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Revisiting the implications of positive germline testing results using multi-gene panels in a cohort of individuals with personal and/or family history of Breast cancer

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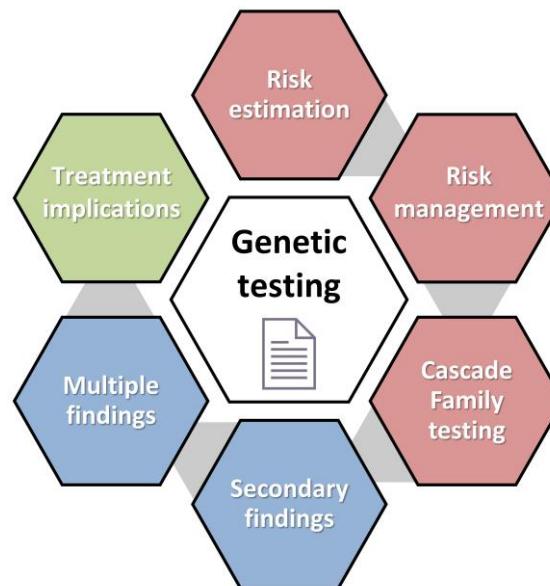
Simple Summary:

Here we present data to demonstrate the different levels of actionability for multi-gene panels used in genetic testing of breast cancer patients and their family members. Selected cases are used to demonstrate the clinical utility of findings in high, moderate, and low breast cancer risk genes. Analysis of our data outlines emerging features in genetic testing reports; moderate and low risk pathogenic variants, secondary information on other cancer risks, double pathogenic variants and treatment implications of germline findings add an anticipated complexity in genetic reports but enable a comprehensive personalized risk estimation and aid clinical decisions. These emerging aspects must be properly incorporated in pre/post testing genetic counseling and test reports.

Abstract: The use of multi-gene panels for germline testing in breast cancer enables the estimation of cancer risk and guides risk-reducing management options for tested individuals and their family members. We performed an analysis in our clinical database to identify breast cancer patients undergoing genetic testing with positive reports. We reviewed positive results with respect to the different levels of information provided in the reports; risk estimation and management, cascade family testing, information from secondary findings and actionable information for treatment decision-making. A total of 415 positive test reports were identified with 57.1%, 18.1%, 10.8% and

13.5% of individuals having pathogenic/likely pathogenic variants in high (*BRCA1, BRCA2, PALB2, PTEN, TP53*), moderate (*ATM, CHEK2, NBN*), low (*BARD1, BRIP1, CHEK2, MLH1, MSH2, MSH6, NF1, RAD51C*) and with insufficient evidence for breast cancer risk genes (*FANCA, FANCM, NBN, MRE11, PMS2, RAD50, RAD51B, XRCC2, MUTYH*), respectively. 6.7% of individuals were double heterozygotes with two pathogenic variants. Germline findings in 92% of individuals are linked to evidence-based treatment information and receive risk estimates for predisposition to breast and/or other cancer types. The use of germline findings for treatment decision making expands the indication of genetic testing to include individuals that could benefit from targeted treatments.

Keywords: germline testing; NGS; breast cancer; genetic counselling; risk assessment;

Note: Authors are encouraged to provide a **Graphical Abstract** as a self-explanatory image to appear alongside with the text abstract in the Table of Contents. Figures should be a high-quality image in any common image format, and must be different from other figures in the main text. Note that images displayed online will be up to 11 by 9 cm on screen and the figure should be clear at this size.

1. Introduction

During the last two decades, genetic testing for breast cancer susceptibility has evolved to an integral part of medical practice [1]. In the early years of genetic testing, only *BRCA1/2* genes were analyzed for pathogenic variants to investigate heredity in breast cancer patients and members of their families. Additional predisposition genes, that are associated with different levels of breast cancer risk, have been characterized and account for approximately 50% of pathogenic variants identified [2,3]. The advent of the Next Generation Sequencing (NGS) technology allowed to incorporate these genes in breast cancer testing through the development of multi-gene panels [4-6]. Sequencing has been made affordable for more patients and this resulted in an expansion of genetic data that facilitates the study of hereditary breast cancer and expands the clinical utility of genetic tests. New data provide important feedback/evidence for the use and translation of additional high, moderate, and low penetrance genes in routine clinical testing for breast cancer. This is evident in the recent versions of the NCCN Genetic/Familial High-Risk Assessment guidelines for Breast, Ovarian, and Pancreatic cancer that have been updated to incorporate the emerging information of newer added cancer suscepti-bility genes and to expand testing criteria for *BRCA1/2* genes to "Testing Criteria for High-Penetrance Breast and/or Ovarian Cancer Susceptibility Genes" [7].

Multi gene testing, although cost-effective, introduces some challenges to the use of the results for decision making in clinical practice. For some genes there are limited data and therefore not clear guidelines for risk determination and management [8]. Secondary findings that give information about the predisposition for other common cancer types discover individuals with increased genetic risk for cancer and consequently add to the actionability of multi gene panels [9].

Advances in the landscape of breast and ovarian cancer treatment mainly through the development, use and drug approvals of poly (ADP-ribose) polymerase (PARP) in-hibitors has added the potential to use germline testing results as a predictive biomarker. Moreover, the correlation of defects in Mismatch repair (MMR) genes with Microsatellite Instability (MSI) enables the use of germline findings in these genes as predictors of the efficacy of cancer immunotherapy using immune checkpoint inhibitors [10,11]. This has expanded the clinical indication of germline testing from the identification of high-risk individuals where it is likely to impact the risk management, to individuals who could also get actionable information for systemic therapy decision making [7].

Interestingly, the accumulated data, show patients with mutations in more than one gene (double heterozygotes) that remain to be interpreted for their association with clin-ical risk and the potential therapeutic use [12]. Today, genetic reports have changed to incorporate cancer risk calculations through the development of statistical models and tools that take into account personal and family history information [13]. Subsequently, test reports act as genetic counseling resources for patients and healthcare professionals.

Here we present data that support the different levels of actionability for multi-gene panels. We describe and discuss the emerging features of genetic testing and reporting in breast cancer based on our data. Furthermore, selected cases outline that the evolution in cancer susceptibility genetic testing has created an increased demand for clarity in clini-cal reporting and communication of genetic tests and their implications.

