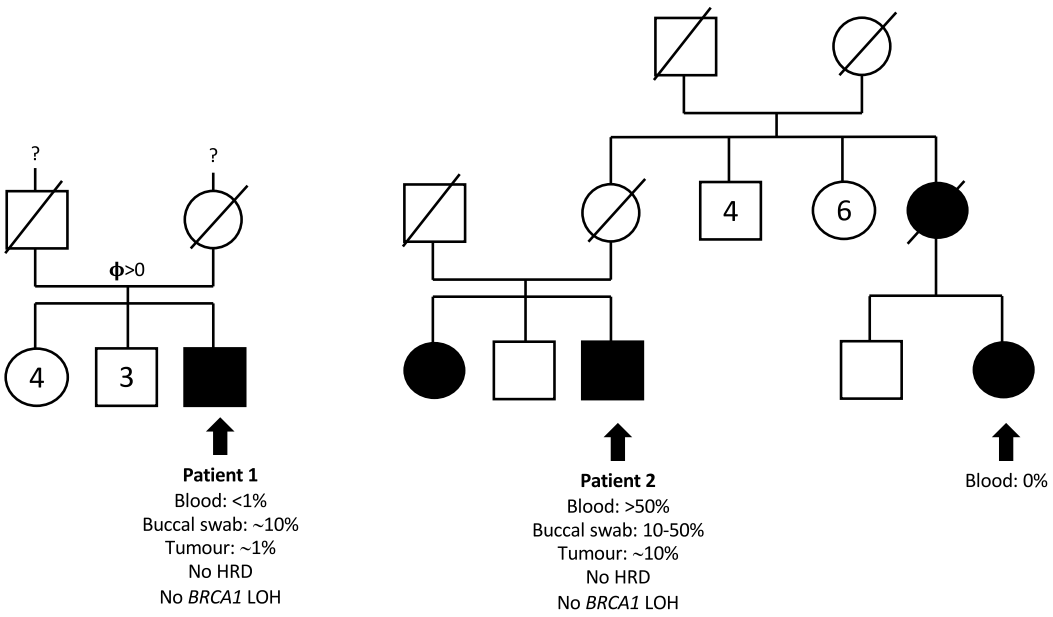


Fig. 2. Pedigrees of the first reported men affected by breast cancer who carry mosaic *BRCA1* promoter methylation. *BRCA1* methylation levels are indicated. Breast cancer patients are shown in black. HRD: homologous recombination deficiency; LOH: loss of heterozygosity.

4. Conclusions and discussion

Overall, this work found no evidence of the involvement of MBPM in male BC predisposition. These negative results appeared surprising regarding the role of MBPM in female BC predisposition. Male and female BCs are molecularly distinct diseases, but they share several predisposing genetic factors, such as gPV in *BRCA1*, *BRCA2*, *PALB2* or *CHEK2* [2]. In women, the relative risk of early-onset BC (before the age of 40 years) is up to 33 for *BRCA1* gPV-carriers, while it is estimated only around 3.5 for MBPM-carriers [6,14]. Consequently, a significant fraction of BCs observed in MBPM-carrying women is not due to MBPM [7]. Here, we report the first two cases of MBPM in males affected by BC. Their BCs were not due to MBPM. Further studies are needed to clarify several pending questions. Larger cohorts could determine if MBPM is a rare predisposition factor that we could not detect in our small population. For instance, MBPM could increase risk of specific rare subtypes of male BC, such as triple-negative BC. Moreover, the role of constitutional epimutations in other genes, such as *BRCA2* or *PALB2*, should be studied in male and female BC predisposition.

Ethics approval

All subjects provided written consent for the use of their samples for genetic studies and research purposes, according to French legislation.

CRediT authorship contribution statement

Mathias Schwartz: Writing - original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Sabrina Ibadioune:** Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Sophie Vacher:** Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marie-Charlotte Villy:** Writing - review & editing, Validation, Resources, Investigation, Data curation. **Olfa Trabelsi-Grati:** Validation, Investigation, Formal analysis. **Jessica Le Gall:** Validation, Investigation, Formal analysis. **Sandrine M. Caputo:** Writing - review & editing, Validation, Resources, Investigation, Data curation. **Hélène Delhomelle:** Validation, Resources, Investigation. **Mathilde Warcoin:** Validation, Resources, Investigation, Data curation. **Virginie Moncoutier:** Validation, Formal analysis, Data curation. **Christine Bourneix:** Validation, Investigation, Formal analysis. **Nadia Boutry-Kryza:** Writing - review & editing, Validation, Resources, Formal analysis. **Antoine De Pauw:** Validation, Resources, Formal analysis. **Marc-Henri Stern:** Writing - review & editing, Investigation, Conceptualization. **Bruno Buecher:** Writing - review & editing, Validation, Resources, Investigation. **Emmanuelle Mouret-Fourme:** Validation, Resources, Investigation, Data curation. **Chrystelle Colas:** Validation, Supervision, Resources, Investigation. **Dominique Stoppa-Lyonnet:** Writing - review & editing, Validation, Supervision, Resources, Project administration. **Julien Masliah-Planchon:** Writing - review & editing, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Lisa Golmard:** Writing - review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Ivan Bieche:** Writing - review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Thank to A.D.T. International for proofreading the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2023.103620.

