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Individuals with *FANCM* biallelic mutations do not develop Fanconi anemia, but show risk for breast cancer, chemotherapy toxicity and may display chromosome fragility.

Running title: Breast cancer cases with *FANCM* biallelic mutations

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Abstract.

Purpose: Monoallelic germline mutations in the *BRCA1*/*FANCS*, *BRCA2*/*FANCD1* and *PALB2*/*FANCN* genes confer high risk of breast cancer. Biallelic mutations in these genes cause Fanconi Anemia (FA), characterized by malformations, bone marrow failure, chromosome fragility and cancer predisposition (*BRCA2*/*FANCD1* and *PALB2*/*FANCN*), or a FA-like disease presenting a phenotype similar to FA but without bone marrow failure (*BRCA1*/*FANCS*). *FANCM* monoallelic mutations have been reported as moderate risk factors for breast cancer, but there are no reports of any clinical phenotype observed in carriers of biallelic mutations.

Methods: Breast cancer probands were subjected to mutation analysis by sequencing gene panels or testing DNA damage response genes.

Results: Five cases homozygous for *FANCM* loss-of-function mutations were identified. They show a heterogeneous phenotype including cancer predisposition, toxicity to chemotherapy, early menopause, and possibly chromosome fragility. Phenotype severity might correlate with mutation position in the gene.

Conclusions: Our data indicate that biallelic *FANCM* mutations do not cause classical FA, providing proof that *FANCM* is not a canonical FA gene. Moreover, our observations support previous findings suggesting that *FANCM* is a breast cancer predisposing gene. Mutation testing of *FANCM* might be considered for individuals with the above-described clinical features.

Keywords (5): Biallelic FANCM mutations, breast cancer risk factors, Fanconi Anemia, FA-like disease, genotype/phenotype correlation.

Text

INTRODUCTION

Breast cancer is the most common female oncological disease worldwide. Up to 15% of all cases have family history for the disease, and the risk of developing breast cancer in individuals with an affected relative is two-fold higher compared to the general population.[1] *BRCA1*, *BRCA2* and *PALB2* are major breast cancer predisposing genes and monoallelic protein-truncating mutations confer risk of developing the disease by age 70 ranging from 35% to 59%.[2,3] These genes are also designated as *FANCS*/*BRCA1*, *FANCD1*/*BRCA2* and *FANCN*/*PALB2,* and together with 18 other genes —including the recently identified *FANCV*/*REV7*[4]— code for proteins involved in a common pathway responsible for DNA interstrand crosslink (ICL) repair, mediated by homologous recombination. Biallelic mutations in genes of this pathway cause the Fanconi Anemia (FA) disease characterized by congenital defects, bone marrow failure, sensitivity to DNA ICLs and cancer susceptibility. FA patients often show chemotherapy sensitivity, abnormal inflammatory response, reproductive/endocrine defects, and increased risk of hematological tumors and other solid tumors such as squamous cell carcinoma (SCC) and breast and ovarian cancer. Biallelic mutations in three of the genes involved in the FA pathway, namely *FANCS*/*BRCA1*, *FANCO*/*RAD51C* and *FANCR*/*RAD51* cause a FA-like disease which is similar to FA but does not include bone marrow failure (reviewed in[5]).

While the *FANCM* gene is a key component of the FA molecular pathway, its role in the etiology of FA disease has been disputed. In 2005, an individual affected with FA was found to carry biallelic mutations in *FAAP250* that was renamed *FANCM* and proposed as a new FA gene.[6] Later, it was discovered that this patient also carried biallelic mutations in the *FANCA* gene.[7] Apart from this case, no other FA patients with *FANCM* biallelic mutations were described to date. Moreover, seven Finnish individuals were found to be homozygous for *FANCM* mutations but had no evidence of blood diseases, increased frequency of cancer events or any other chronic disease.[8] While these observations raised the question of whether or not *FANCM* was a bona-fide FA gene, recent case-control studies indicated that monoallelic truncating mutations located in the C-terminus of the gene might be risk factors for breast cancer. The *FANCM* c.5101C>T mutation (p.Gln1701*, rs147021911) is relatively frequent in Finland, where it was shown to be statistically associated with breast cancer risk, particularly in familial cases (odds ratio, OR=2.11) and individuals with triple negative tumors (OR=3.56).[9] The *FANCM* c.5791C>T mutation (p.Gly1906Alafs*12; rs144567652), also known as p.Arg1931*, was tested in a large series of familial breast cancer cases and controls from different populations, resulting in a statistically significant association with disease risk (OR=3.93).[10] These observations are supported by a very recent analysis of truncating *FANCM* mutations found by sequencing German familial cases and controls.[11]

