Germline pathogenic variants in metaplastic breast cancer patients: a monocentric study and literature review

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Abstract

Metaplastic breast cancer (MpBC) is a rare, aggressive type of breast cancer, often classified as triple negative (TN). Scarce information is available about germline testing in MpBC. We retrospectively reviewed MpBC patients counseled at our Institute and found to harbor germline pathogenic variants (PVs), and we revised literature data. We identified a germline PV in 15 MpBC patients: 13 in *BRCA1* (86.7%), one in *TP53* (6.7%), one in *MLH1* (6.7%) genes. Eight MpBC PV carriers in *BRCA1* have been previously described, including a patient with a PV in both *BRCA1* and *TP53*. MpBC histological subtype in PV carriers was heterogeneous. All MpBCs were TN but 13.3% in our series showed HER2 overexpression.

We described the largest series of MpBCs with germline PVs. As previously reported, we observed that *BRCA1* is the mainly involved gene in MpBC patients who underwent germline testing according to specific selection criteria. Additional studies on unselected patients are required to assess the authentic role of germline *BRCA1* PVs in MpBCs and to explore the possible involvement of other genes in MpBC predisposition. Unraveling a specific MpBC molecular landscape is a starting point for the definition of new therapeutic strategies, since these tumors have a poor prognosis.

Introduction

Metaplastic breast cancer (MpBC) is an aggressive malignancy characterized by the presence of two or more cell types, most commonly an admixture of epithelial and mesenchymal elements [1]. MpBC is a rare condition, accounting for about 0.2-5% of all breast cancers (BCs) [2]. However, it carries the worst prognosis in comparison to other BC types and plays a significant role in global BC mortality [3]. Indeed, almost all MpBCs are classified as triple-negative breast cancers (TNBCs), meaning that they are characterized by ≤ 1% nuclear expression of hormone receptors (HR) and HER2 negative status, with or without gene amplification [4–5]. TNBC is overall more aggressive than HR positive BC, being responsible for 5% of all cancer-related deaths annually [6]. Despite the recent achievements in the treatment of BC, the current preferred approach for TNBC remains prevalently chemotherapy-based [7], or nothing in some low-risk TNBCs [8]. The majority of TNBCs (95%) are histologically classified as high-grade invasive carcinomas of no special type (NST) and tend to spread very early to lymph nodes and/or distant organs [9–10]. Also, MpBC is tipically high-grade but has a more aggressive behavior compared to TNBC with NST, showing great propensity for recurrence and specific chemoresistance, in particular in neo-adjuvant settings [11–12].

The TNBC phenotype appears with an aggressive pattern [13] and to be closely associated with a hereditary cause of the disease [14], most frequently the Hereditary Breast and Ovarian Cancer (HBOC) syndrome. HBOC is an autosomal dominant condition caused by germline mutations in *BRCA1* and *BRCA2* genes. The latest National Comprehensive Cancer Network (NCCN) clinical practice guidelines recommend *BRCA1/2* genetic testing for all TNBC patients aged ≤ 60 years [15]. More specifically, *BRCA1* mutation carriers are more likely to develop TNBC than *BRCA2* carriers [16]. In addition to HBOC, early-onset BC is a well-known phenotype also in Li Fraumeni syndrome (LFS), a rare hereditary disorder due to germline mutations in *TP53* gene and responsible for epithelial and mesenchymal tumors. Accordingly, genetic testing of *TP53* gene is recommended in all patients diagnosed with BC under the age of 31 years, regardless of family history [17].

As far as MpBC is concerned, only a few studies have been published exploring the association with germline PVs in cancer-related genes. On the whole, due to its rarity, histological diversity and aggressive nature, scarce information is available about genetic predisposition to MpBC. In this study, we revised literature data on MpBC genetic predisposition and we describe our series of MpBC patients found to carry germline PVs.