2. Materials and Methods

We conducted a retrospective analysis of individuals referred for germline genetic testing using a multi-gene NGS panel in our laboratory. We used our clinical database to select referrals of breast cancer patients or healthy individuals that undertook cancer susceptibility genetic testing due to family history of breast cancer. Positive reports with pathogenic (P)/likely pathogenic (LP) variants and/or large genomic rearrangements were further analyzed. Prior to genetic testing all individuals had provided informed consent and permission for the anonymous use of their data for research purposes and/or scientific publications. Information on demographics, clinical history, and family history

of cancer was collected from test requisition forms, and pedigrees that had been provided by the ordering clinicians at the time of testing in our data archive.

All selected individuals had been referred for germline genetic testing using a multi-gene NGS panel as described in our previous study [14]. Genes were further classified as high, moderate, low, and unknown penetrance genes based on their relative risk for cancer development that they confer to pathogenic variant carriers as described in [14] and [7] (Table S1).

Statistics were performed with R (version 3.5.3). The p-values were based on Fisher's Exact Test. A p-value <0.05 was considered statistically significant.

The study was approved by the ethics committee of Hellenic Breast Surgeons Society on the 7th of May 2021.

3. Results

We reviewed a total of 415 cases where a pathogenic (P) or likely pathogenic (LP) variant was identified in a breast cancer patient or an unaffected individual with family history of breast cancer. P and LP variants were identified in 24 out of the 36 genes tested. Reports with positive findings were grouped according to the associated breast cancer risk of the gene in the following subgroups: high, moderate, low risk and insufficient evidence for breast cancer risk genes (unknown risk) as described in Methods (Figure 1). Moreover, a special category of positive findings were cases where two P/LP variants were detected in two different genes or the same gene (double heterozygotes). 391 out of the 415 (94.2%) individuals had a personal history of breast cancer, and the remaining 24 individuals were unaffected with at least one first degree relative with personal history of breast cancer. Average age of diagnosis and testing were 44 and 46 years old, respectively.

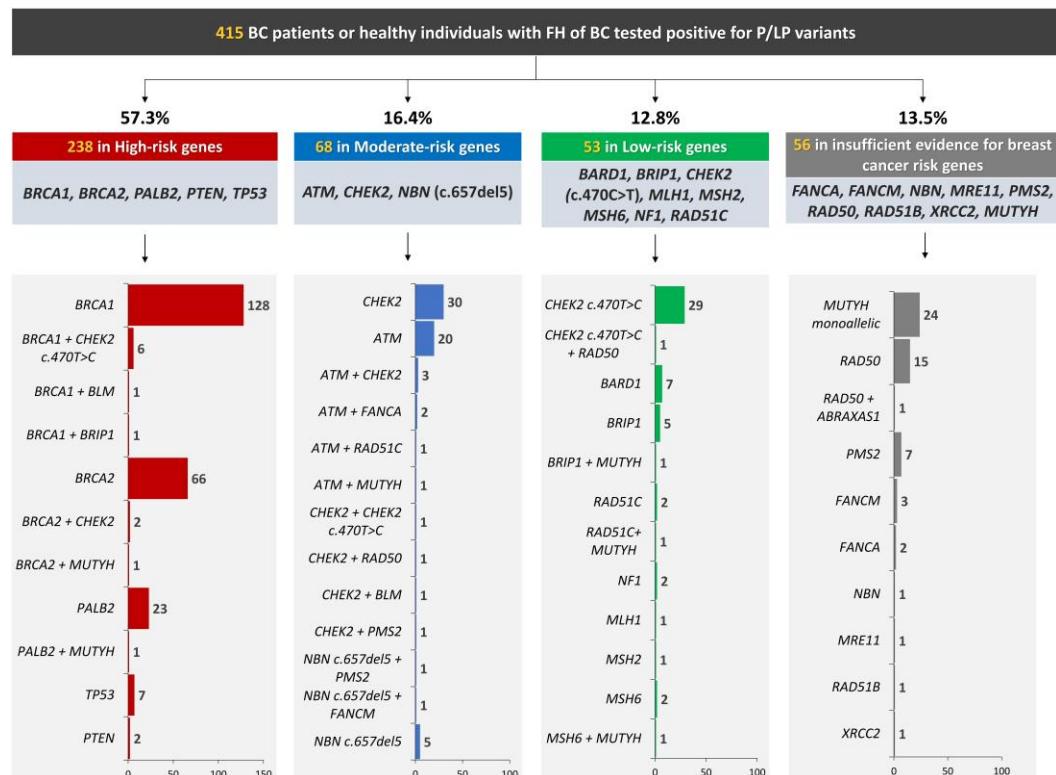



Figure 1. Summary of Individuals with pathogenic/likely pathogenic variants categorized by breast cancer risk. In each group the gene incidence of pathogenic/likely pathogenic variants and double heterozygotes are shown. *CHEK2*:c.470C>T and *NBN*:c.657del5 results are separated with variant specific breast cancer risk levels.

3.1. High risk genes for Breast Cancer

A P/LP variant in a risk high breast cancer gene was identified in 238/415 (57.3%) of the positive cases and specifically in *BRCA1* (136/415, 32.8%), *BRCA2* (69/415, 16.6%), *PALB2* (24/415, 5.8%), *PTEN* (2/415, 0.5%), and *TP53* (7/415, 1.7%). P/LP variants in *BRCA1/2* genes account for 49.4% (205/415) of positive findings and 86.5% (205/238) of positive findings in high-risk breast cancer genes.

3.2. Moderate risk genes for Breast cancer

A P/LP variant in a moderate risk for breast cancer gene was identified in 68/415 (16.4%) of the positive cases and specifically in *CHEK2* (37/415, 8.9%) and *ATM* (27/415, 6.5%). The moderate risk pathogenic variant c.657del5 (p.Lys219Asnfs*16) in *NBN* was identified in 1.7% (7/415) of positive cases.

3.3. Low risk genes for Breast cancer

A P/LP variant in a low risk for breast cancer gene was identified in 53/415 (12.8%) of positive cases. The majority of cases (56.6%, 30/53) carried the low penetrance c.470T>C (p.Ile157Thr) pathogenic variant *in CHEK2*. The remaining cases had P/LP variants in *BARD1, BRIP1, MLH1, MSH2, MSH6, NF1* and *RAD51C*.

3.4. Genes with insufficient evidence for breast cancer risk

In 56/415 (13.5%) of positive results, tested individuals carried a mutation in a gene where there is limited or insufficient evidence for the association with breast cancer risk (*FANCA, FANCM, NBN, MRE11, PMS2, RAD50, RAD51B, XRCC2, MUTYH*). The majority of the cases in this category were *MUTYH* heterozygotes (monoallelic) (42.9%, 24/56) and RAD50 pathogenic variant carriers (28.6%, 16/56).

