

## Supplementary Material

**Table S1: Personal and family history of cancer (general and group).**

Variable	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	Group 4 N (%)	Total N (%)	p-value*
<b>Age at diagnosis (index)</b>						<b>&lt;0.001</b>
≤30	8 (15.7)	24 (44.4)	30 (30.3)	0 (0.0)	62 (21.7)	
>30 e ≤50	34 (66.7)	25 (46.3)	56 (56.6)	40 (48.8)	155 (54.2)	
>50	9 (17.6)	5 (9.3)	13 (13.1)	42 (51.2)	69 (24.1)	
<b>Other cancer (index)</b>						<b>0.005</b>
Yes	20 (40.8)	16 (30.2)	28 (29.5)	11 (13.4)	75 (26.9)	
No	29 (59.2)	37 (69.8)	67 (70.5)	71 (86.6)	204 (73.1)	
<b>Number of affected generations</b>						**
1	9 (17.6)	11 (20.4)	16 (16.2)	45 (62.5)	81 (29.3)	
2	20 (39.2)	25 (46.3)	50 (50.5)	24 (33.3)	119 (43.1)	
3	19 (37.3)	18 (33.3)	30 (30.3)	3 (4.2)	70 (25.4)	
4	3 (5.9)	0 (0.0)	3 (3.0)	0 (0.0)	6 (2.2)	
<b>Bilateral breast cancer in family history</b>						<b>&lt;0.001</b>
No	41 (80.4)	49 (90.7)	95 (95.0)	71 (100.0)	256 (92.8)	
Yes	10 (19.6)	5 (9.3)	5 (5.0)	0 (0.0)	20 (7.2)	
<b>Male breast cancer in family history</b>						0.071
No	51 (100.0)	52 (96.3)	100 (100.0)	71 (100.0)	274 (99.3)	
Yes	0 (0.0)	2 (3.7)	0 (0.0)	0 (0.0)	2 (0.7)	
<b>Ovarian cancer in family history</b>						<b>&lt;0.001</b>
No	38 (74.5)	50 (92.6)	89 (89.0)	71 (100.0)	248 (89.9)	
Yes	13 (25.5)	4 (7.4)	11 (11.0)	0 (0.0)	28 (10.1)	
<b>Number of cancer cases</b>						<b>&lt;0.001</b>
≤3	8 (15.7)	18 (33.3)	40 (40.0)	54 (75.0)	120 (43.3)	
>3	43 (84.3)	36 (66.7)	60 (60.0)	18 (25.0)	157 (56.4)	
<b>Number of breast cancer cases</b>						<b>&lt;0.001</b>
≤3	23 (45.1)	44 (81.5)	77 (77.8)	72 (100.0)	216 (78.3)	
>3	28 (54.9)	10 (18.5)	22 (22.2)	0 (0.0)	60 (21.7)	
<b>Number of cancer deaths</b>						<b>&lt;0.001</b>
≤3	32 (62.7)	43 (79.6)	90 (90.9)	69 (97.2)	234 (85.1)	
>3	19 (37.3)	11 (20.4)	9 (9.1)	2 (2.8)	41 (14.9)	
<b>Number of breast cancer deaths</b>						<b>0.004</b>
≤3	46 (90.2)	51 (94.4)	98 (99.0)	71 (100.0)	266 (96.7)	
>3	5 (9.8)	3 (5.6)	1 (1.0)	0 (0.0)	9 (3.3)	

Group 1: women with germline mutations in the *BRCA1/BRCA2* genes; Group 2: women with VUS in the *BRCA1/BRCA2* genes; Group 3: women WT for the *BRCA1/BRCA2* genes; Group 4: control, sporadic group.

\*Chi-square/Fisher's exact

\*\* Given the very low sample size in some of the groups, it could not perform the statistical test.

Note: Values in bold indicate statistical significance (p<0.05).