Methods

In the present study, we retrospectively searched for patients affected by MpBC who were addressed to genetic counseling at the Division of Cancer Prevention and Genetics of the European Institute of Oncology (IEO) between 2002 and 2022. They underwent germline genetic testing for one or more genes (including *BRCA1*, *BRCA2*, *TP53*, and others) according to their personal/family history of cancer and were found to be carriers of a PV. Histological diagnosis had been performed or reviewed at IEO by pathologists with extensive experience in breast cancer. Variant pathogenicity was assessed based on the ACMG guidelines and using the *ClinVar* database (https://www.ncbi.nlm.nih.gov/clinvar/). Personal and family history, clinical, histopathological and genetic data were collected and stored in a dedicated institutional database. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the European Institute of Oncology (UID 0833, date of approval 23/05/2018). Informed consent was obtained from all subjects involved in the study.

We then compared our series of MpBC PV carriers with those reported in the literature. We performed a PubMed search (up to Dec 31, 2022) of the following keywords in the title/abstract of the articles: [germline variant OR germline mutation] AND [metaplastic breast cancer]. Additional papers were identified by a manual search of references from original articles and reviews. Studies not reporting molecular details of the germline PVs identified in MpBCs and/or family history (FH) of breast/ovarian cancers and/or not specifying the selection criteria used to address MpBC patients to genetic testing were excluded from this review.

Results

We identified 15 female MpBC carriers of a germline PV: 13 in *BRCA1* (86.7%), one in *TP53* (6.7%) and one in *MLH1* (6.7%), as shown in Table 1. All variants were classified as pathogenic (C5) or likely pathogenic (C4). PVs were defined as follows: seven small deletions, two small insertions, two large rearrangements, three nonsense, and one missense. All PVs in *BRCA1* and *TP53* genes were truncating (*i.e.*, predicted to result in a truncated protein product), while the one in *MLH1* gene was a missense substitution.

Mean age at MpBC diagnosis was 41.5 years (range 27–58). MpBCs were histologically heterogeneous, with the majority showing mesenchymal differentiation (47%) or displaying squamous features (27%). Notably, the *TP53* PV carrier developed MpBC with sarcomatoid elements. All patients were diagnosed with a TNBC, except for two cases of HER2 overexpressed BC.

Nine out of 15 patients (60%) were also diagnosed with other tumors, before or after MpBC: eight *BRCA1* PV carriers developed other BCs, while the *MLH1* PV carrier was also affected by colorectal cancer.

Eight patients showed family history of BC and/or OC (53%), while the remaining seven patients (47%) did not report any relative affected by BC or OC. Pedigrees of the 15 MpBC patients of our series are shown in Fig. 1.

We also performed a detailed literature search to identify previous papers about germline PVs in MpBC, describing also family history of BC/OC and selection criteria used for genetic testing. Eight case reports have been published so far on MpBC patients harboring germline PVs [18–25]. All of them were found to carry a C5 variant in *BRCA1* gene; notably, one was also carrier of a C4 splicing variant in *TP53* gene (Table 2).

The nucleotide changes in *BRCA1* gene included five small deletions or insertions leading to frameshift, one nonsense mutation, one large deletion and one missense substitution. Except for this last variant, all PVs were expected to result in a premature termination of the protein.

Mean age at MpBC diagnosis of PV carriers was 30 years (range 15–49). All BCs were of TN subtype; however, the histological subtype of MpBC varied greatly among patients.

Five out of eight cases (62.5%) were affected by other BCs in addition to the MpBC: all of them were invasive ductal carcinomas but the patient described by Breuer *et al*., who was diagnosed with a metachronous bilateral MpBC with squamous differentiation.

A positive family history of BC and/or OC was described in five out of eight (62.5%) of the screened MpBC cases.

Discussion

To the best of our knowledge, we report here the largest monocentric series of MpBC patients harboring a germline PV and fully described in terms of clinical and molecular characteristics, as well as family history of BC/OC. Indeed, only eight single case reports have been published so far with all information. These eight MpBC cases reported in the literature carried PVs almost exclusively in *BRCA1* gene, except for the unusual case of a woman affected by both HBOC and LFS [25]. An additional 54-year-old woman affected by TN MpBC with osseous differentiation has been reported [26]: a genetic alteration in *BRCA1* was identified in the tumor (c.1530del, p.Gly511AlafsTer21), but the patient refused to undergo germline testing and she did not have any family history of malignancies.