3.5. Double heterozygotes

In 30/415 (7.2%) of the positive cases a P/LP variant was identified in two different genes (double heterozygotes). The majority of double heterozygotes (16/30, 53.3%) carried a variant in the *CHEK2* gene, with the low penetrance c.470T>C (p.Ile157Thr) pathogenic variant being the most common alteration found in this category (8/30, 26.7%). In six cases (6/30, 20.0%) the second P/LP alteration was a heterozygous variant in *MUTYH* (*MUTYH* monoallelic). Other genes with pathogenic alterations in double heterozygotes were *ATM, BRCA1, BRCA2, BLM, BRIP1, ABRAXAS1, NBN, FANCA, FANCM, MSH6, PALB2, PMS2, RAD50 and RAD51C*. Detailed information is included in Table S2.

3.6. Large genomic rearrangements (LGRs)

Notably, in 36/415 (8.7%) of individuals a large genomic rearrangement, referring to the deletion of one or more exons of a gene, was detected. Of the 36 LGRs detected, 21 occurred in *BRCA1*, 7 in *CHEK2*, 3 in *PMS2*, 2 in *FANCA*, and 1 in each of the *ATM*, *BRCA2* and *MSH2* genes. In four cases (2 with *CHEK2*, 1 with *FANCA* and 1 with *PMS2*) the individual carried a second P/LP variant (double heterozygote).

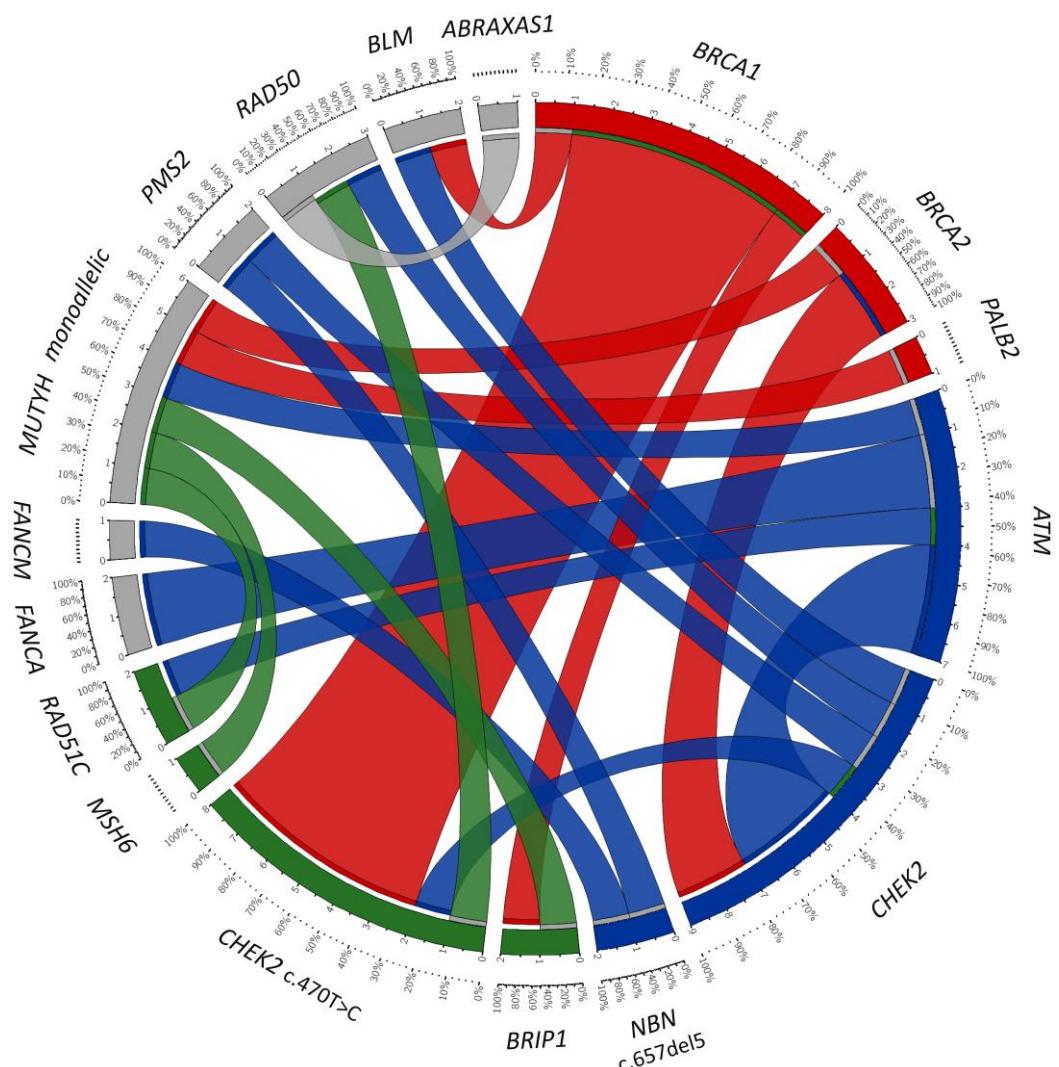



Figure 2. Combinations of genes in cases with two pathogenic variants (Double heterozygotes). High-, moderate-, low- and insufficient evidence for breast cancer risk genes are highlighted with red, blue, green, and grey ribbons/tracks, respectively. Visualization was performed using Circos [15].Additional information about these cases is available in Table S2.

4. Discussion

4.1.Testing Selection criteria

Although the updated guidelines describe the clinical indication of genetic testing to a much broader group of individuals, still approximately 10% (41/415) of positive for pathogenic variants individuals would have been missed if strict selection were applied (as described in NCCN guidelines [16]). Despite the recent updates, selection criteria based on personal and family history information perform better in identifying *BRCA1/2* and other high breast cancer risk positive individuals compared to individuals with pathogenic variants in other breast cancer associated moderate and low risk genes (Table 1.). These families, due to the fact that they carry pathogenic variants in genes with lower penetrance, fail to exhibit the characteristics of high-risk families and therefore be selected for genetic testing due to family history information [17].

Meeting NCCN selection criteria?	All tested positive	*BRCA1/2*	High risk	Moderate risk	Low risk	Insufficient evidence

Yes	374 (90.1%)	196 (95.6%)	227 (95.4%)	65 (86.7%)	38 (84.4%)	44 (77.2%)
No	41 (9.9%)	9 (4.4%)	11 (4.6%)	10 (13.3%)	7 (15.6%)	13 (22.8%)
p-value*	0.0217	-	0.9195	0.0086	0.0056	< 0.0001

Table 1. The performance of NCCN selection criteria (Version 2.2021) for genetic testing in breast cancer to identify individuals with pathogenic/likely pathogenic variants in each gene risk group.