In this study, we describe the phenotype of five breast cancer probands incidentally found to carry homozygous *FANCM* loss-of-function mutations. These findings contribute to elucidate the role of this gene in both FA disease and breast cancer predisposition.

INDIVIDUALS AND METHODS

Breast cancer probands and mutation testing strategies. The five female individuals included in this study were originally eligible for mutation testing in breast cancer predisposing genes based on the facts that they were affected with breast cancer, and they had either early onset or family history for the disease, or were affected with hormone receptor negative cancer. All probands underwent mutation testing through a diagnostic or a research protocol at four different centers in Italy, Germany, Sweden and Spain. Mutation testing consisted of next generation sequencing (NGS) of custom or commercial gene panels including established and candidate breast cancer genes (Table S1). In addition, probands from Italy and Germany were subjected to whole exome sequencing (WES) and of all annotated variants we considered only those located within the genes involved in the DNA damage response (Table S2). To establish genotype/phenotype correlations, all the variants found in probands' DNA were annotated and prioritized for causality using different pipelines that classified variants considering i) their effect on the protein product, ii) their frequency reported in public databases, and iii) their clinical classification, if available in public databases or literature. All individuals included in this study and herein described signed an informed consent to the use of their biological samples and clinical data for research projects. This study was approved by local Ethics Committees.

RESULTS

In this collaborative study, we report the clinical phenotype of five female breast cancer probands that were tested for mutations in breast cancer related genes and found to be homozygous for loss-of-function mutations in the *FANCM* gene (Table 1 and Figure 1). The presence of homozygous *FANCM* mutations was confirmed in all probands DNA by Sanger sequencing (Figure S1). Proband 1 was Italian, she developed early onset breast cancer at age 29 followed by several other oncological diseases and was found to

carry the homozygous mutation *FANCM* c.1972C>T (p.Arg658*). She was born to first cousin parents and we speculate they both inherited the same *FANCM* alleles from a common ancestor. Proband 2 was born in Germany. Also this woman developed breast cancer at an early age (31) and inherited one *FANCM* c.1972C>T (p.Arg658*) allele from each of her heterozygous parents. The two probands from Sweden had a family history for breast or ovarian cancer and were homozygous for *FANCM* c.5101C>T (p.Gln1701*) that is a relatively common mutation in Finland.[9] Finally, the Spanish proband developed a triple negative breast cancer and was found to be homozygous for the *FANCM* c.5791C>T (p.Arg1931*) mutation that has been reported as possibly more frequent in Southern-Western Europe.[10] As we could not specifically test for the presence of single exon rearrangements, we cannot formally exclude that the homozygosity we observed is due to exonic deletions in the *FANCM* locus.

While these individuals were firstly diagnosed with breast cancer, the majority of them showed other clinical signs including chemotherapy toxicity and early menopause (Table 1 and Table S3). These phenotypes, together with the presence of biallelic mutations in a FA pathway gene, prompted us to measure sensitivity to DNA ICLs agents by a chromosome fragility test that was performed as previously described.[12] We could only obtain fresh blood cells from probands 2, 3 and 5, and observed sensitivity to DNA ICLs only in proband 2 (Table S4).

Apart from the *FANCM* homozygous mutations, we only found one other known breast cancer associated variant, *CHEK2* c.470C>T (p.Ile157Thr) detected in the proband 4. As this variant is reported as a low/moderate risk factor for breast cancer,[13] we cannot exclude it could have contributed to the phenotype observed in this proband. Nor can we exclude that other mutations possibly influencing the clinical phenotypes might remain undetected. However, since our gene panels included known variants associated with a moderate to high risk for developing breast cancer, with the only exception of *CHEK2*

c.470C>T in proband 4 we believe that the homozygous *FANCM* mutations were most likely to be considered the only disease-associated mutations identified in the five probands.

