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ORIGINAL ARTICLE

Cascade testing in Italian Hereditary Breast Ovarian Cancer families: a missed opportunity for cancer prevention?

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Abstract
Healthy carriers of *BRCA1/2* pathogenic variants (PVs) may benefit from risk-reducing measures of proven efficacy. The main approach to identify these individuals is cascade testing, and strategies to support this complex process are under investigation. In Italy, cascade testing has received little attention; therefore, we analyzed the uptake and characteristics of *BRCA1/2* cascade testing in families diagnosed with HBOC between 2017 and 2019 at two Italian genetics centers. All blood relatives aged 18 years or older at September 2022 and who could be involved in the first step of cascade testing (i.e., all the living relatives closest to the proband) were included. In addition to first-degree relatives, individuals who were second-, third- or fourth-degree relatives were included if the closest relative(s) was/were deceased. Overall, 213 families were included (103, Genoa; 110, Bologna). Most probands were women affected by breast and/or ovarian cancer (86.4%, Genoa; 84.5%, Bologna), and the branch segregating the PV was known/suspected in 62% of families (62.1%, Genoa; 60.9%, Bologna). Overall, the uptake of cascade testing was 22.8% (25.8%, Genoa; 19.9%, Bologna; OR = 0.59: 95%CI 0.43–0.82). It was strongly associated with female gender (OR = 3.31, 95%CI 2.38–4.59), age ≤ 70 years (< 30 years OR = 3.48, 95%CI 1.85–6.56; 30–70 years OR = 3.08, 95%CI 2.01–4.71), first-degree relationship with the proband (OR = 16.61, 95%CI 10.50-26.28) and segregation of the PV in both the maternal (OR = 2.54, 95%CI 1.72–3.75) and the paternal branch (OR = 4.62, 95%CI 3.09–6.91). These real-world data may be important to inform the design and implementation of strategies aimed at improving the uptake of HBOC cascade testing in Italy.

Keywords *BRCA1/2* · HBOC · Pathogenic variant · Cascade testing uptake · Degree of relationship

Introduction

The Hereditary Breast-Ovarian Cancer (HBOC) syndrome is the most common hereditary cancer syndrome and is generally associated with pathogenic variants (PV) in the *BRCA1* and *BRCA2* genes. Because PVs in these genes confer a substantial risk of developing cancers of the breast, ovary, prostate, and pancreas, identifying healthy *BRCA1/2* carriers and offering them risk-reducing measures of proven efficacy is an important cancer prevention intervention (both primary and secondary prevention) [1]. Indeed, female *BRCA1/2* PV carriers have a risk of breast cancer that is several times that of their non-*BRCA1/2* peers when they are younger than 40 years, well before the youngest recommended age for breast cancer screening in the general population [2]. Moreover, female *BRCA1/2* PV carriers have such a high risk of developing ovarian cancer (40% for *BRCA1*, 20% for *BRCA2*) that, absent proven secondary prevention

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options, salpingo-oophorectomy is recommended around 40 years of age, once the desired family size is reached [2]. For male carriers of *BRCA2* PVs, PSA screening from age 40 has been suggested, given their higher risk of aggressive prostate cancer [3–5]. Screening for pancreatic cancer, instead, is only recommended if there is a family history of the disease (at least one first- or second-degree relative) [5].

Currently, *BRCA1/2* testing is mainly performed in cancer patients to guide their treatment and because for primary or secondary cancer prevention interventions - mainly prophylactic surgery or screening procedures - testing a cancer patient is more informative than testing a healthy relative in the same family.

Different approaches have been proposed to identify healthy *BRCA1/2* PV carriers. One strategy is population screening, which has been strongly advocated as an effort that will "save women's lives and provide a model for other public health programs in genomic medicine" [6]. Recently, population genomic screening has been shown to be likely cost-effective in US adults aged ≤ 40 years, provided the cost of testing is relatively low and cancer prevention interventions are accessible [7]. However, it raises a range of concerns in terms of, e.g., inequitable access, complexity of results interpretation, informed consent, and psychological sequelae [8].

The main strategy remains cascade testing, which is the process of sequentially testing the relatives of the first recognized carrier within a given family, i.e., the proband, starting from her/his closest relatives. This process is now increasingly viewed as an emerging opportunity for population-wide cancer prevention [9]. It has been estimated that assuming a 7% prevalence of PVs across cancer types, an average family size of 3 per generation, and 15% of incident patients with cancers in the United States undergoing germline testing, 10 years would be enough to identify all individuals with a PV in 18 cancer susceptibility genes if 70% of all first-, second- and third-degree at risk relatives were tested for familial PVs [8].