* Evaluation of the statistical significance of the statistical significance of the difference on the selection performance (% yes) of each group compared to the *BRCA1/2* group.

4.2. High risk genes for Breast cancer

Approximately half of individuals tested positive had pathogenic variants in genes with very strong or strong evidence for increased risk of breast cancer and specifically >60% absolute lifetime risk. Consequently, these genes (*BRCA1*, *BRCA2*, *PALB2*, *PTEN* and *TP53*) have specific clinical management guidelines which often include the discussion of risk reducing surgeries (RRM) for breast cancer. Some of these genes are associated with cancer syndromes (*PTEN* - Cowden Syndrome, *TP53* – Li Fraumeni Syndrome) which have specific management recommendations. Moreover, these patients are informed about the additional evidence for high, moderate or low risk association with other cancer types (e.g. Ovarian, Pancreatic, Prostate, Colorectal, Endometrial) based on their test results. Therefore, genetic testing in these cases provides actionable information that is used for the guidance of risk reduction management decisions. Additionally, such results assist towards the identification of at-risk family members who would benefit from cascade family testing since the higher risk association and the specific management guidelines can act as convincing factor and an additional motive for other members of the family to undergo genetic testing.

An interesting example of a family in this category is depicted in Fig.3. A 43-year-old female (III:2) was diagnosed with Breast cancer with no family history of breast cancer. Genetic testing was clinically indicated as she developed breast cancer at age <45 years. Multi-gene panel testing revealed the *BRCA1* pathogenic variant NM_007294.4(*BRCA1*):c.181T>G (p.Cys61Gly) and the low penetrance pathogenic variant NM_007194.4(*CHEK2*):c.470T>C (p.Ile157Thr) in *CHEK2*. The proband receives important information about her breast cancer management with the discussion of risk-reducing options and/or increased screening as she has an increased contralateral breast cancer risk [18] (45% lifetime risk up to the age of 85).

In addition, her *BRCA1* positive result provides treatment recommendations as international guidelines suggest treatment with PARP inhibitors for patients with germline or somatic *BRCA1/2* mutations. Moreover, she receives information for her increased risk of other cancer types as ovarian and pancreatic cancer. In specific she has a high risk of ovarian cancer with available risk-reductions management recommendations and a moderate risk for pancreatic cancer that could advice an increased surveillance although there is no family history of these cancer types. The low penetrance variant in *CHEK2* may contribute to her increased risk for breast cancer and increases the risk for colorectal cancer suggesting earlier screening for colorectal cancer than the age of 50. First degree relatives of the patient have up to 50% risk of having the same variants and genetic counseling was provided to the family. The mother of the patient (II:3) was tested for the same variants but carried only the pathogenic variant in *BRCA1*. This information enables more aggressive screening of this family member for breast, ovarian and pancreatic cancer. Moreover, the combination of the absence of the *CHEK2* variant and her health status may suggest a synergistic effect of the multiple pathogenic variants in her daughter. Experimental studies have shown that this missense variant in *CHEK2* reduces the binding of the CHEK2 protein to Cdc25A, BRCA1 and p53 proteins in vitro and may have a dominant-negative effect in cells, although it does not have an effect on CHEK2 protein kinase activity [19-23]. Genetic testing in this family affects multiple family

members and the results provide evidence for an increased risk of additional cancer types in the family and assist towards cancer prevention actions.

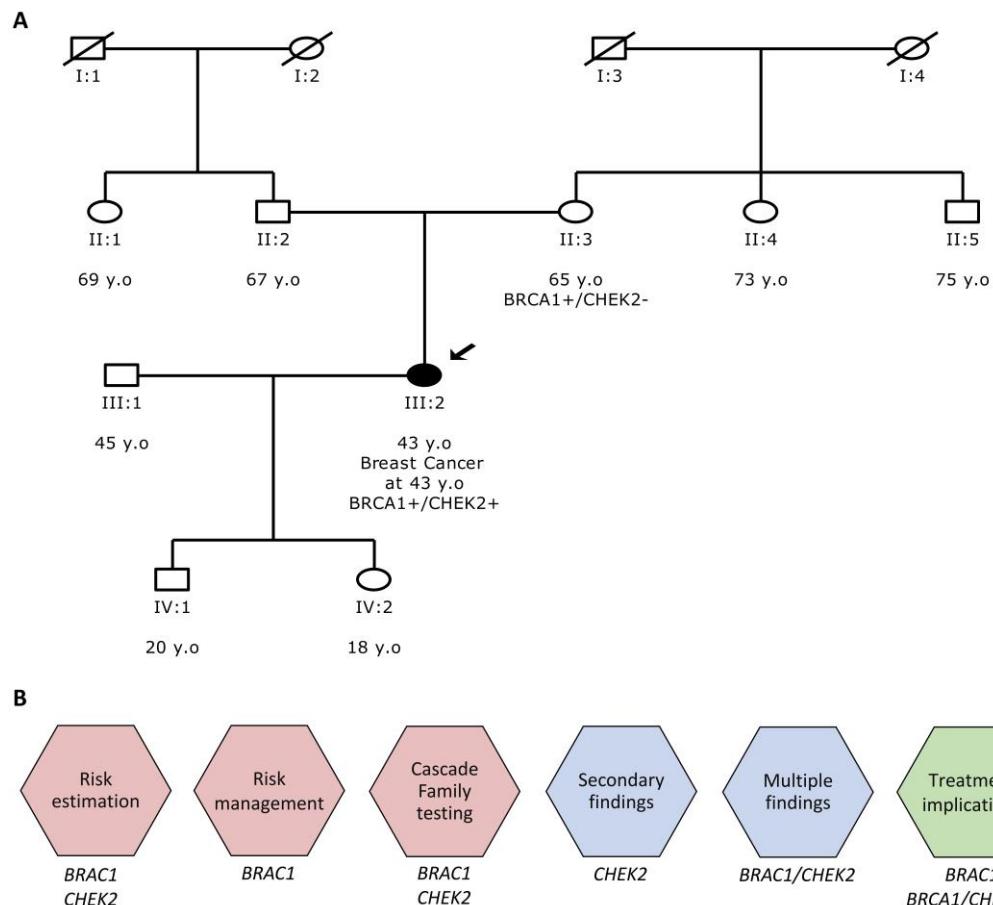



Figure 3. Example of a patient (double heterozygote) with a pathogenic variant in a high-risk gene (*BRCA1*) and a low penetrance pathogenic variant in a moderate risk gene (*CHEK2*). A. The pedigree of the family, B. The clinical actionability of germline testing in this family through the different levels of information received after the disclosure of the results. Due to the identification of two pathogenic variants this information differentiates between the two findings and/or their co-occurrence.