DISCUSSION

Our data document for the first time the possible association of biallelic *FANCM* mutations with a clinical phenotype. The five probands included in this study were all originally recruited as breast cancer probands, and none was clinically diagnosed with FA nor FA-like diseases. While none suffered from congenital abnormalities, bone marrow failure or hemato-oncological diseases, some of them showed clinical signs which are included in FA phenotype spectrum. In particular, four of the five probands showed chemotherapy related hematological side effects. Moreover, proband 1 developed three head and neck squamous cell carcinomas (HNSCC) and another solid cancer. Probands 2, 4, and 5 showed sign of early menopause, and proband 3 suffered from autoimmune disease (Sjögren's syndrome). Finally, proband 2 tested positive for chromosome fragility indicating sensitivity to DNA ICLs (Table 1). These clinical observations indicate that *FANCM* is not a bona fide FA gene. The small number of carriers of biallelic *FANCM* mutations that we describe in this study does not allow performing any statistical analysis, and neither to claim that the *FANCM* genotypes we detected are causative of the observed clinical phenotypes. However, on a less conservative view, we cannot exclude that homozygosity for truncating mutations in *FANCM* may have clinical consequences that are more pronounced than for heterozygous mutation carriers. The phenotypes in our five probands are consistent with *FANCM* being a gene associated with FA-like cancer susceptibility. This appears to be supported by molecular data from human cell lines and by phenotypic observations from *Fancm*-deficient mice. During the early steps of the physiological repair of DNA ICLs, FANCM and the protein products encoded by the seven

bona fide FA genes *FANCA*, *B*, *C*, *E*, *F*, *G*, and *L* assemble in the "core complex". This complex monoubiquitinates the ID2 heterodimer which mediates the recruitment of "effector proteins" responsible for DNA ICLs repair (reviewed in [5]). While biallelic mutations in all the core complex genes abolish monoubiquitination of the ID2 complex, lack of FANCM only reduces the efficiency of this mechanism. Moreover, studies in which FANCM was depleted in cell lines showed that this loss neither affect the levels of other FA core complex proteins, such as FANCA and FANCG, nor the ability of FANCL to co-immuniprecipitate with them, suggesting that FANCM is not essential for FA core complex formation and stabilization (reviewed in [14]). With respect to FA mice models, mice deficient for *Fancm* showed increased cancer incidence,[15] an excess of ovarian, mammary and uterine cancers, and sex independent gonadal defects.[16]

Monoallelic loss-of-function mutations in *FANCM* have been suggested to be associated with moderate/low risk for breast cancer.[9-11] The identification of breast cancer probands with biallelic *FANCM* mutations corroborates the role of this gene as a breast cancer predisposing factor. Two of these probands showed early onset breast cancer and one proband developed bilateral breast cancer, suggesting that *FANCM* biallelic mutations may have a stronger effect on breast cancer risk compared to monoallelic mutations. Such an effect has been previously observed for the moderate breast cancer risk factor *CHEK2* c.1100delC. Homozygote carriers of this variant have been estimated to have a twofold and a fourfold higher risk compared to heterozygotes and women of the general population, respectively.[17] Moreover, of the nine *CHEK2* c.1100delC homozygous familial cases reported to date,[17,18] three developed breast cancer before age 35 and four developed bilateral breast cancer, which is comparable to the clinical phenotypes observed in some of the *FANCM* homozygous probands.