**Table S2: Pathological features related to breast cancer (group and general).**

Variable	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	Group 4 N (%)	Total N (%)	p-value *
<b>Histological grade</b>						<b>0.010</b>
Grade I	1 (2.6)	4 (8.5)	10 (11.9)	6 (8.2)	21 (8.6)	
Grade II	21 (53.8)	29 (61.7)	39 (46.4)	49 (67.1)	138 (56.8)	
Grade III	17 (43.6)	14 (29.8)	35 (41.7)	18 (24.7)	84 (34.6)	
<b>Stage</b>						
<b>Size and/or extent (T)</b>						<b>0.040</b>
T1	14 (28.6)	18 (34.6)	33 (37.1)	29 (39.2)	94 (35.6)	
T2	16 (32.7)	15 (28.8)	31 (34.8)	28 (37.8)	90 (34.1)	
T3	9 (18.4)	11 (21.2)	15 (16.9)	9 (12.2)	44 (16.7)	
T4	10 (20.4)	8 (15.4)	10 (11.2)	8 (10.8)	36 (13.6)	
<b>Lymph Node (N)</b>						0.493
N0	26 (52.0)	25 (48.1)	49 (54.4)	48 (65.8)	148 (55.58)	
N1	17 (34.0)	22 (42.3)	26 (28.9)	16 (21.9)	81 (30.6)	
N2	4 (8.0)	3 (5.8)	11 (12.2)	6 (8.2)	24 (9.1)	
N3	3 (6.0)	2 (3.8)	4 (4.4)	3 (4.1)	12 (4.5)	
<b>Metastasis (M)</b>						0.105
M0	45 (91.8)	52 (100.0)	86 (95.6)	72 (98.6)	255 (96.6)	
M1	4 (8.2)	0 (0.0)	4 (4.4)	1 (1.4)	9 (3.4)	
<b>Triple Negative</b>						<b>&lt;0.001</b>
Yes	24 (51.1)	7 (13.2)	18 (19.6)	8 (9.8)	57 (20.9)	
No	23 (48.9)	46 (86.8)	74 (80.4)	74 (90.2)	217 (79.1)	

\*Chi-square

\*\* Samples whose FISH analysis was not performed owing to lack of tumor paraffin block.

Note: Values in bold indicate statistical significance (p &lt; 0.05).

**Table S3: Main characteristics of the breast tumors**

Variable	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	Group 4 N (%)	p-value*
<b>Estrogen receptor</b>					<b>&lt;0.001</b>
Negative	28 (58.3)	19 (35.8)	32 (34.8)	19 (23.2)	
Positive	20 (41.7)	34 (64.2)	60 (65.2)	63 (76.8)	
<b>Progesterone receptor</b>					<b>0.020</b>
Negative	28 (58.3)	18 (34.0)	44 (46.8)	33 (40.2)	
Positive	20 (41.7)	35 (66.0)	50 (53.2)	49 (59.8)	
<b>HER2</b>					<b>0.012</b>
Negative	42 (89.4)	37 (72.5)	59 (64.8)	63 (76.8)	
Positive	4 (8.5)	14 (27.5)	30 (33.0)	19 (23.2)	
Inconclusive**	1 (2.1)	0 (0.0)	2 (2.2)	0 (0.0)	
<b>CK5/6</b>					<b>0.002</b>
Negative	17 (60.7)	24 (92.3)	40 (74.1)	58 (90.6)	
Positive	11 (39.3)	2 (7.7)	14 (25.9)	6 (9.4)	
<b>CK14</b>					<b>0.016</b>
Negative	22 (71.0)	28 (90.3)	43 (75.4)	59 (81.9)	
Positive	9 (29.0)	3 (9.7)	13 (22.8)	13 (18.1)	
<b>Ki67</b>					<b>&lt;0.001</b>
≤14%	3 (8.1)	3 (7.7)	12 (16.0)	23 (31.9)	
>14%	34 (91.9)	36 (92.3)	63 (84.0)	49 (68.10)	

\*Chi-square

\*\* Samples whose FISH analysis was not performed due to lack of tumor paraffin block.