4.3. Moderate risk genes for Breast cancer

A substantial percentage of individuals tested positive in breast cancer (approximately 16%) have P/LP variants in moderate penetrance genes (*ATM, CHEK2, NBN*). In particular for the *ATM* and *CHEK2* genes there is strong evidence for the risk association but with a lower absolute lifetime risk ranging from 15-40%. Screening and risk-reduction management in international guidelines is mainly extrapolated in these cases by *BRCA1/2* data based on the levels of risk. Management takes into account family history information and further clinical data that are available to physicians in each case. However, there is an adequate amount of data from case-controls studies to calculate personalized risk levels for carriers of *ATM* and *CHEK2* pathogenic variants.

In addition, there are specific alleles, especially the c.470T>C variant in *CHEK2* that are associated with a lower risk for breast cancer and should be considered when reporting such variants and using them to guide management. Although not present in our cohort, there are also certain *ATM* pathogenic variants that are associated with an increased (high) risk for early onset breast cancer and bilateral breast cancer [24,25].

The majority of the cases with pathogenic variants identified in *NBN* (7 out of 8) carried the 657del5 frameshift causing variant (p.Lys219Asnfs*16) that has been described as a pathogenic founder variant of Slavic and Eastern European origin and is the most common pathogenic variant in patients with the related autosomal recessive con-

dition called Nijmegen Breakage syndrome. There is evidence for increased breast cancer risk for carriers of the c.657del5 variant in *NBN* compared to a lower or non-significant risk for carriers of other pathogenic/likely pathogenic variants in this gene [8]. Therefore, these variant reports should include variant specific information about the associated risk along with information about the mutated gene as in this category there are certain examples of variants or variant types that have adequate data to calculate variant-level associated breast cancer risks. Variant specific cancer risks should be taken into consideration when interpretating test results for moderate penetrance genes.

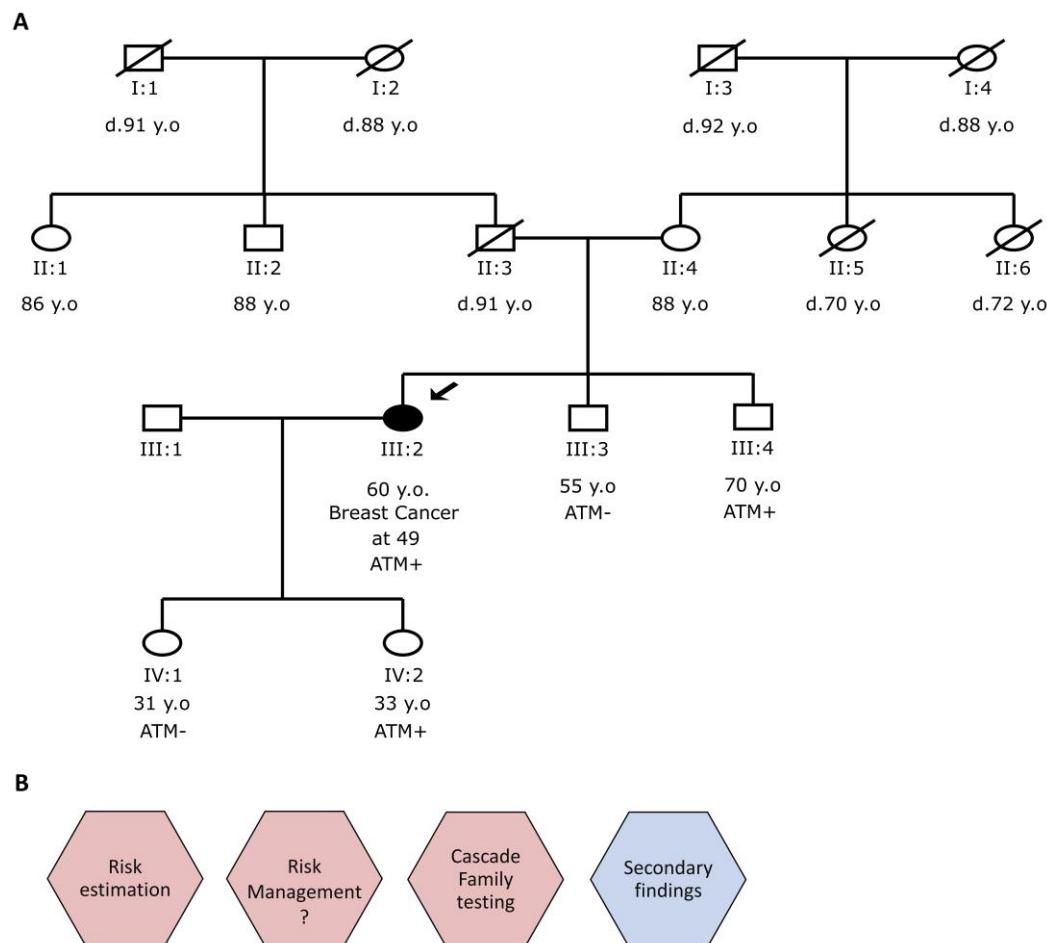



Figure 4. Example of a patient with a pathogenic variant in a moderate risk gene (*ATM*). **A.** The pedigree of the family, **B.** The clinical actionability of germline testing in this family through the different levels of information received after the disclosure of the results.

In Figure 4 we describe the example of a family with no family history of breast cancer and genetic testing not clinically indicated according to international guidelines. The female proband was diagnosed with breast cancer at the age of 49 and was tested at the age of 60. Multi-gene panel testing identified the NM_000051.3(*ATM*):c.8988-1G>C pathogenic variant. This variant is expected to result in incorrect splicing and removal of the entire exon in the resulting ATM protein and has been described in the international literature in association with ataxia-telangiectasia [26]. This test result may explain her personal history of breast cancer and better estimates her risk for breast cancer providing evidence to guide her management options. Moreover, she is informed about her slightly increased risk of ovarian cancer and a moderate risk (~4% to the age of 85) of pancreatic cancer. The association of *ATM* with colorectal cancer is not well established so her associated colorectal cancer risk is uncertain. After genetic counselling provided to the family, four members proceeded with cascade family testing. Her two brothers (III:3 and III:4) and her two daughters (IV:1 and IV:2) were tested for the identified pathogenic *ATM* variant and individuals III:4 and IV:2 were found positive. Individuals IV:1 and

III:3 are informed that they do not have an elevated cancer risk at least for the portion of the *ATM*-associated cancer risk in their family. On the other hand, her brother (III:4) is getting information about his increased *ATM*-associated risk of pancreatic and prostate cancer and her daughter (IV:2) about her moderately increased risk of breast and pancreatic cancer. Such information could guide screening of these individuals towards cancer prevention.