Our data shows that biallelic *FANCM* mutations might be associated with a clinical phenotype including breast cancer predisposition, chemotherapy toxicity and possibly

early menopause and chromosome fragility. In the accompanying manuscript,[19] Bogliolo *et al.* describe additional individuals with *FANCM* homozygous mutations who were affected with different types of early onset cancer. Altogether, these observations support the hypothesis that biallelic mutations in *FANCM* may cause a heterogeneous cancer susceptibility phenotype. This heterogeneity and the breast cancer severity in term of age of onset that we observed in our probands, might be influenced by the position of the mutations in the gene (Figure S2). All three carriers of the p.Gln1701* and p.Arg1931* mutations, which are located in the gene C-terminus, developed breast cancer at typical ages and, at least the two who were tested, did not display chromosome fragility. Both carriers of the p.Arg658* mutation, which is expected to produce a protein lacking the domains of interaction with the DNA-binding mediators MHF1 and MHF2, and with the Bloom syndrome complex,[20] developed early onset breast cancer and one showed high chromosome fragility. Similarly, the patients described by Bogliolo *et al*. who suffered from severe early onset cancers and showed chromosome fragility, carried homozygous mutations expected to truncate FANCM at aminoacid 503 and 863.[19]

In conclusion, our data point out that biallelic mutations in *FANCM* do not cause classical FA, providing evidence that *FANCM* is not a canonical FA gene. Moreover, our observations reinforce the hypothesis that *FANCM* is a breast cancer predisposing gene with biallelic mutations possibly conferring higher risk. Finally, we suggest that *FANCM* biallelic mutations might be associated with a FA-like cancer susceptibility, characterized by increased risk for breast and other types of cancer, chemotherapy toxicity, and possibly early menopause and chromosome fragility. *FANCM* mutation testing may be offered, in the context of research protocols, to individuals with the above clinical features. Additional genotype/phenotype correlations are necessary to better clarify the clinical impact of *FANCM* biallelic mutations.

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Supplementary materials

Supplementary information is available at the *Genetics in Medicine* website.

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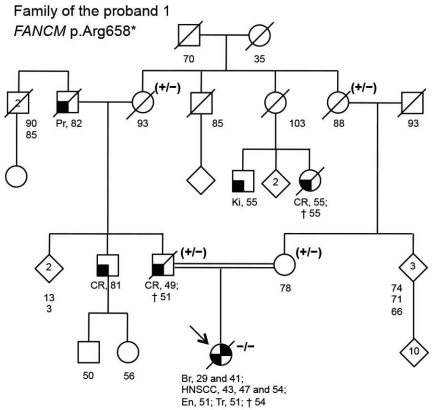
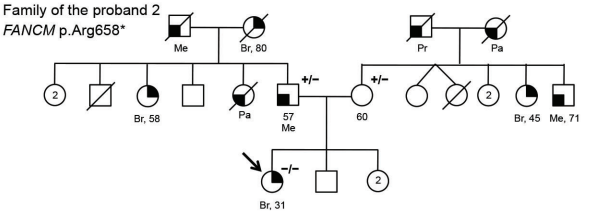
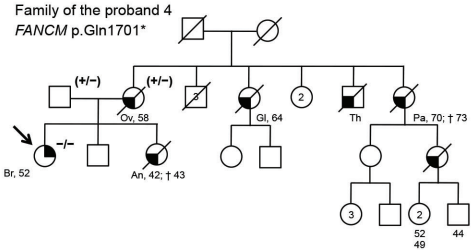
Figure Legend

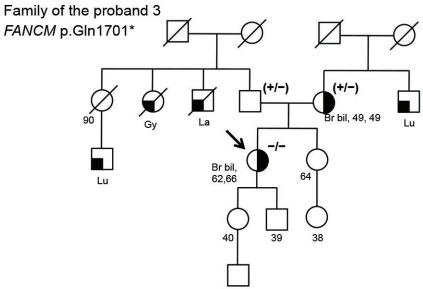
Figure 1. Family pedigrees of the five probands homozygous for the *FANCM* c.1972C>T (p.Arg658*), the c.5101C>T (p.Gln1701*) and the c.5791C>T (p.Arg1931*) mutations. Probands are indicated by the arrows. Cancer type, age at diagnosis and age of death are reported when known. Age of healthy individuals, if known, was at date of genetic counseling. Events occurred after genetic counseling, if known, are annotated. Mutation status is reported as it follows: +/+, normal; +/−, heterozygote; −/−, homozygote; (+/−), obligate carrier. Cancer type is reported as it follows: An, anal; Br, breast; Br bil, bilateral breast cancer; CR, colorectal; En, endometrial; Gl, glioblastoma; Gy, gynecologic non-specified cancer; HNSCC, head and neck squamous cells cancer; Ki, kidney; La, larynx; Ly, lymphoma; Lu, lung; Me, melanoma; Ov, ovarian; Pa, pancreas; Pr, prostate; Th, Throat; Tr, trichilemmoma.