It is not surprising, therefore, that cascade testing of the *BRCA1/2* genes is unvaryingly recommended by clinical guidelines [10, 11]. Yet, this strategy remains vastly underutilized: a recent systematic review and meta-analysis showed that, when information about genetic risk was shared with relatives by the proband, only 30% (24–37, 95% CI) of those relatives underwent *BRCA1/2* cascade testing [12].

Considering these difficulties and the potential role that cascade testing could play in improving access to cancer prevention interventions by individuals with a hereditary risk, several studies have proposed strategies to support it and improve testing uptake [13]. Guidelines have been developed in some countries aimed at improving procedures to inform family members [14], but no standard protocols have been established.

In Italy, cascade testing seems to have received little attention [15, 16] and no recommendations exist to guide clinical practice. The routine approach, when genetic test results are positive, is for genetics professionals to explain their significance for at risk-relatives to probands, encourage them to discuss those results with their family and suggest that they, in turn, seek genetic counseling. In the absence of specific professional recommendations, however, no standardized strategy has been developed to guide the process of intrafamilial communication of genetic risk that cascade testing involves. For instance, many genetics centers give probands a family letter to help with sharing test results and discussing their implications with relatives, but no shared approach has been developed regarding the use and content of the letter.

In order to provide real-world data on the rates of cascade testing in Italy and inform future efforts, we analyzed the uptake of cascade testing of the *BRCA1/2* genes and its characteristics in 213 families diagnosed with HBOC between 2017 and 2019 at two Italian genetics centers.

Materials and methods

Study design and setting

This is an observational, retrospective, multicenter study that took place at two Italian genetic centers: the Unit of Hereditary Cancer (UHC) of the IRCCS Ospedale Policlinico San Martino (HSM), Genoa, and the Unit of Medical Genetics (UMG) of the IRCCS Azienda Ospedaliero-Universitaria of Bologna.

Genetic counseling protocol

The process of genetic counseling included in-person pre-test and post-test counseling, according to standard procedures. Of notice, part of the genetic test disclosure session was dedicated to discussing the importance of the genetic test result for relatives and identifying at-risk family members eligible for the first step of cascade testing. If intrafamilial communication problems were reported, and if considered helpful by the proband, an information letter for the family members was also provided to support information sharing. Although the practice of the two centers was not substantially different, a common protocol was not implemented during the study period.

Study population

Probands

We enrolled cancer patients and cancer-free individuals who were found to carry a PV after undergoing a complete *BRCA1* and *BRCA2* genetic test between May 2017 and December 2019 at the UHC, Genoa or at the UMG, Bologna. The observation period ended on September 30, 2022, to allow a minimum period of observation of three years (range three to five years) for test uptake by the at-risk blood-relatives of the probands enrolled in the study.

At the Genoa center, only probands who consented to participate in the Ligurian *BRCA* Registry (Ligurian Ethics Committee, approval n. 002REG2017), who spoke Italian, and who had the *BRCA* test and post-test counseling at the UHC were included in the study. At the Bologna center, only probands who consented to participate in the REGIO Registry (the registry of individuals undergoing cancer risk assessment at the Bologna center, approved by the CE-AVEC Ethics Board n.272/2022/Oss/AOUBo on 14th April 2022) and who had the *BRCA* test and post-test counseling at the UMG were included in the study.

From the clinical records of probands, we retrieved the following information: gender; date of birth; disease status; type of cancer(s); age at cancer diagnosis; genetic test result; date of genetic test result disclosure; pedigree; cancer family history; branch of the family suspected for HBOC.

Relatives

Relatives of probands included in the study were identified from the pedigrees that were built during counseling sessions. All blood relatives aged 18 years or older at the time of data collection (September 30, 2022) and who could be involved in the first step of cascade testing (i.e., the living relatives closest to the proband) were included. In addition to first-degree relatives (parents, offspring, siblings), individuals who were second-, third- or fourth-degree relatives were included if the closest relative(s) was/were deceased.

Individuals of both maternal and paternal branches of the family were included if no indication of PV segregation was available. When the family branch segregating the PV was known or suspected, only relatives from that side of the family were included.

For the families in which PV segregation was unknown, a 50% probability of carrying the family PV was used for each proband's parent. When the family branch segregating the variant was known/suspected, a 100% probability of carrying the family PV was used for the proband's parent who was a known (or presumed) carrier. Examples of individuals included in the study and their risk of carrying the family PV for pedigrees with unknown or known/suspected family segregation branch are reported in Supplementary Fig. 1 (Supplementary Fig. 1A for pedigrees with unknown family segregation branch, Supplementary Fig. 1B for pedigrees with known/suspected family segregation branch).

For each relative included in the study, the following information was collected from the proband's pedigree: gender; date of birth; disease status; degree of relationship with the proband. When only the year of birth was known, June 30th was used as the day of birth. When the year of birth was also unknown, it was estimated from other information reported in the pedigree (e.g., the age of closest relatives).