In addition to these case reports, systematic review of the existing literature revealed several other studies concerning germline PVs of different cancer genes in MpBC patients. However, they did not include molecular details about the detected variants and/or on FH of cancer, and/or did not specify which selection criteria they used to address patients to genetic testing. Therefore, we decided not to include them in our review and we only cite them here.

In a recent paper on neoadjuvant chemotherapy in MpBC, Wong *et al*. (27) reported that two out of 31 (6%) MpBC patients who underwent genetic testing were found to carry a *BRCA1* germline mutation, one of which being a large deletion of exons 23–24. Additional studies on target therapies or specific clinical management for *BRCA*-positive MpBC patients have also been published [28–29]. Moukarzel et al. [30] identified Homologous Recombination DNA Repair deficiency (HRD) in 15 out of 33 MpBCs (45%). Of relevance, six of the 15 HRD-defective cases harbored a germline PV in *BRCA1* (6/15 = 40%) and one in *BRCA2* (1/15 = 7%).

Moreover, eight additional case reports described MpBC development in patients with a clinical diagnosis of Neurofibromatosis type 1 [31–38]. However, only one of these patients underwent detailed germline analysis and proved to be carrier of a deleterious variant in *NF1* gene [38]. Finally, Rodríguez-Fernández and colleagues [39, 40] found a statistically significant difference in frequencies of histological types between *BRCA1* carriers vs. non carriers. In particular, they reported 3.2% MpBCs in 93 *BRCA1*-positive BCs *vs.* 0.8% MpBCs in 3157 non *BRCA*-positive BCs.

Taken together, all these evidences from case reports, retrospective reviews and studies on target therapies point to a possible involvement in MpBC genetic predisposition for different cancer-related genes, but mainly for *BRCA1* gene.

Our results are consistent with literature data: in a series of 15 MpBC patients, the great majority of the identified germline PVs lied in the *BRCA1* gene (86.7%), confirming that it could have a crucial role in MpBC predisposition. We also identified a *TP53* PV carrier without any alteration in *BRCA1* gene. The patient was diagnosed with multiple BCs over time, including a TN MpBC with sarcomatoid features at 52 years of age (Fig. 1, patient ID 14). Interestingly, no FH suggestive for LFS syndrome was observed, even if family was relatively small.

Of relevance, we also describe for the first time a germline C4 variant of *MLH1* gene in a patient who developed both CRC at 51 and MpBC at 58 years of age (Fig. 1, patient ID 15). Constitutive mutations of mismatch repair genes (including *MLH1*, *MSH2*, *MSH6* and *PMS2*) are known to cause the Lynch syndrome (LS), an inherited condition characterized by an increased risk of developing many tumor types, mainly colorectal and endometrial cancers [40–41]. Whether BC belongs to the LS spectrum is a long-standing question [42–43]; at any rate, *MLH1* germline defects have never been reported before in MpBC patients. Proband's father was affected by both CRC and pancreatic cancer at 72 years of age, and grandmothers from both paternal and maternal sides of the family were reported to be diagnosed with BC (at 40 and 65 years, respectively). Notably, the patient also harbored other two genetic variants (class C3 according to the ACMG classification): the c.5986G > A (p.Ala1996Thr) variant in *BRCA2* gene and the c.3313G > A (p.Gly1105Arg) in *MSH6* gene.

We cannot exclude that the missense substitution in *BRCA2* could act as a low penetrance variant for BC risk nor that additional genetic/environmental factor could modulate BC risk in this family.

Almost all PVs detected in MpBC patients were expected to result in a truncated protein product, but the c.181T > G C5 missense variant of *BRCA1* and the c.375 + 2T > C C4 splicing variant of *TP53* reported in the literature, and the c.244A > G C4 missense variant of *MLH1* gene described in our series. The most common mutation types were small deletions/insertions leading to frameshift, followed by nonsense.

The germline PVs we identified were different compared with those already reported, except for the *BRCA1* c.5266dup variant, described both in our series and in a previous report [24]. In addition, we identified the c.5030_5033del *BRCA1* variant in two different MpBC patients. These variants are among the most frequent mutations detected in *BRCA1* gene, in Europe or worldwide [44].

A potential limitation of our study is that we focused on molecular findings only in a small group of patients, making it difficult to perform statistical analysis or extrapolation to other individuals with MpBC.