Figure 1


Family of the proband 1
FANCM p.Arg658*
70
35
90
85
Pr, 82
(+/−)
93
85
103
(+/−)
88
93
Ki, 55
2
CR, 55;
† 55
2
13
3
CR, 81
CR, 49;
† 51
(+/−)
(+/−)
78
3
74
71
66
−/−
Br, 29 and 41;
HNSCC, 43, 47 and 54;
En, 51; Tr, 51; † 54
50
56
10


Family of the proband 2
FANCM p.Arg658*
Me
Br, 80
Pr
Pa
2
Br, 58
Pa
+/−
57
Me
+/−
60
2
Br, 45
Me, 71
−/−
Br, 31
2


Family of the proband 4
FANCM p.Gln1701*
(+/−)
(+/−)
Ov, 58
3
Gl, 64
2
Th
Pa, 70; † 73
−/−
Br, 52
An, 42; † 43
3
2
52
49
44


Family of the proband 3
FANCM p.Gln1701*
90
Gy
La
(+/−)
(+/−)
Br bil, 49, 49
Lu
Lu
−/−
Br bil,
62,66
64
40
39
38

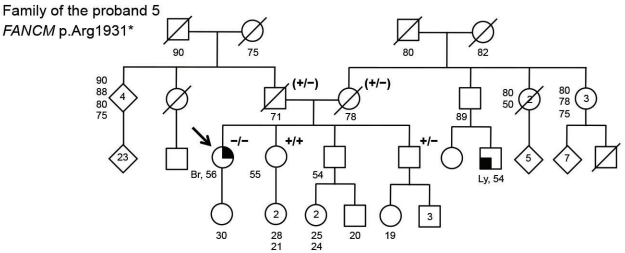

Family of the proband 5
FANCM p.Arg1931*
90
75
80
82
90
88
80
75
4
(+/−)
71
(+/−)
78
89
80
50
80
78
75
3
23
−/−
Br, 56
55
+/+
54
+/−
Ly, 54
5
7
30
2
28
21
2
25
24
20
19
3

Table S1. Description of five breast cancer probands and mutation testing protocol.

Ascertainment Center	(id) Proband	Protocol	Gene-panel/sequencing instrument	(Number) and names of genes tested
Istituto Nazionale dei Tumori, Milan, Italy	(1) early-onset case	Research	TruSeq Custom Amplicon assay (Illumina)/ MiSeq (Illumina)	(20): ATM, BAP1, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FANCM, MRE11A, NBN, PALB2, PTEN, RAD50, RAD51C, RAD51D, RECQL, STK11, TP53 and XRCC2.
University Hospital of Cologne, Germany	(2) early-onset and familial case	Diagnostic	Custom GC-HBOC/ Hiseq 4000 (Illumina)	(34): ATM, BRCA1, BRCA2, CDH1, CHEK2, FANCM, NBN, PALB2, RAD51C, RAD51D, TP53 and other genes of interest.
The Nation-wide SWEA study, Lund University, Sweden	(3, 4) familial cases	Diagnostic	Modified version of SureSelectXT Target Enrichment System (Agilent Technology)/ Hiseq 2500 (Illumina)	(64): FANCA, FANCB, FANCC, BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, BRIP1, FANCL, FANCM, PALB2, RAD51C, SLX4, BRCA1, ATM, BARD1, CDH1, CHEK2, MRE11A, NBN, PTEN, RAD51D, STK11, TP53 and other breast cancer related genes.
Centro Nacional de Investigaciones Oncological, Madrid, Spain	(5) case with triple-hormone receptor negative cancer	Diagnostic	Onco-Gene SGKit LV2511 (Sistemas Genomicos)/ MiSeq (Illumina)	(80): APC, ATM, AXIN2, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, BUB1, CDH1, CDKN2A, CHEK2, DDB2, DKC1, ELANE, EPCAM, ERCC1 , ERCC2, ERCC3, ERCC4, ERCC5, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FLCN, GFI1, GPC3, HAX1, HOXB13, KIF1B, MAX, MEN1, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NOP10, NSD1, NUDT1, OGG1, PALB2, PMS1, PMS2, POLH, PRSS1, PTEN, RAD50, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SLX4, SMAD4, STK11, TERT, TMEM127, TP53, TSC1, TSC2, VHL, WAS, WT1, XPA, XPC and XRCC2