Information about genetic testing uptake is presented for relatives who had genetic testing at the same center as the proband. Included individuals who did not undergo testing at the study centers were assumed to have not undergone testing unless a note in the proband chart specifically reported this information. No attempt was made to contact probands to survey testing uptake in their family.

For relatives who underwent genetic testing at the study centers, personal charts were used to amend pedigree information and to collect information about genetic test result and date of genetic test result disclosure.

Data analysis

Data were entered anonymously into a dedicated database and were analyzed using the SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0).

Frequencies, percentages (for categorical variables), means or medians, as appropriate, and interquartile ranges (for continuous variables) were used as descriptive statistics. The Chi-square test was used to compare differences among categorical variables. A binary multivariate logistic analysis was applied to estimate the probability of *BRCA* testing among relatives and the odds ratio (OR) and its 95% confidence intervals (95% CI) were calculated. When the 95% CI of the reported ORs did not include 1.0, the association with the outcome of each specific category, as compared to the reference stratum, was considered statistically significant. The OR estimates were adjusted (adjOR) for the following variables: center, gender, age of the relative (<30 years, 30–70 years, >70 years), age of the proband at testing (<40, 40–49, 50–59, 60–69, ≥ 70 years), proband's reason for testing (prevention, medical treatment, prophylactic mastectomy), relative degree (first, second, third-fourth), family segregation branch (maternal, paternal, unknown).

To estimate the number of PV carriers who were undetected, we focused only on individuals with a 50% and 25% risk of carrying the family PV as these risk figures are undoubtedly to be considered for the first step of cascade

testing while lower risks may be not a priority. Only first- and second-degree relatives have a 50% and 25% risk of carrying the PV (respectively) in families with unknown segregation of the PV. However, these risk figures assume a different distribution in families with a known/suspected segregation of the PV: aunts/uncles have a 50% risk, and first-cousins and great aunts/uncles have a 25% risk (Supplementary Fig. 1).

Results

Between May 2017 and December 2019, all probands who attended the UHC of HSM and carried a *BRCA* PV (80 in *BRCA1* and 49 in *BRCA2*) were enrolled in the *BRCA* registry of the Liguria Region at the Genoa center (n = 129). Of them, 26 did not meet enrollment criteria for the present study: 21 had had *BRCA* testing years before the *BRCA* registry was established, three had not had genetic counseling at HSM, one carried a *de novo BRCA* PV, one did not speak Italian.

In the same period, at Bologna center, all the 110 probands found to carry a *BRCA* PV (54 in *BRCA1* and 56 in *BRCA2*) were included in the REGIO registry; all were eligible for the study.

Overall, 213 families were included in the study: 114 carried a PV in *BRCA1* and 99 in *BRCA2*. The main characteristics of the probands are reported in Table 1. In both centers, most probands (85.4%) were women affected by breast and/or ovarian cancer, and the branch segregating the family PV was known/suspected in 62% of families. In all, 27.1% of the female probands had *BRCA* testing to inform the decision about prophylactic mastectomy at primary surgery for breast cancer (24.0% in Genoa and 27.2% in Bologna).

Including parents, 1,413 relatives were reported (681 at the Genoa center and 732 at the Bologna center). The 213 probands reported 145 living parents, 62 of whom (42.8%) had targeted *BRCA* testing after a PV was identified in the

Table 1 Main characteristics of probands according to gender in the two study populations

	Genoa			Bologna			Total		
Characteristics	Females	Males	Total	Females	Males	Total	Females	Males	Total
	N = 96	N = 7	N = 103	N = 103	N = 7	N = 110	N = 199	N = 14	N = 213
Median age at testing (IQR)	52.0 (40.2–63.7)	69.0 (62.0–78.0)	53.0 (41.0–66.0)	52.0 (43.0–64.0)	64.0 (58.0–71.0)	54.0 (44.0–64.0)	52.0 (43.0–64.0)	65.0 (61.0-73.5)	54.0 (43.0–64.0)
Cancer[1]									
None	6	1	7	1	0	1	7	1	8
Breast	54[a]	0	54	56	4	60	110	4	114
Breast + ovary	8	NA	8	11	NA	11	19	NA	19
Ovary	27[b]	NA	27	26	NA	26	53	NA	53
Pancreas	1	2[c]	3	2	2	4	3	4	7
Prostate	NA	4[d]	4	NA	1	1	NA	5	5
BRCA mutation									
BRCA1	58	2	60	54	0	54	112	2	114
BRCA2	38	5	43	49	7	56	87	12	99
Family segregation branch									
Maternal	37	1	38	30	2	32	67	3	70
Paternal	25	2	27	33	2	35	58	4	62
Unknown	34[e]	4	38	40	3	43	72	7	81
Reason for testing									
Secondary prevention	32	2	34	45	4	49	77	6	83
Prophylactic mastectomy	23	0	23	31	0	31	54	0	54
Medical treatment	41	5	46	27	3	30	68	8	76
N. relatives (mean/family)	644 (6.70)	37 (5.28)	681 (6.61)	664 (6.44)	41 (5.86)	705 (6.40)	1308 (6.57)	78 (5.57)	1413 (6.63)
Females	343 (3.57)	22 (3.14)	365 (3.54)	351 (3.41)	22 (3.14)	373 (3.39)	694 (3.29)	44 (3.14)	738 (3.46)
Males	301 (3.13)	15 (2.14)	316 (2.81)	313 (3.04)	19 (2.71)	332 (3.02)	614 (3.08)	34 (2.43)	648 (3.04)
Gender not reported					0	27 (0.24)		0	27 (0.13)