At any rate, no hotspot mutations specific for MpBC seem to be identifiable in *BRCA1* gene. Several PVs clustered in exon 11 of *BRCA1*, just because it is the largest one of the gene (3426 base pairs). On the whole, the germline variants found in MpBC patients – both in our series and in published case reports - fall within the three regions most frequently mutated in cancer patients. These include the RING (Really Interesting New Gene) domain (exons 2–7), a region encoded by exons 11–13, and the BRCT (BRCA1 C-terminus) domain (exons 16–24) [Clark 2012] (Fig. 2). RING is responsible for the interaction of *BRCA1* with *BARD1.* The region encoded by exons 11–13 contains multiple binding sites for a number of diverse proteins; it includes for example the SCD (serine containing domain), which mediates interaction with *PALB2.* Finally, the BRCT domain is again critical for tumor suppression, since its main function is modulating phosphoprotein interactions between *BRCA1* and proteins phosphorylated by *ATM* and *ATR*, two kinases activated by DNA damage.

As regards histological classification, MpBCs of PV carriers reported in the literature were extremely heterogeneous, displaying epithelial, mesenchymal and mixed features. Our series showed the same variability: although the most prevalent phenotype was MpBC with mesenchymal differentiation (47%), we did not observe a specific histological subtype in patients with *BRCA1* PVs. Notably, the *TP53* PV carrier developed MpBC with sarcomatoid elements. All the eight MpBCs described in previous case reports were of TN subtype, as expected from literature data. In our series, TN phenotype was the most frequent subtype as well (86.7%), even though 13.3% of MpBCs showed HER2 overexpression (one with a *BRCA1* germline PV and the other with the germline *MLH1* PV).

In the eight case reports reviewed, family history of *BRCA*-positive MpBC cases was ascertained: three of them were apparently sporadic, three presented positive FH of BC, one of OC, and one of both BC and OC.

Our case series reflects literature data, since about 50% of MpBC cases seem to be sporadic. However, it should be stressed that these are highly selected cases, addressed to genetic counselling and germline testing due to specific clinical criteria, which include but are not limited to FH (*i.e.* development of early-onset BC and/or of TNBC ≤ 60 years and/or of bilateral BC and/or presence of family history of BC/OC).

Additional studies on larger unselected series of cases are required to better characterize MpBC genetic predisposition. These studies, if conducted on unselected MpBC patients and through careful data collection (family history, histological classification, etc.), are expected to unravel the authentic detection rate of PVs in *BRCA1* gene, as well as to explore the possible involvement of additional genes in increasing the risk of developing MpBC.

There are multiple evidences suggesting that MpBC and TNBC of NST are two independent and extremely heterogeneous BC subtypes, particularly in their clinical manifestations. Such different features require the urgent definition for new systemic therapeutic strategies. Further studies are needed to uncover the specific genetic landscape of MpBCs, since it would be an interesting starting point for the definition of new therapeutic strategies.

Conclusions

Taken together, our findings and literature data point to the following conclusions: 1) in MpBC patients described so far, who are highly selected for germline testing of specific genes, *BRCA1* seems to play a crucial role in increasing the risk of MpBC development; 2) additional studies on larger, unselected series of patients are necessary to elucidate the authentic role of germline *BRCA1* PVs in MpBCs and to explore the possible implication of other genes in MpBC predisposition; 3) almost all PVs detected in MpBC carriers were clearly deleterious and predicted to result in a truncated protein product; 4) no hotspot mutations specific for MpBC seem to be detectable in *BRCA1* gene, since the identified PVs are frequently found in HBOC patients or affect the most commonly mutated regions of the protein in cancer patients; 5) *BRCA1* PVs do not seem to be associated with a specific histological subtype of MpBC; 6) almost all MpBCs in PV germline carriers are TN, with a few exceptions of HER2 overexpressed cases; 7) unraveling MpBC genetic predisposition and its specific molecular landscape is important for the clinical management of affected patients and families and could be a starting point for the definition of new therapeutic strategies.

Declarations

Author Contributions: Concept and design, XX; Supervisor board, XX; Iconography and graphic design, XX; Acquisition of data, analysis, and interpretation of data, critical revision of the manuscript for important intellectual content, final approval of manuscript-all authors. Drafting of the manuscript, XX, with input of all authors. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1. Germline PVs, family history and clinical, histological characteristics of our series of MpBC patients.