4.4. Low risk genes for Breast cancer

Pathogenic variants identified in *BARD1, BRIP1, MLH1, MSH2, MSH6, NF1* and *RAD51C* are associated with a potential lower absolute lifetime risk for breast cancer (<15%) but with insufficient evidence in most cases for an accurate estimation. In these cases, management associated with breast cancer risk is mainly based of personal and family history characteristics. However, genes in this category are often associated with other cancer types with enough evidence to make specific management recommendations, such as ovarian and colorectal cancer. In our cohort, 17% (9/53) of cases in this category had a P/LP variant in high-risk genes for ovarian cancer (*BRIP1, RAD51C*) and 9.4% (5/53) had a P/LP variant in high-risk colorectal cancer genes (*MLH1, MSH2, MSH6*). These individuals, although they do not receive specific actionable information for breast cancer, they are presented with information about other cancer types and their personalized risk, so as to make informed decisions and begin screening surveillance. This potential scenario, of test results in this category, should be discussed in detail during their pre-test genetic counselling.

An example of a family in this category is described in Fig.5. A 38-year-old female (III:1) diagnosed with breast cancer was referred for testing and reported family history of ovarian and pancreatic cancer from her mother's family side. The pathogenic variant NM_032043.3(*BRIP1*):c.2392C>T (p.Arg798Ter) was identified. *BRIP1* is described as a low-risk breast cancer gene and guidelines suggest breast cancer management based on family history as there are insufficient data for an accurate risk estimation. This variant has been described as a recurrent disease-causing mutation in both Fanconi anemia type-J (FA-J) and breast cancer patients [27-31]. However, this patient is also introduced with an additional increased risk of ovarian cancer as there is strong evidence for high risk (>10%) of ovarian cancer in carriers of *BRIP1* pathogenic/likely pathogenic variants. Multi gene panels identify pathogenic variants in genes not primarily associated with the referred phenotype. However, such genes provide information for predisposition to other cancer types. In this regard, the *BRIP1* variant in this individual is regarded as a secondary finding. We argue that this term should not imply an unexpected finding but rather additional information that is the benefit of using multi-gene panels. In this case, the *BRIP1* pathogenic finding is clinically significant, and actionable information as international guidelines suggest the consideration of risk-reducing salpingo-oophorectomy at the age of 45-50y for the prevention of ovarian cancer. Moreover, since *BRIP1* is a gene involved in the homologous recombination pathway she receives information about potential response to treatment with PARPi [32] as their use is approved for *BRIP1* and other HR genes with somatic variants in prostate cancer [33] and is under investigation for HR genes in several ongoing clinical trials (Table). First degree relatives of the patient have up to 50% risk of having the same variants and genetic counseling was provided to the family. The mother of the patient (II:5) was tested and found positive for this pathogenic variant in *BRIP1*. As being a healthy individual, she receives valuable information for increased surveillance for breast and ovarian cancer based on her family history of cancer and may consider the option of risk-reducing interventions for the prevention of ovarian cancer. Genetic testing in this family affects multiple family members and illustrates the effectiveness of testing other members at risk.

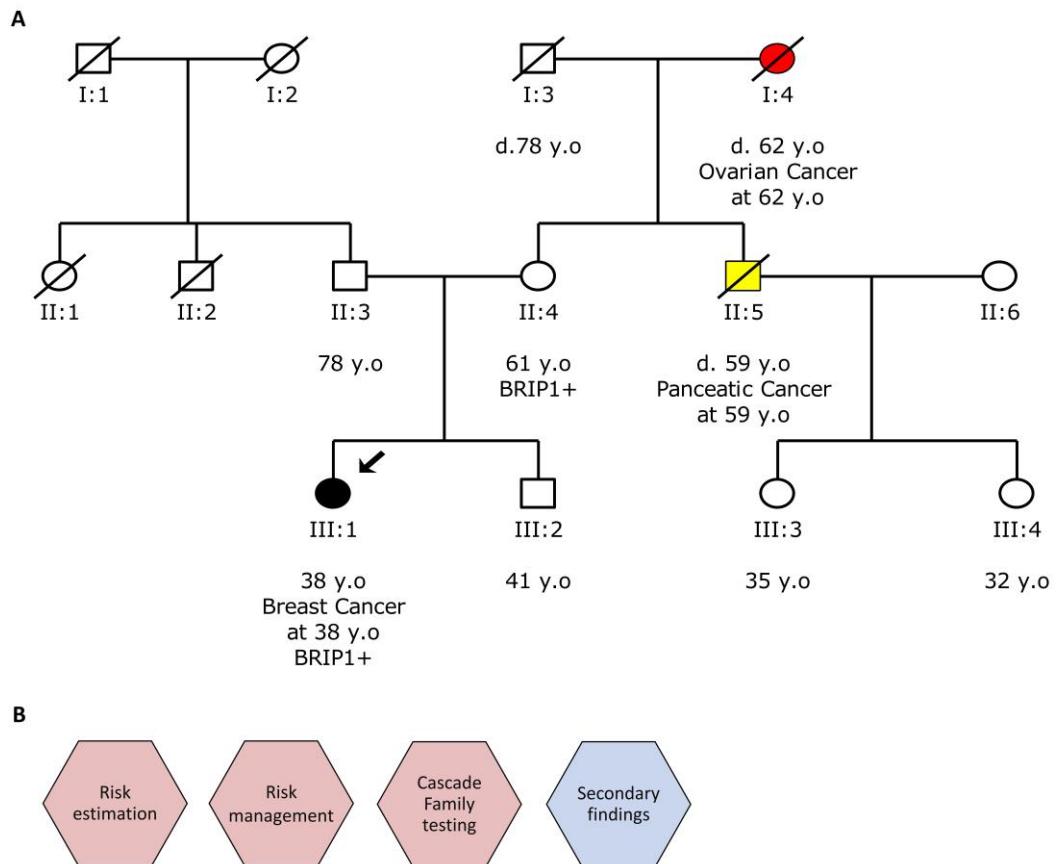



Figure 5. Example of a patient with a pathogenic variant in a low-risk gene (*BRIP1*). **A.** The pedigree of the family, **B.** The clinical actionability of germline testing in this family through the different levels of information received after the disclosure of the results.