**Note:** Values in bold indicate statistical significance (p<0.05).

**Table S4:** Family history, histopathological and immunohistochemical characteristics of mutated *BRCA1* versus groups 2, 3 and 4.

Variable	BRCA1 mutated	Group 2	Group 3	Group 4	p-value*
<b>Bilateral breast cancer</b>					<b>&lt;0.001</b>
Yes	23.3	9.3	5.0	0.0	
No	76.7	90.7	95.0	100.0	
<b>Ovarian cancer</b>					<b>&lt;0.001</b>
Yes	30.0	7.4	11.0	0.0	
No	70.0	92.6	89.0	100.0	
<b>Number of affected generations</b>					<b>&lt;0.001</b>
1	13.3	20.4	16.2	62.5	
2	40.0	46.3	50.5	33.3	
3	43.3	33.3	30.3	4.2	
4	3.4	0.0	3.0	0.0	
<b>Number of breast cancer cases</b>					<b>&lt;0.001</b>
≤3	40.0	81.5	77.8	100.0	
>3	60.0	18.5	22.8	0.0	
<b>Total cases of cancer</b>					<b>&lt;0.001</b>
≤3	6.7	33.3	40.0	75.0	
>3	93.3	66.7	60.0	25.0	
<b>Number of breast cancer deaths</b>					<b>0.011</b>
≤3	90.0	94.4	99.0	100.0	
>3	10.0	5.6	1.0	0.0	
<b>Total cancer deaths</b>					<b>&lt;0.001</b>
≤3	66.7	70.6	90.9	97.2	
>3	33.3	20.4	9.1	2.8	
<b>Age at diagnosis</b>					<b>&lt;0.001</b>
≤30 years	20.0	44.4	30.3	0.0	
>30 and ≤50 years	63.3	46.3	56.6	48.8	
>50 years	16.7	9.3	13.1	51.2	
<b>Location of the tumor</b>					0.379
Unilateral	96.7	94.4	96.0	100.0	
Bilateral	3.3	5.6	4.0	0.0	
<b>Histopathological type</b>					1.000
Invasive ductal carcinoma	96.0	85.1	94.4	92.1	
Others	4.0	14.9	5.6	7.9	
<b>Histological grade</b>					0.090
1	0.0	8.5	11.9	8.2	
2	43.5	61.7	46.4	67.1	
3	56.5	29.8	41.7	24.7	
<b>Estrogen receptor</b>					<b>&lt;0.001</b>
Negative	80.0	35.8	34.8	23.2	
Positive	20.0	64.2	65.2	76.8	
<b>Progesterone receptor</b>					0.046
Negative	80.0	34.0	46.8	40.2	
Positive	20.0	66.0	53.2	59.8	

<b>HER2</b>					0.030
Negative	96.6	72.5	64.8	76.8	
Positive	0.0	27.5	33.0	23.2	
Uncertain	3.4		2.2	0.0	
<b>Ki-67</b>					0.006
<=14	15.0	7.7	16.0	31.9	
>14	85.0	92.3	84.0	68.1	
<b>CK 5/6</b>					0.001
Negative	46.7	92.3	74.1	90.6	
Positive	53.3	7.7	25.9	9.4	
<b>CK 14</b>					0.212
Negative	55.6	90.3	76.8	81.9	
Positive	44.4	9.7	23.2	18.1	
<b>Triple negative</b>					<0.001
Yes	75.9	13.2	19.6	9.8	
No	24.1	86.8	80.4	90.2	
<b>Molecular subtype</b>					0.001
Luminal A	38.5	2.6	11.0	26.0	
Luminal HER2 negative	0.0	57.9	34.2	42.5	
Luminal HER2 positive	0.0	23.7	26.0	16.4	
Basal-like	0.0	2.6	13.7	5.5	
HER2 overexpressed	61.5	13.2	15.1	9.6	

\* Comparison analysis with Bonferroni correction

**Note:** Values in bold indicate statistical significance (p<0.05).

**Table S5:** Family history, histopathological and immunohistochemical characteristics of mutated *BRCA2* versus groups 2, 3 and 4.