Patient ID	Gene	Germline defect[a]	Location[b]	Variant description	Age at diagnosis	Laterality	Histopathological Classification[C]	Sub-type	Other tumors (age)	FH[d]
1	*BRCA1*	c.(?_-119)_(80+1_81-1)del p.(?)	5'UTR, exons 1 and 2	Large rearr.	44	Left	Squamous cell carcinoma	TN	Right IDC (40); left IDC (42)	no
2	*BRCA1*	c.798_799del p.(Ser267LysfsTer19)	Exon 11	Deletion	36	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (matrix-producing)	TN	Right IDC (24)	no
3	*BRCA1*	c.816_825dup p.(Thr276AlafsTer14)	Exon 11	Insertion	37	Left	Squamous cell carcinoma	TN	_	BC/OC
4	*BRCA1*	c.2934T>G p.(Tyr978Ter)	Exon 11	Non-sense	48	Right	Squamous cell carcinoma	TN	Left IDC (30); right IDC (37)	BC
5	*BRCA1*	c.3904G>T p.(Glu1302Ter)	Exon 11	Non-sense	47	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (matrix-producing carcinoma)	TN	_	BC
6	*BRCA1*	c.3228_3229del p.(Gly1077AlafsTer8)	Exon 11	Deletion	35	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (chondroid)	TN	Right IDC (39)	no
7	*BRCA1*	c.1088del p.(Asn363IlefsTer11)	Exon 11	Deletion	52	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (matrix-producing carcinoma)	TN	_	no
8	*BRCA1*	c.4186-3156_4357+94dup p.(?)	Exon 13	Large rearr.	47	Right	Spindle cell carcinoma	TN	_	no
9	*BRCA1*	c.4964_4982del p.(Ser1655TyrfsTer16)	Exon 16	Deletion	40	Left	Mixed metaplastic carcinoma	HER2+	Left DCIS (40)	BC
10	*BRCA1*	c.5030_5033del p.(Thr1677IlefsTer2)	Exon 17	Deletion	40	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (matrix-producing carcinoma)	TN	Right IDC (35)	OC
11	*BRCA1*	c.5030_5033del p.(Thr1677IlefsTer2)	Exon 17	Deletion	28	Left	Mixed metaplastic carcinoma	TN	_	no
12	*BRCA1*	c.5179A>T p.(Lys1727Ter)	Exon 19	Non-sense	27	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (matrix-producing carcinoma and chondroid)	TN	_	BC
13	*BRCA1*	c.5266dup p.(Gln1756ProfsTer74)	Exon 20	Insertion	32	Right	Spindle cell carcinoma	TN	Left IDC (33)	BC/OC
14	*TP53*	c.635_636del p.(Phe212SerfsTer3)	Exon 5	Deletion	52	Left	Spindle cell carcinoma	TN	Left IDC (36, 39, 54);	no

									right IDC (37); right DCIS (45, 47); multiple BCCs	
15	*MLH1*	c.244A>G p.(Thr82Ala)	Exon 3	Missense	58	Left	Metaplastic carcinoma with heterologous mesenchymal differentiation (rhabdomyoid)	HER2+	CRC (51)	BC

BC: breast cancer; BCC: basal cell carcinoma; CRC: colorectal cancer; DCIS: ductal carcinoma in situ; FH: family history; IDC: infiltrating ductal carcinoma; MpBC: metaplastic breast cancer; N/A: not applicable; NOS: not otherwise specified; OC: ovarian cancer; TN: triple negative.

[a]cDNA and protein changes are named according to HGVS nomenclature. Reference sequences: *BRCA1* NM_007294.4; *TP53* NM_000546.5; *MLH1* NM_000249.4

[b]After exon 3, subsequent exon numbers of *BRCA1* gene are increased by one, due to historical misannotation of an additional "exon 4"

[c]Classification into seven sub-categories following the WHO guidelines [1; 3]

[d]Family history of BC or OC in 1st and 2nd degree relatives on the maternal side or until 3rd degree relatives on the paternal side (pathology-confirmed or self-reported cancer diagnoses)

Table 2. Germline PVs, family history and clinical, histological characteristics of MpBC patients reported in the literature.