Note: [1]When two or more cancers had been diagnosed in one patient, the most recent was listed because it was the reason for referral to genetic counseling

[a]10 cases of bilateral breast cancer and 1 case of breast cancer + condrosarcoma

[b]1 case of ovarian cancer + colorectal cancer

[c]1 case pancreatic cancer + prostate cancer and 1 case of pancreatic cancer + breast cancer

[d]1 case of prostate cancer + breast cancer

[e]Both family branches were suspected in 1 case

proband: they were 7/23 fathers (30.4%), 15/38 mothers (39.5%) and 20/42 (47.6%) couples of parents.

Overall, the mean number of relatives per family was 6.63 and was similar at the two centers (6.61 Genoa; 6.40 Bologna). The mean number of tests was 1.48 and a higher mean number of tests per family was reported for the Genoa families (1.71) compared with the Bologna families (0.94). The number of relatives, the total number of tests performed and PV detection rates according to center, degree of relationship with the proband (first- to fourth- degree) and gender are reported in Table 2. Overall, the uptake of cascade testing was 22.8% (29.4% Genoa; 15.3% Bologna). Among first degree relatives, 252 of 568 (44.4%) had cascade tests (55.8% at the Genoa center and 31.3% at the Bologna center) and the highest rate was observed for offspring (63.3%). The rate of cascade testing was 8.6% among second-degree relatives (10.8% in Genoa and 5.7% in Bologna) and 7.1% among third-fourth degree relatives (11.4% in Genoa and 2.9% in Bologna). Overall, females had a higher rate than males (29.4% vs. 15.3%).

Supplementary Table 1 reports the uptake of testing and test results among female relatives aged 30–70 years according to their degree of relationship with the proband: 63.2% of first-degree female relatives aged 30–70 years was tested [78 of 107 (72.9%) in Genoa and 51 of 97 (52.6%) in Bologna] but only 15.4% of second-degree female relatives of the same age range was, in both centers.

Table 3 shows the probability of having cascade testing among relatives: in Bologna the probability was 40% lower than in Genoa (OR = 0.59; 95%CI 0.43–0.82). The probability of having cascade testing among relatives was positively associated with female gender (OR = 3.31, 95%CI 2.38–4.59), age below 70 years (< 30 years, OR = 3.48, 95%CI 1.85–6.56; 30–70 years OR = 3.08, 95%CI 2.01–4.71), first-degree and second-degree relationship with the proband (OR = 16.61, 95%CI 10.50-26.28; OR = 1.79, 95%CI 1.01–3.16, respectively) and segregation of the PV in both the maternal (OR = 2.54; 95%CI 1.72–3.75) and the paternal (OR = 4.62; 95%CI 3.09–6.91) branch of the family.

The relatives of the probands who had the test to decide about prophylactic mastectomy at primary surgery in the Genoa center had a slightly non-significant increased probability of having cascade testing compared to the relatives of the probands who had the test for preventive reasons (OR = 1.35; 95% CI 0.65–2.68); the opposite was observed at the Bologna center (OR = 0.35; 95%CI 0.19–0.65) (Supplementary table 2 S).

The distribution of the time elapsed between the disclosure of test results to the proband and cascade testing in relatives is shown in Fig. 1. The median time elapsed was 3.35 months (IQR 1.11–7.98 months), at the Genoa center and 4.94 months (IQR 2.03–8.60) at the Bologna center) (Supplementary Fig. 2).

Among individuals with a 50% and 25% risk of carrying the family PV, an estimate of PV carriers who were undetected due to the low cascade testing uptake is shown in Table 4. Among the 50% risk individuals (i.e., first-degree and siblings of the parent carrying the family PV in the group of families where the PV segregation was known/suspected; first-degrees only in families where the PV segregation was unknown), a large fraction of PV carriers was missed: 86/192 (44.8%) and 130/292 (44.5%) at the Genoa and Bologna centers, respectively. Given that enrichment of non-carrier women due to breast and ovarian cancer deaths among carriers is expected, the observed detection rate was used to estimate the number of missed carriers among women who were not tested.