SWEA, the Swedish BRCA1 & BRCA2 study collaborators extended analysis; GC-HBOC, German Consortium for Hereditary Breast and Ovarian Cancer

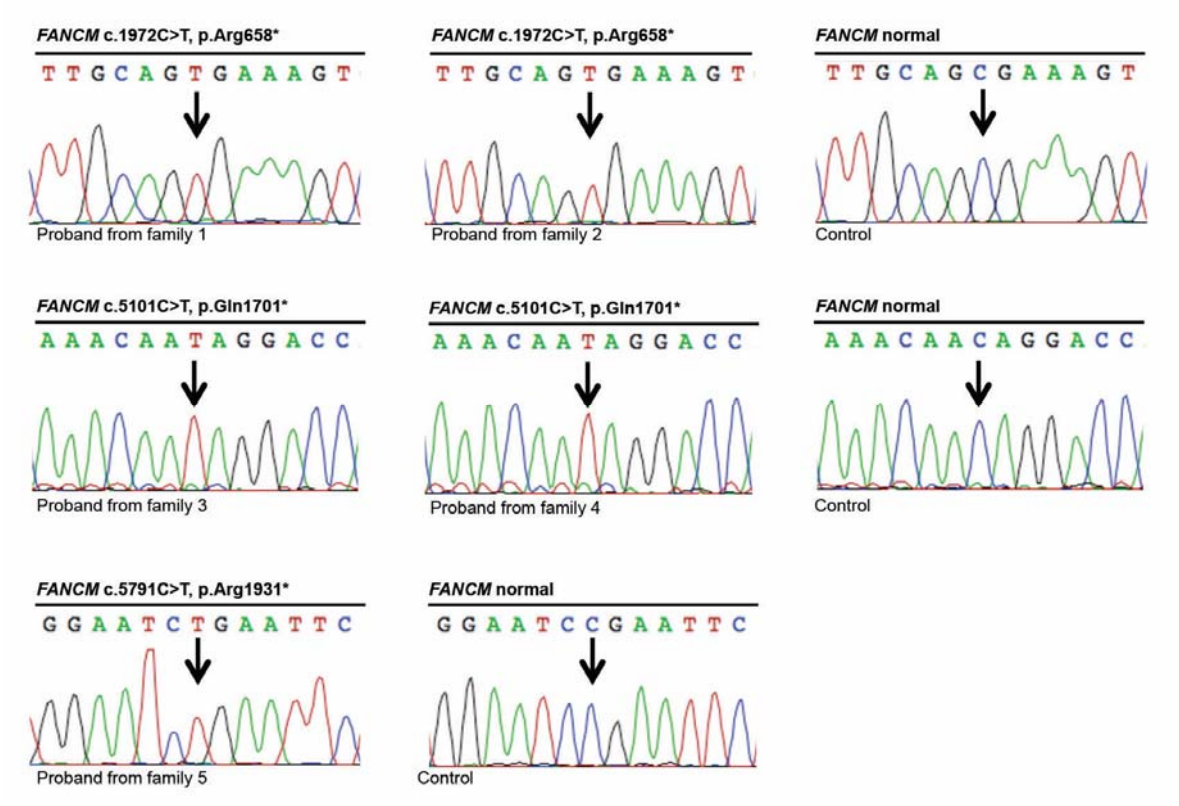


Figure S1. Electropherograms showing the sequences of the probands homozygous for the *FANCM* c.1972C>T (p.Arg658*), c.5101C>T (p.Gln1701*) and c.5791C>T (p.Arg1931*), and normal individuals. The nucleotide changes are indicated by arrows.