Reference	Gene	Germline defect[a]	Location[b]	Variant description	Age at diagnosis	Laterality	Histology	Sub-type	Other tumors (age)	FH[c]
Rashid 2011	*BRCA1*	c.68_69del p.(Glu23ValfsTer17)	Exon 2	Deletion	22	Left	Mixed metaplastic carcinoma (epithelial and mesenchymal components)	TN	_	No
Nöel 2010	*BRCA1*	c.66dup p.(Glu23ArgfsTer18)	Exon 2	Insertion	49	Right	Low-grade adenosquamous carcinoma	TN	Right IDC (36)	No
Breuer 2007	*BRCA1*	c.181T>G p.(Cys61Gly)	Exon 5	Missense	25	Right	Squamous cell carcinoma	TN	Left MBC with squamous cells (28)	BC
Yamashita 2021	*BRCA1*	c.188T>A p.(Leu63Ter)	Exon 5	Non-sense	39	Left	Mixed metaplastic carcinoma (invasive ductal carcinoma and mesenchymal component with chondroid differentiation)	TN	Left IDC (39)	BC/OC
Vohra 2022	*BRCA1*	c.1961dup p.(Tyr655ValfsTer18)	Exon 11	Insertion	15	Right	Metaplastic carcinoma with heterologous mesenchymal differentiation	TN	_	No
Ghilli 2017	*BRCA1*	c.4754_4755del p.(Pro1585ArgfsTer36)	Exon 16	Deletion	35	Right	Mixed metaplastic carcinoma (epithelial and mesenchymal components	TN	Right IDC (35)	BC
Suspitsin 2011	*BRCA1*	c.5266dup p.(Gln1756ProfsTer74)	Exon 20	Insertion	35	Left	Mixed metaplastic carcinoma (epithelial and mesenchymal components	TN	_	OC
Bell 2014	*BRCA1*	c.81-?_134+?del p.(Cys27Ter)	Exon 3	Large rearr.	20	Right	Metaplastic carcinoma, NOS	TN	Right IDC (20)	BC
	TP53	c.375+2T>C p.(?)	Intron 4	Splicing						

BC: breast cancer; FH: family history; IDC: infiltrating ductal carcinoma; MpBC: metaplastic breast cancer;N/A: not applicable; NOS: not otherwise specified; OC: ovarian cancer; TN: triple negative.

[a]cDNA and protein changes are named according to HGVS nomenclature. Reference sequences: *BRCA1* NM_007294.4; *TP53* NM_000546.5

[b]After exon 3, subsequent exon numbers of *BRCA1* gene are increased by one, due to historical misannotation of an additional “exon 4”

[c]Classification into seven sub-categories following the WHO guidelines [1; 3]