Discussion

The main finding of this study is that, overall, 77% of the relatives who were eligible for *BRCA* testing did not have cascade testing. Uptake remained low among first- and second-degree relatives as only 252 of 568 (44%) and 34 of 397 (8.6%) had testing, respectively. Although these results are concerning, they are consistent with the literature [12]. Moreover, our figure is probably an underestimation of the actual family uptake, as we limited our analysis to relatives who were tested at the same genetic center as the proband, but other relatives may have been tested elsewhere.

A significant difference in the uptake of cascade testing was observed at the Genoa and Bologna centers, with 26% and 20% of eligible relatives having had the test, respectively. This finding suggests that some features of the practice and/or of the families at the two centers had an impact on testing uptake. Due to the retrospective nature of the study, we cannot compare specific components of the genetic counseling process at the two centers (e.g., time spent discussing cascade testing, content of the discussion). The only difference that may explain at least in part the different testing uptake was the catchment area of the centers: the Genoa center is the only referral genetic center for HBOC in the region of Liguria, while the Bologna center is one of four HBOC referral genetics centers of the region of Emilia-Romagna [17].

In our study, the probability of having cascade testing among relatives was associated with female gender, first-degree relationship with the proband, paternal segregation of the PV, and age < 30 years. Female gender and first-degree relationship with the proband are known to influence HBOC cascade testing, as reported in the recent systematic review by Frey et al. [12]. In families affected by HBOC,

Table 2 Number of tests performed by the relatives, pathogenic variants detected, and mean number of relatives and test per proband

Center	Age (yrs)	Females			Males			Total		
Family degree										
	(median,IQR)	N.	N. test (%)	N. pathogenic variants	N.	N. test (%)	N. pathogenic variants	N.	N. test (%)	N. pathogenic variants
First degree relatives										
Genoa	**54.0 (41.0–54.0)**	**161**	**105 (65.2)**	**42**	**112**	**39 (34.8)**	**22**	**273**	**144 (52.7)**	**64**
Parents	71.0 (64.0–77.0)	41	25 (61.0)	9	26	14 (53.8)	9	67	39 (58.2)	18
Offspring	35.0 (36.0–43.0)	49	40 (81.6)	19	28	13 (46.4)	7	77	53 (68.8)	26
Siblings	55.0 (46.0–65.0)	71	40 (56.4)	14	58	12 (20.7)	6	129	52 (40.3)	20
Mean N. per proband		1.56	1.02		1.09	0.36		2.65	1.40	
Bologna		**142**	**64 (45.1)**	**24**	**153**	**44 (28.8)**	**33**	**295**	**108 (36.6)**	**57**
Parents	70.5 (65.0–78.0)	42	12 (28.6)	0	36	11 (30.5)	11	78	23 (29.5)	11
Offspring	34.0 (24.0–44.0)	45	32 (71.1)	18	47	22 (46.8)	15	92	54 (58.7)	33
Siblings	54.0 (44.0–66.0)	55	20 (36.4)	6	70	11 (15.7)	7	125	31 (24.8)	13
Mean N. per proband		1.29	0.58		1.39	0.40		2.68	0.98	
Total	**54.0 (39.0–68.0)**	**303**	**169 (55.8)**	**66**	**265**	**83 (31.3)**	**55**	**568**	**252 (44.4)**	**121**
Parents	71.0 (64.7–77.2)	83	37 (44.6)	9	62	25 (40.3)	20	145	62 (42.8)	29
Offspring	34.0 (25.0–43.0)	94	72 (76.6)	37	75	35 (46.7)	22	169	107 (63.3)	59
Siblings	54.0 (45.0-65.2)	126	60 (47.6)	20	128	23 (18.0)	13	254	83 (32.7)	33
Mean N. per probands degree relatives		**1.42**	**0.85**		**1.24**	**0.39**		**2.66**	**1.18**	
Second degree relatives										
Genoa	**71.0 (54-80.75)**	**78**	**10 (12.8)**	**3**	**70**	**4 (5.7)**	**2**	**148**	**14 (9.5)**	**5**
Grandparent	87.0 (83.0–87.0)				3	1 (33.3)	1	3	1 (33.3)	1
Aunt/uncle	77.0 (68.0-82.2)	57	3 (5.3)	0	49	2 (4.1)	1	106	5 (4.7)	1
Niece/nephew	47.0 (41.0–52.0)	20	6 (30.0)	3	17	1 (5.9)	0	37	7 (18.9)	3
Half-sib	67 F, 65 M	1	1 (100.0)	0	1	0 (0.0)		2	1 (50.0)	0
Mean N. per proband		0.76	0.10		0.68	0.04		1.44	0.14	
Bologna	**60.0 (71.0–69.0)**	**145**	**14 (9.6)**	**4**	**104**	**6(5.8)**	**5**	**249**	**20 (8.0)**	**9**
Grand-parent	89.5 (86.7–93.0)	3	0		3	0		6	0	
Aunt/uncle	73.5 (65.0–80.0)	115	5 (4.3)	2	85	4 (4.7)	3	200	9 (4.5)	5
Niece/nephew	50.5 (41.7–59.0)	23	6 (26.1)	0	15	2 (13.3)	2	38	8 (21.0)	2
Half-sib	40.0 (37.5–47.5)	4	3	2	1	0		5	3 (60.0)	2
Mean N. per proband		1.32	0.13		0.94	0.05		2.26	0.18	
Total	**70.0 (59.5–80.0)**	**223**	**24 (10.8)**	**7**	**174**	**10 (5.7)**	**7**	**397**	**34 (8.6)**	**14**
Grand-parent	88.0 (86.5–90.5)	3	0		6	1 (16.7)	1	9	1 (11.1)	1
Aunt/uncle	74.5 (66.0-80.2)	172	8 (4.6)	2	134	6 (4.5)	4	306	14 (4.6)	6
Niece/nephew	48.0 (41.0–54.0)	43	12 (27.9)	3	32	3 (9.4)	2	75	15 (20.0)	5
Half-sib	41.0 (38.0–65.0)	5	4 (80.0)	2	2	0		7	4 (27.1)	2
Mean N. per proband		**1.05**	**0.11**		**0.82**	**0.04**		**1.86**	**0.16**	
Third-fourth degree										
Genoa	**59.0 (52-67.7)**	**126**	**13 (10.3)**	**2**	**134**	**5 (3.7)**	**3**	**260**	**18 (6.9)**	**5**
Great-aunt/uncle	82.0 (79.0–85.0)	3	1 (33.3)	1	2	0 (0.0)	0	5	1 (20.0)	1