Table S2. The Italian and German probands were subjected to whole exome sequencing using Agilent SureSelect Human All Exon V5 and V6, respectively. In these analyses we only tested the genes involved in the DNA damage response using the Molecular Signatures Database (MSigDB, http://software.broadinstitute.org/gsea/index.jsp).

"Gene sets"	REACTOME_DNA_REPAIR, KEGG_NUCLEOTIDE_EXCISION_REPAIR, KEGG_MISMATCH_REPAIR, KEGG_BASE_EXCISION_REPAIR_HALLMARK_DNA_REPAIR, DNA_REPAIR and DOUBLE_STRAND_BREAK_REPAIR
Genes tested	AAAS, ABL1, ADA, ADCY6, ADRM1, AK1, AK3, ALKBH1, ALKBH2, ALKBH3, APEX1, APEX2, APRT, APTX, ARL6IP1, ASF1A, ATM, ATR, ATRX, ATXN3, BCAM, BCAP31, BLM, BOLA2, BRCA1, BRCA2, BRF2, BRIP1, BRP44, BTG2, C17orf70, C19orf40, CANT1, CCNH, CCNO, CDA, CDK7, CDKN2D, CEBPG, CETN2, CIB1, CLP1, CMPK2, COBRA1, COX17, CSNK1D, CSNK1E, CSTF3, CUL4A, CUL4B, DAD1, DCTN4, DDB1, DDB2, DFNA5, DGCR8, DGUOK, DUT, EDF1, EIF1B, EIF2C4, ELL, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, ERCC8, EXO1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCL, FANCM, FEN1, GADD45A, GADD45G, GMPR2, GPX4, GTF2A2, GTF2B, GTF2F1, GTF2H1, GTF2H2, GTF2H2B, GTF2H3, GTF2H4, GTF2H5, GTF3C5, GUK1, H2AFX, HCLS1, HMGB1, HMGB1P1, HMGB1P10, HMGB1P40, HMGB2, HPRT1, HUS1, IGHMBP2, IMPDH2, ITPA, KAT5, LIG1, LIG3, LIG4, LOC389901, LOC648152, LOC651610, LOC651921, LOC652672, LOC652857, MAD2L2, MBD4, MDC1, MGMT, MLH1, MLH3, MMS19, MNAT1, MPG, MRE11A, MRPL40, MSH2, MSH3, MSH5, MSH6, MUTYH, NBN, NCBP2, NEIL1, NEIL2, NEIL3, NFX1, NHEJ1, NME1, NME3, NME4, NPR2, NT5C, NT5C3, NTHL1, NUDT21, NUDT9, OGG1, PALB2, PARP1, PARP2, PARP3, PARP4, PCNA, PDE4B, PDE6G, PMS1, PMS2, PMS2P1, PNKP, PNP, POLA1, POLA2, POLB, POLD1, POLD2, POLD3, POLD4, POLE, POLE2, POLE3, POLE4, POLG, POLH, POLI, POLL, POLQ, POLR1C, POLR1D, POLR2A, POLR2B, POLR2C, POLR2D, POLR2E, POLR2F, POLR2G, POLR2H, POLR2I, POLR2J, POLR2K, POLR2L, POLR3C, POLR3GL, POM121, PRIM1, PRKCG, PRKDC, RAD1, RAD17, RAD21, RAD23A, RAD23B, RAD50, RAD51, RAD51B, RAD51C, RAD52, RAD54B, RAD54L, RAD9A, RAE1, RALA, RBBP8, RBX1, RDBP, RECQL, RECQL4, RECQL5, REV1, REV3L, RFC1, RFC2, RFC3, RFC4, RFC5, RNMT, RPA1, RPA2, RPA3, RPA4, RPAIN, RPS27A, RPS27AP11, RRM2B, RUVBL2, SAC3D1, SDCBP, SEC61A1, SETX, SF3A3, SMAD5, SMC1A, SMUG1, SNAPC4, SNAPC5, SOD1, SRSF6, SSBP1, SSRP1, STX3, SUMO1, SUPT4H1, SUPT5H, SURF1, TAF10, TAF12, TAF13, TAF1C, TAF6, TAF9, TARBP2, TCEA1, TCEB3, TDG, TDP1, TH1L, THOC4, TK2, TMED2, TNP1, TP53, TP53BP1, TP73, TREX2, TSG101, TYMS, UBA52, UBE2A, UBE2B, UBE2N, UBE2T, UBE2V1, UBE2V2, UMPS, UNG, UPF1, UPF3B, USP1, USP11, UVRAG, VCP, VPS28, VPS37B, VPS37D, WRNIP1, XAB2, XPA, XPC, XRCC1, XRCC2, XRCC3, XRCC4, XRCC5, XRCC6, XRCC6BP1, ZBTB32, ZNF707, ZNRD1, ZWINT.