Variable	<i>BRCA2</i> mutated	Group 2	Group 3	Group 4	p-value*
<b>Bilateral breast cancer</b>					<b>&lt;0.01</b>
Yes	14.3	9.3	5.0	0.0	
No	85.7	90.7	95.0	100.0	
<b>Male breast cancer</b>					<b>0.09</b>
Yes	0.0	0.0	0.0	0.0	
No	100.0	100.0	100.0	100.0	
<b>Ovarian cancer</b>					<b>0.011</b>
Yes	19.9	7.4	11.0	0.0	
No	81.0	92.6	89.0	100.0	
<b>Number of affected generations</b>					*
1	23.8	20.4	16.2	62.5	
2	38.1	46.3	50.5	33.3	
3	28.6	33.3	30.3	4.2	
4	9.5	0.0	3.0	0.0	
<b>Number of breast cancer cases</b>					<b>&lt;0.001</b>
≤3	52.4	81.5	77.8	100.0	
>3	47.6	18.5	22.8	0.0	
<b>Total cases of cancer</b>					<b>&lt;0.001</b>
≤3	28.6	33.3	40.0	75.0	
>3	71.4	66.7	60.0	25.0	
<b>Number of breast cancer deaths</b>					<b>0.011</b>
≤3	90.5	94.4	99.0	100.0	
>3	9.5	5.6	1.0	0.0	
<b>Total cancer deaths</b>					<b>&lt;0.001</b>
≤3	57.1	70.6	90.9	97.2	
>3	42.9	20.4	9.1	2.8	
<b>Age at diagnosis</b>					<b>&lt;0.001</b>
≤30 years	9.5	44.4	30.3	0.0	
>30 and ≤50 years	71.4	46.3	56.6	48.8	
>50 years	19.0	9.3	13.1	51.2	
<b>Location of the tumor</b>					<b>0.136</b>
Unilateral	100.0	94.4	96.0	100.0	
Bilateral	0.0	5.6	4.0	0.0	
<b>Histopathological type</b>					<b>0.329</b>
Invasive ductal carcinoma	90.0	85.1	94.4	92.1	
Others	10.0	14.9	5.6	7.9	
<b>Histological grade</b>					<b>0.221</b>
1	6.3	8.5	11.9	8.2	
2	68.8	61.7	46.4	67.1	

3	25.0	29.8	41.7	24.7	
<b>Estrogen receptor</b>					<b>&lt;0.246</b>
Negative	22.2	35.8	34.8	23.2	
Positive	77.8	64.2	65.2	76.8	
<b>Progesterone receptor</b>					<b>0.173</b>
Negative	22.2	34.0	46.8	40.2	
Positive	77.8	66.0	53.2	59.8	
<b>HER2</b>					<b>0.507</b>
Negative	77.8	72.5	64.8	76.8	
Positive	22.2	27.5	33.0	23.2	
Uncertain	0.0		2.2	0.0	
<b>Ki-67</b>					<b>&lt;0.001</b>
<=14	0.0	7.7	16.0	31.9	
>14	100.0	92.3	84.0	68.1	
<b>CK 5/6</b>					<b>0.045</b>
Negative	76.9	92.3	74.1	90.6	
Positive	23.1	7.7	25.9	9.4	
<b>CK 14</b>					<b>0.337</b>
Negative	92.3	90.3	76.8	81.9	
Positive	7.7	9.7	23.2	18.1	
<b>Triple negative</b>					<b>0.302</b>
Yes	11.1	13.2	19.6	9.8	
No	88.9	86.8	80.4	90.2	
<b>Molecular subtype</b>					<b>0.041</b>
Luminal A	66.7	2.6	11.0	26.0	
Luminal HER2 negative	25.0	57.9	34.2	42.5	
Luminal HER2 positive	0.0	23.7	26.0	16.4	
Basal-like	0.0	2.6	13.7	5.5	
HER2 overexpressed	8.3	13.2	15.1	9.6	

\* Comparison analysis with Bonferroni correction

Note: Values in bold indicate statistical significance (p<0.05).