Table 2 (continued)

Center	Age (yrs)	Females			Males			Total		
Family degree										
	(median,IQR)	N.	N. test (%)	N. pathogenic variants	N.	N. test (%)	N. pathogenic variants	N.	N. test (%)	N. pathogenic variants
First cousin	59.0 (52.0–67.0)	111	8 (7.2)	0	124	4 (3.3)	3	235	12 (5.1)	3
Second cousin	59.0 (53.2–68.0)	12	4 (33.3)	1	8	1 (12.5)	0	20	5 (25.0)	1
Mean N. per proband		1.22	0.12		1.30	0.04		2.36	0.17	
Bologna	**56.0 (49.0–64.0)**	**85**	**11 (12.9)**	**5**	**75**	**1 (1.3)**	**0**	**160**	**12**	**5**
Great-aunt/uncle	85.0 (81.7–88.2)	0			10	0		10	0	
First cousin	56.0 (49.0–64.0)	83	10 (12.0)	4	65	1 (1.5)	0	148	11 (7.4)	4
Second cousin	48, 61	2	1 (50.0)	1	0			2	1 (50.0)	1
Mean N. per proband		0.77	0.10		0.68	0.009	3	1.45	0.11	
Total	**58.0 (50.0–66.0)**	**211**	**24 (11.4)**	**7**	**209**	**6 (2.9)**	**0**	**420**	**30 (7.1)**	**10**
Great-aunt/uncle	84.0 (81.0–87.0)	3	1 (33.3)	1	12	0	3	15	1 (6.7)	1
First cousin	58.0 (50.0–66.0)	194	18 (9.3)	4	189	5 (2.6)	0	383	23 (6.0)	7
Second cousin	59.0 (53.2–68.0)	14	5 (35.7)	2	8	0	0	22	6 (27.3)	2
Mean N. per proband		0.99	0.11		0.98	0.03		1.97	0.14	
All degrees										
Genoa	59.0 (49.0–71.0)	365	128 (35.0)	47	316	48 (15.2)	27	681	176 (25.8)	74
Mean N. per proband		3.54	1.24		3.07	0.47		6.61	1.71	
Bologna	61.0 (47.0–72.0)	372	89 (23.9)	33	332	51 (15.4)	38	704	140 (19.9)	71
Mean N. per proband		3.38	0.81		3.02	0.46		6.4	0.94	
Total	**60.0 (48.0–72.0)**	**737**	**217 (29.4)**	**80**	**648**	**99 (15.3)**	**65**	**1.385**	**316 (22.8)**	**145**
Mean N. per proband		**3.46**	**1.01**		**3.04**	**0.46**		**6.5**	**1.48**	

Table 3 Relative and proband characteristics associated with the uptake of *BRCA* testing