[d]Pathology-confirmed or self-reported cancer diagnoses

Figures

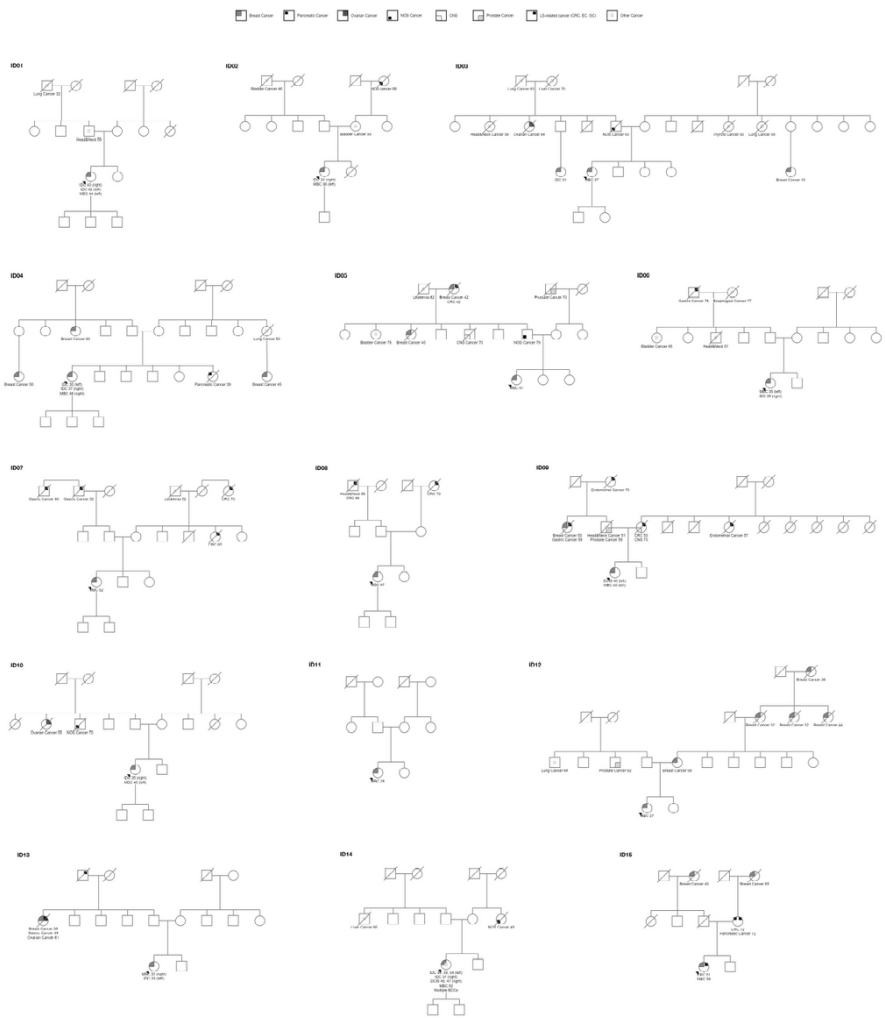

Figure 1

Pedigrees of MpBC PV carriers described in our series.

ID number of the proband is shown on the top left corner of each family tree. Index case is indicated by an arrow. Tumors and ages at diagnosis are displayed under affected individuals.

BC: breast cancer; CNS: central nervous system; CRC: colorectal cancer; GC: gastric cancer; EC: endometrial cancer; NOS: not otherwise specified; OC: ovarian cancer; PanC: pancreatic cancer; PrC: prostate cancer.

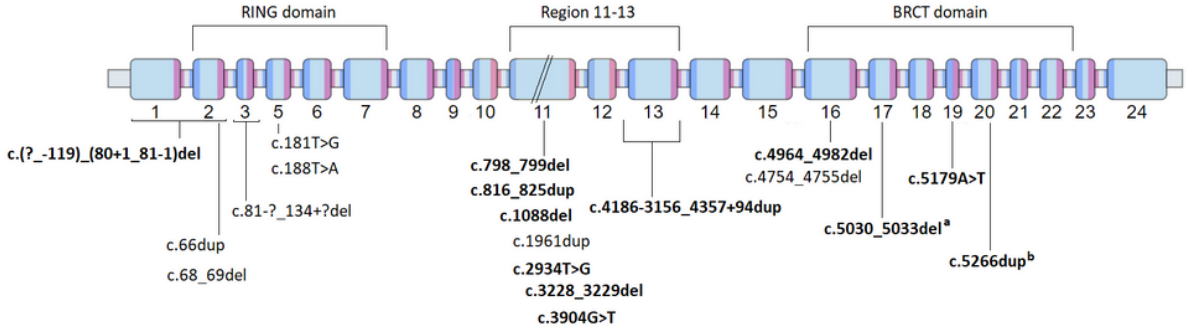

Figure 2

Distribution of unique germline *BRCA1* PVs identified in MpBC patients (modified from *BRCA* Exchange https://brcaexchange.org/). Alternate exons are shown as rectangles, with the corresponding number underneath. Donor splice sites are depicted in violet, acceptor splice site in blue. After exon 3, subsequent exon numbers are increased by one, due to historical mis-annotation of an additional "exon 4". Exon 11 is not to scale, since it covers >65% of *BRCA1* sequence.

PVs described in our series are indicated in bold; PVs reported in previous case reports are in normal type.

[a]reported twice in our series

[b]reported both in our series and in [24]

The three regions of BRCA1 protein that are mutated in cancer patients with a higher frequency [44] are indicated above the graph.