4.5. Genes with insufficient evidence for breast cancer risk

A small percentage of P/LP variants (56/415) are identified in genes (*FANCA, FANCM, NBN, MRE11, PMS2, RAD50, RAD51B, XRCC2, MUTYH*) with unknown risk for breast cancer and insufficient evidence of further association. These genes are often included in routine genetic testing although they cannot give clear information about the associated breast cancer risk, but these data often act as a pool for further risk association studies and metanalysis. Here, is raised a problem with communication and genetic counselling of such results as they add an inherited uncertainty to positive results without giving answers to the initial reason of referral.

4.6. Double heterozygotes

A considerable number of tested individuals (~9%) carried two P/LP variants. Half of the cases, involved *MUTYH* heterozygotes and carriers of the low penetrance c.470T>C (p.Ile157Thr) pathogenic variant in *CHEK2*. In both cases there is limited evidence to support an increased risk for breast cancer. However, in other double heterozygotes, P/LP variants were identified in genes with risk associations for multiple cancer types or even association with breast cancer risk at different risk levels (e.g. family in Fig.3). Similar results have been reported in the literature and have been described as Multilocus Inherited Neoplasia Alleles Syndrome [12]. However, there are not enough data to determine if there is an additive or synergistic effect of gene defects in these cases [34]. Further studies need to evaluate if they impose an increased risk compared to carriers of P/LP variants in the same single genes and whether they can be used as predictive biomarkers to PARPi especially in the case of double heterozygotes of HR genes. Nevertheless, they provide a good reason why reflex testing for germline mutations should be avoided. In some cases, the different mutated genes give information about multiple

cancer types and may explain the genetic history of different sides of the family history and the occurrence of diverse cancers in the family [35].

4.7. Therapeutic implications

An important new level of information in hereditary cancer testing results is the association with potential therapies especially in cases with metastatic disease. This is mainly feasible since an important number of genes included in genetic testing have been used as predictive biomarkers for response to targeted therapies and in specific to PARP inhibitors. These genes are included in the Homologous Recombination (HR) pathway which is involved in the repair of DNA double-strand breaks and include: *ATM, BARD1, BRCA1/2, BRIP1, CHEK2, FANCA, FANCM, MRE11, NBN, PALB2, RAD50, RAD51C* and *RAD51D*. Moreover, protein defects in Lynch Syndrome (LS)-associated mismatch repair genes (*MLH1, MSH2, MSH6, PMS2, EPCAM*) are associated with Microsatellite instability (MSI) and/or mismatch repair deficiency (MMR-D) and can subsequently be used as predictive biomarkers for anti-PD-1/PD-L1 immunotherapy efficacy [10,11]. Interestingly, 92% (382/415) of individuals with positive results are linked to additional therapy selection information with different levels of clinical and/or experimental evidence (Figure S1). In specific, 49% (205/415) of results include biomarkers that predict response to FDA-approved therapies for breast cancer mainly due to pathogenic variants in *BRCA1/2* genes. 6% (14/415) of results include biomarkers that could predict response to immunotherapy with immune checkpoint inhibitors (ICI) based on well-powered studies with consensus from experts in the field. The remaining of the positive results (31%, 130/415) include genes that can be used as potential biomarkers for response to PARP inhibitors in breast cancer (off label evidence-based drug use) since they include genes involved in the HR pathway shown to predict response to approved therapies in a different cancer type (prostate cancer).

These data suggest that, in addition to risk assessment, breast cancer patients could benefit from genetic testing by receiving useful information to guide treatment selection. It is important to note that such information is available through the spectrum of different breast cancer risk association of genes and it is an added value when testing moderate/low risk genes for breast cancer. In the case of genes with limited information and association with an increased risk for breast cancer, we observe that the majority of them link to ongoing clinical trials that examine the response of these carriers to PARP inhibitors and may add actionable information in these results in the future.

Table 2. Treatment implications of germline findings in hereditary cancer predisposition genes with P/LP variants in our cohort

Gene/Biomarker	Cancer type	Drug	Evidence level
BRCA1/2	Breast	Olaparib, Talazoparib	Approved [36,37]
BRCA1/2	Pancreatic	Olaparib	Approved [38]
BRCA1/2	Ovarian	Olaparib, Rucaparib, Niraparib, Talazoparib	Approved [39-42]
BRCA1/2, ATM, BRIP1, BARD1, CHEK2, PALB2, RAD51C, RADB1D	Prostate	Olaparib	Approved [33]
BRCA1/2	Breast	Carboplatin	Well-powered studies [43]
BRCA1/2	Ovarian	Carboplatin	Well-powered studies [44]
MLH1, MSH2, MSH6, PMS2, EPCAM	All tumors	Pembrolizumab, Nivolumab, Atezolizumab, Durvalumab	Well-powered studies [10,11]
POLE, POLD1	All tumors	Pembrolizumab, Nivolumab, Atezolizumab, Durvalumab	Well-powered studies [10,11]

ATM	Gastric	Olaparib	Preclinical studies [45]
ATM	Pancreatic	Olaparib	Preclinical studies [46]
BRCA1/2, ATM, BARD1, BRIP1, CHEK2, MRE11, PALB2, RAD51C, RAD51D	Ovarian	Platinum-based agents	Preclinical studies [47]
BARD1, BRIP1, FANCA, NBN, PALB2, RAD51C, RAD51D	All tumors	Rucaparib	Clinical trials; [NCT04171700]
ATM, BRIP1, BARD1, BLM, CHEK2, MRE11, NBN, PALB2, POLD1, PTEN, RAD50	All tumors	Niraparib	Clinical trials; [NCT03207347]
ATM, BARD1, BRIP1, CHEK2, FANCA, FANCM, MRE11, NBN, PALB2, RAD50, RAD51C, RAD51D	Breast	Olaparib	Clinical trials; [NCT03344965]
PTEN, PALB2, CHEK2, ATM, NBN, BARD1, BRIP1, RAD50, RAD51C, RAD51D, MRE11, FANCA	Breast	Talazoparib	Clinical trials; [NCT02401347]
BRCA1/2, PALB2, CHEK2, ATM, BARD1, BLM, BRIP1, FANCA, FANCM, MRE11, NBN, PTEN, RAD50, RAD51C, RAD51D	Breast	HX008 (anti-PD-1) + Niraparib	Clinical trials; [NCT04508803]

4.8. Genetic counselling

Historically, genetic counselling is the communication process which deals with the human problems associated with the occurrence or risk of occurrence of a genetic disorder in a family. Nowadays, when communicating the results of the genetic test there are multiple levels of information that should be included and involve: (1) information about the way heredity contributes to breast cancer and how are the genes tested associated with different risk levels for breast cancer but also for other cancer types, (2) information about how results could affect the management of breast cancer in the patient, (3) actions associated with the reduction of the risk of occurrence/recurrence to the tested individual and specified relatives and (4) evidence based information on how testing results can be used for treatment selection using PARP inhibitors and/or immunotherapy.