Covariates	N. tested/total (%)	adjOR	95% CI	P
Center				
Genoa	171/669 (25.6)	1 (REF)		
Bologna	141/705 (20.0)	0.59	0.43–0.82	0.001
Gender				
Males	99/644 (15.4)	1 (REF)		
Females	213/730 (29.2)	3.31	2.38–4.59	0.000
Age class of relatives				0.000§
< 30	39/83 (47.0)	3.48	1.85–6.56	0.000
30–70	233/915 (25.0)	3.08	2.01–4.71	0.000
70+	40/374 (10.7)	1 (REF)		
Age class of probands				0.006§
< 40	62/206 (30.6)	0.82	0.43–1.54	0.53
40–49	52/292 (17.8)	0.43	0.24–0.77	0.005
50–59	92/400 (23.0)	0.72	0.42–1.23	0.23
60–69	61/303 (20.1)	0.47	0.27–0.81	0.007
70+	44/173 (25.4)	1 (REF)		
Proband's reason for testing				0.089§
Prevention	131/524 (25.0)	1 (REF)		
Medical treatment	104/492 (21.1)	0.79	0.55–1.15	0.22
Prophylactic mastectomy	77/358 (21.5)	0.62	0.40–0.96	0.032
Relative degree				0.000§
First	252/568 (44.4)	16.61	10.50-26.28	0.000
Second	31/394 (7.9)	1.79	1.01–3.16	0.045
Third - fourth	29/412 (7.0)	1 (REF)		
Family segregation branch				0.000§
Unknown	90/606 (14.9)	1 (REF)		
Maternal	100/396 (25.3)	2.54	1.72–3.75	0.000
Paternal	122/372 (32.8)	4.62	3.09–6.91	0.000

§chi-square test for heterogeneity over the covariate classes

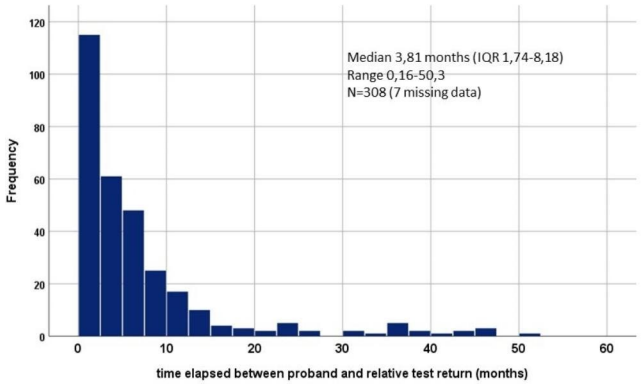

Fig. 1 Time elapsed between the proband test and that one of their relatives

the gender difference in test uptake is explained to some extent by the fact that the benefits of being recognized as a *BRCA* carrier have long been known for women, while men were primarily involved in cascade testing for the benefit of their daughters (if they had any). Also, the importance of the degree of relationship with the proband is somewhat explained by the fact that cascade testing is mediated by the proband, who may have less difficulty initiating conversations about genetic risk with closer relatives. In a survey of young adults, the majority (58.5%) reported having received the information about the family PV from one or both parents in an unplanned conversation [18]. Interestingly, both in this and in other studies [19, 20] offspring had testing significantly more often than siblings. In our study, the rate of testing among second-degree relatives was far lower than reported in other studies [21–23], suggesting that information sharing outside the nuclear family may be particularly difficult in our country. Also, both in our analysis and in the study by Gauna Cristaldo and colleagues [24], the uptake of cascade testing was higher when the family PV segregated in the paternal branch. This finding is opposite to what reported by other studies in which the uptake was higher when the family PV segregated in the maternal branch [25, 26]. Social and cultural beliefs shape perceived social pressures creating subjective norms that influence the intention to communicate in a negative (e.g., male stoicism) or positive (e.g., fatherly protection) way [26]. One hypothesis might be that, in some cultural contexts (e.g., in Italy), masculinity is more associated with notions of

Table 4 Number of tests performed among relatives according to the risk of being a *BRCA* pathogenic variant carrier and estimate of the pathogenic variants potentially undetected among relatives who did not have the *BRCA* test

	Genoa				Bologna				Total			
	Females[a]		Males		Females[a]		Males		Females		Males	
	50%	25%	50%	25%	50%	25%	50%	25%	50%	25%	50%	25%
n. individuals	203	92	140	95	208	140	203	104	410	229	344	199
n. tests	109	14	42	5	72	17	47	3	180	28	89	8
n. pathogenic variants	43	3	24	3	28	4	35	3	71	7	59	6
n. individuals not tested	94	78	98	90	136	123	156	101	230	201	255	191
n. pathogenic variants potentially lost	37	16	49	22	52	28	78	25	90	50	127	47

[a]For female relatives the observed percentage of positive tests was used to estimate the number of missed carriers (figures are rounded down)

family protection, leading to male family members strongly encouraging relatives to seek out testing.