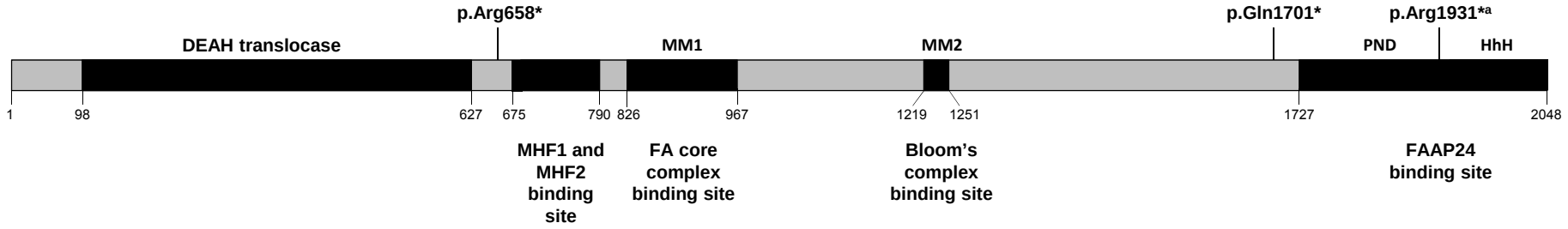


Figure S2. Schematic diagram of the FANCM protein showing functional domains, as reported previously.[20] and the position of the mutations identified in the five probands. PND, pseudonuclease domain ERCC4; HhH, helix-hairpin-helix domain. The p.Arg1931* mutation was later re-annotated as p.Gly1906Alafs*12 [10] and has been drawn accordingly.

Table S3. Description of the chemotherapy treatments or protocols received by the five breast cancer probands.

Proband id	Nationality	Chemotherapy treatments
1	Italian	2 cycles of cyclophosphamide, methotrexate and fluorouracil (CMF); interrupted for toxicity.
2	German	4 cycles of docetaxel and trastuzumab. 1 cycle of epirubicin, cyclophosphamide and trastuzumab; interrupted for neutropenia. 1 cycle of carboplatinum and trastuzumab; interrupted for pancytopenia.
3	Swedish	1 cycle of fluorouracil, epirubicin and cyclophosphamide (FEC); interrupted for mucositis and neutropenia. 1 cycle of epirubicin and cyclophosphamide (EC); interrupted for pancytopenia.
4	Swedish	3 cycles of fluorouracil, epirubicin and cyclophosphamide (FEC). 1 cycle of docetaxel following by a 80% dose reduction due to adverse effect (not including cytopenia).
5	Spanish	4 cycles of doxorubicin and cyclophosphamide. 1 cycle of carboplatin and docetaxel; interrupted for pancytopenia. 9 cycles of taxol.

Table S4. Results of the chromosome fragility tests in cells from probands 2, 3, and 5.

Probands id	DEB [0.1 ug/ml]	Nº cells	% Aberrant cells	Breaks/ cell	Breaks/ aberrant cell	% Multiaberrants	Breaks/ multiaberrant cell	Radial figures
2	Untreated	50	24	0.28	1.17	4	2	0
	Treated	100	76	3.06	4.03	62	4.71	11
3	Untreated	50	8	0.08	2	8	2	0
	Treated	100	22	0.26	1.18	4	2	0
5	Untreated	50	26	0.3	1.15	4	2	0
	Treated	100	31	0.46	1.48	12	2.25	0

DEB, diepoxybutane