References

[1] Miao H, Verkooijen HM, Chia K-S, Bouchardy C, Pukkala E, Larønningen S, et al. Incidence and outcome of male breast cancer: an international population-based Study. J Clin Orthod 2011;29:4381–6. https://doi.org/10.1200/JCO.2011.36.8902.

[2] Campos FAB, Rouleau E, Torrezan GT, Carraro DM, Casali da Rocha JC, Mantovani HK, et al. Genetic landscape of male breast cancer. Cancers 2021;13:3535. https://doi.org/10.3390/cancers13143535.

[3] Hitchins MP, Alvarez R, Zhou L, Aguirre F, Dámaso E, Pineda M, et al. MLH1-methylated endometrial cancer under 60 years of age as the "sentinel" cancer in

female carriers of high-risk constitutional MLH1 epimutation. Gynecol Oncol 2023; 171:129–40. https://doi.org/10.1016/j.ygyno.2023.02.017.
[4] Wong EM, Southey MC, Terry MB. Integrating DNA methylation measures to improve clinical risk assessment: are we there yet? The case of BRCA1 methylation marks to improve clinical risk assessment of breast cancer. Br J Cancer 2020;122: 1133–40. https://doi.org/10.1038/s41416-019-0720-2.
[5] Lønning PE, Nikolaienko O, Pan K, Kurian AW, Eikesdal HP, Pettinger M, et al. Constitutional *BRCA1* methylation and risk of incident triple-negative breast cancer and high-grade serous ovarian cancer. JAMA Oncol 2022. https://doi.org/10.1001/jamaoncol.2022.3846.
[6] Wong EM, Southey MC, Fox SB, Brown MA, Dowty JG, Jenkins MA, et al. Constitutional methylation of the *BRCA1* promoter is specifically associated with *BRCA1* mutation-associated pathology in early-onset breast cancer. Cancer Prev Res 2011;4:23–33. https://doi.org/10.1158/1940-6207.CAPR-10-0212.
[7] Prajzendanc K, Domagała P, Hybiak J, Ryś J, Huzarski T, Szwiec M, et al. *BRCA1* promoter methylation in peripheral blood is associated with the risk of triple-negative breast cancer. Int J Cancer 2020;146:1293–8. https://doi.org/10.1002/ijc.32655.
[8] Schwartz M, Ibadioune S, Chansavang A, Vacher S, Caputo SM, Delhomelle H, et al. Mosaic BRCA1 promoter methylation contribution in hereditary breast/ovarian cancer pedigrees. J Med Genet 2023. https://doi.org/10.1136/jmg-2023-109325. jmg-2023-109325.
[9] Lønning PE, Berge EO, Bjørnslett M, Minsaas L, Chrisanthar R, Høberg-Vetti H, et al. White blood cell *BRCA1* promoter methylation status and ovarian cancer risk. Ann Intern Med 2018;168:326. https://doi.org/10.7326/M17-0101.
[10] Wojdacz TK, Dobrovic A. Methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation. Nucleic Acids Res 2007;35:e41. https://doi.org/10.1093/nar/gkm013. –e41.
[11] Machaj F, Sokolowska KE, Borowski K, Retfiński S, Strapagiel D, Sobalska-Kwapis M, et al. Analytical sensitivity of a method is critical in detection of low-level BRCA1 constitutional epimutation. Sci Rep 2023;13:16102. https://doi.org/10.1038/s41598-023-43276-7.
[12] Brugge P. Homologous recombination deficiency derived from whole-genome sequencing predicts platinum response in triple-negative breast cancers. Nat Commun 2023;14:1958. https://doi.org/10.1038/s41467-023-37537-2.
[13] Eeckhoutte A, Houy A, Manié E, Reverdy M, Bièche I, Marangoni E, et al. ShallowHRD: detection of homologous recombination deficiency from shallow whole genome sequencing. Bioinformatics 2020;36:3888–9. https://doi.org/10.1093/bioinformatics/btaa261.
[14] Antoniou A, Pharoah PDP, 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;72:1117–30. https://doi.org/10.1086/375033.
[15] Nikolaienko O, Eikesdal HP, Ognedal E, Gilje B, Lundgren S, Blix ES, et al. Prenatal BRCA1 epimutations contribute significantly to triple-negative breast cancer development. Genome Med 2023;15:104. https://doi.org/10.1186/s13073-023-01262-8.