In contrast with previous studies [22, 24, 27], we found that relatives younger than 30 years old were prone to have cascade testing. In a study on intrafamilial communication of genetic information in Italian women belonging to families affected by HBOC, younger women were more likely than other probands to attend genetic counseling sessions with a family member and to talk about those sessions with their relatives [15]. Moreover, qualitative interviews conducted with young adults undergoing cascade testing in Bologna showed that the appointment for pre-test counseling was often made by their parents, suggesting a more active role of the family in promoting test uptake in the younger population [28].

Finally, the mean time elapsed between the disclosure of test results to the proband and cascade testing of relatives was 3–5 months. A similar finding was reported by others [22], suggesting that most of the probands' efforts at sharing genetic risk information with their relatives take place relatively soon after the disclosure of test results.

Because this is an observational, retrospective study, we were not able to explore what the actual barriers to HBOC cascade testing may have been in the families seen at our two centers. However, to the best of our knowledge, this is the first study to report on the uptake of *BRCA* cascade testing in Italy. In addition, as far as we are aware, most Italian genetics centers where clinical *BRCA* testing is performed adopt the same approach followed at our two centers. Therefore, this analysis provides real-world data that may be important to inform the design and implementation of strategies aimed at improving the uptake of HBOC cascade testing in Italy. However, the validity of our observations needs to be confirmed by studies that evaluate the uptake of HBOC cascade testing by collecting information about all the *BRCA* tests conducted in families, regardless of where they were performed. In addition, prospective studies that include the adoption of support interventions are needed to identify organizational and socio-cultural factors that may influence the uptake of HBOC cascade testing at Italian centers. Qualitative studies would be useful to generate understandings of how Italian probands view their role as information givers and help design of tools/strategies to support them in this role. In addition, future qualitative and quantitative studies may also focus on decision-making about *BRCA* testing [29] specifically against the background of cascade testing. For example, the Swiss multicenter CASCADE study is producing evidence on probands' intention to inform relatives, the preference for patient-mediated versus provider direct communication, and reasons for forgoing cascade testing in a cohort of HBOC and Lynch syndrome at-risk relatives [30–32].

In addition to strategies centering around proband-led communication of genetic risk with relatives, proactive interventions in which genetics professionals directly contact the proband's relatives to invite them to genetic counseling deserve attention as evidence showing that, compared to the traditional proband-mediated approach, direct communication with at-risk relatives by healthcare professionals improves uptake rates [12] has been reported. At the same time, however, Menko et al. have found that the implementation of the Dutch guidelines on cascade testing that included several support strategies (i.e., family letter, periodic active follow-up, direct contact of relatives by healthcare professionals) did not result in a significant increase of the uptake of HBOC cascade testing and that only 50% of relatives who were directly contacted had testing [33]. This finding suggests that future Italian studies should focus not only on the support of intrafamilial communication but also on the communication process between professionals and at-risk relatives. In addition to genetic professionals, such studies should include oncologists involved in mainstreaming programs for treatment-oriented *BRCA* testing and other professionals involved in HBOC high-risk prevention (e.g., breast screening/surgeon, gynecologists) as networking approaches in which geneticists share the responsibility of cascade testing programs with other professionals may be a strategy that will allow to overcome barriers (e.g., understaffing at cancer genetics centers) and exploit synergies (e.g., periodic follow-up at oncology, breast and gynecological clinics).

In conclusion, based on the low uptake among first- and second-degree relatives observed in this study, most individuals belonging to the hundreds of HBOC families identified in Italy every year will not be able to access genetic counseling and testing, and the ones who carry *BRCA* PVs will miss the opportunity of potentially life-saving preventive measures. Therefore, it is crucial that research efforts and innovations in clinical practice be directed at improving cascade testing in our country, possibly within the framework of shared processes involving the interested national scientific societies e.g., SIGU (the Italian Society of Human Genetics) and AIFET (the Italian Association of Familial and Hereditary Cancer) and Italian patient advocacy groups (e.g., aBRCAdabra, a national association of *BRCA* PV carriers and their families).

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Data availability The data underlying this article will be shared on reasonable request to the corresponding author.

Declarations

Ethical approval At the Genoa center, no specific ethics approval was required for this study as, by providing their written consent to be included in the Ligurian *BRCA* registry, individuals gave permission to retrieve information from their clinical records for research purposes related to HBOC. The Ligurian *BRCA* registry was approved by the Ligurian Ethics Committee (approval n. 002REG2017). At the Bologna center, theREGIO (Registro mono-istituzionale di individui sottoposti a valutazione del rischio genetico oncologico) Registry was approved by the CEAVEC Ethics Board (approval n.272/2022/Oss/AOUBo, 14th April 2022).

Competing interests Eva Blondeaux reports research grant (to the Institution) from Gilead Science outside the submitted work. All the other authors declare no conflict of interest.

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