All this information should be extracted from our current scientific knowledge in line with international guidelines and well powered studies and counselling should be provided in the pre- and post-testing setting. Cascade family testing should be encouraged, especially in the cases of positive findings for high-risk genes. Our data suggest that only approximately 10% of families tested positive continue with genetic testing of selected relatives and the process of family testing is underutilized. This has been shown to be irrelevant of the cost of testing [48,49].

When a trained and certified genetic counselor is not involved in the process, all this information should be adequately communicated to the tested individual by the physician and/or the testing laboratory. The latter should include relevant information and proper language in the report of the results so that the report can act as a resource of our current actionable knowledge.

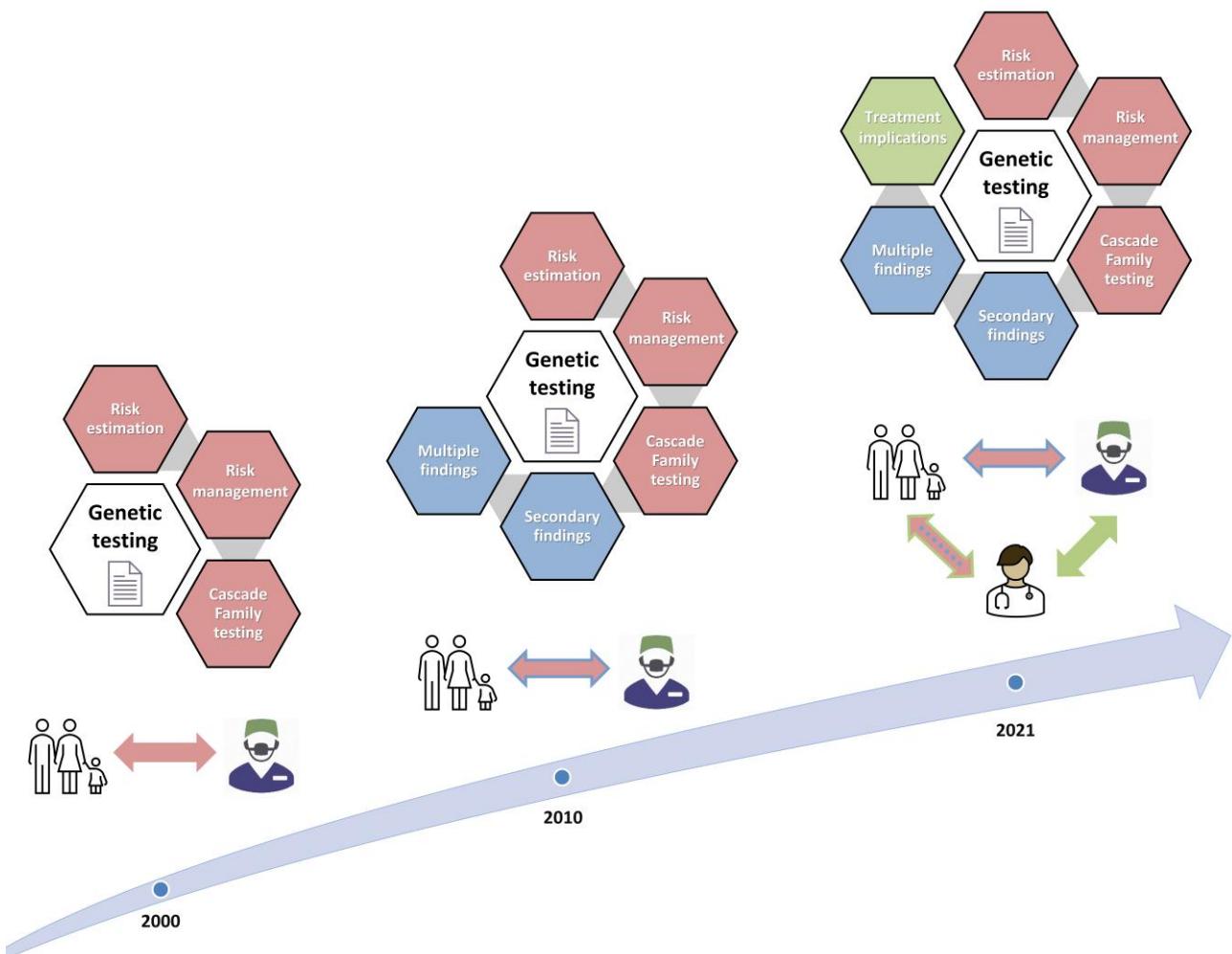



Figure 6. The evolution of information retrieved from genetic testing in the last two decades. The different levels of information add quantitative and qualitative changes to the clinical utility of genetic testing. The interaction model nowadays includes the oncologist, the surgeon, and the family.

Evidence based information included in the reports and the standardization of the reporting language can help towards this direction [13]. Special efforts should be made to clearly describe findings in moderate/low risk genes and to describe the cases of double heterozygotes. In such cases the results, as discussed above, could be linked to increased risk for additional cancer types that would require additional management and/or referral to another expert.

5. Conclusions

The identification of pathogenic/likely pathogenic variants in moderate- and low-risk genes impose challenges in risk estimation but provide actionable information for other cancer risk associations. Moreover, they are potential biomarkers for targeted therapies using PARPi and immunotherapies using ICIs. Therapeutic implications of germline findings are an additional level of information produced by genetic tests and could be included in a separate section in clinical reports. Reporting and pre-/post-genetic counseling should take into account these features. Improvement efforts should be focused to the actual use of genetic testing results for the management of patients.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, **Figure S1**: Treatment implications of germline findings in the 415 individuals with P/LP variants grouped by the level of breast cancer risk., **Table S1**: Clinical utility and different levels of information associated with pathogenic/likely pahtogenic variants in genes tested positive in our cohort., **Table**

S2: Information for the 415 individuals with personal and/or family history of breast cancer (BC) tested positive with a multi-gene panel for pathogenic/likely pathogenic variants.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data presented in this study are available in the supplementary material and are also openly available in ClinVar.

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