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Male breast cancer: genetics, epigenetics, and ethical aspects

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Background and study design: Male breast cancer (MBC) is a rare disease compared with female BC and our current understanding regarding breast carcinogenesis in men has been largely extrapolated from the female counterpart. We focus on differences between the ethical issues related to male and female BC patients. A systematic literature search by using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), was carried out to provide a synopsis of the current research in the field of MBC genetics, epigenetics and ethics. Original articles and reviews published up to September 2012 were selected by using the following search key words to query the PubMed website: 'male breast cancer', 'male breast cancer and genetic susceptibility', 'male breast cancer and epigenetics', 'male breast cancer and methylation', 'male breast cancer and miRNA', 'male breast cancer and ethics'.

Results and conclusions: As in women, three classes of breast cancer genetic susceptibility (high, moderate, and low penetrance) are recognized in men. However, genes involved and their impact do not exactly overlap in female and male BC. Epigenetic alterations are currently scarcely investigated in MBC, however, the different methylation and miRNA expression profiles identified to date in female and male BCs suggest a potential role for epigenetic alterations as diagnostic biomarkers. Overall, much still needs to be learned about MBC and, because of its rarity, the main effort is to develop large consortia for moving forward in understanding MBC and improving the management of MBC patients on a perspective of gender medicine.

Key words: epigenetic alterations, ethics, genetic susceptibility, male breast cancer, methylation, miRNAs

introduction

Although rarely, breast cancer (BC) affects men. To date, in Western countries, male BC (MBC) makes up <1% of all BCs and <1% of all cancers in men [1–3]. Its incidence is estimated at <1 per 100 000 men-years [4]. Overall, recent epidemiologic studies suggest that the incidence of MBC is increasing by 1.1% yearly [1, 2]. MBC incidence is generally low when compared with female BC (FBC), but substantial variability between countries exists. The highest overall age-adjusted rates occurred in Israel (1.08 per 100 000 man-years), whereas the lowest rates were recorded in Thailand (0.14 per 100 000 man-years) [4]. Such variability in rates may be due to population-specific genetic susceptibility.

Common BC risk factors, such as genetic, hormonal, and environmental factors, are involved in the pathogenesis of BC in women as in men. The major MBC predisposition factor is a positive family history (FH) of BC. Patients with a positive first-degree FH have a 2.0-fold increased risk, which increases to more than 5.0-fold with the number of affected relatives and early onset relatives, thus suggesting a relevant role of genetic factors in MBC risk [5].

From an epidemiological point of view, MBC resembles postmenopausal FBC and generally MBC treatment follows the same indications as postmenopausal FBC. However, clinical and pathological characteristics of MBC do not exactly overlap FBC and this could explain why mortality and survival rates have improved significantly less in male than in female BC patients [6]. Thus, identification of specific MBC subgroups is essential for developing an appropriate therapeutic approach.

There is growing evidence that methylation play an important role in BC development and that identification of tumor-specific methylation profiles may allow the identification of specific biomarkers for characterizing BC subtypes [7]. In addition to methylation, the involvement of micro RNAs (miRNAs) in modulating gene expression has been recently reported in BC development [8]. Altered expression of miRNA, predicted to regulate key BC genes, is frequently observed in breast tumors [9, 10]; and significant differences in miRNA expression profiles related to hormonal status have been reported, thus allowing definition of distinct molecular subgroups of BC.

The contribution of epigenetic mechanisms in MBC is still largely unknown. In this review, we will focus on the most

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relevant genetic and epigenetic alterations in the development of MBC. Ethical issues related to MBC management will be also discussed.

study design

We did a systematic literature search by using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), to provide a synopsis of the current understanding and future directions of research in MBC field. We selected original articles and reviews published up to September 2012. The following search key words were used to query the PubMed website: 'male breast cancer', 'male breast cancer and genetic susceptibility', 'male breast cancer and epigenetics', 'male breast cancer and methylation', 'male breast cancer and miRNA', 'male breast cancer and ethics'. The abstracts resulting from these queries were individually analyzed for relevance.

genetics of MBC

It is estimated that up to 10% of all MBC are hereditary forms caused by inherited germline mutations in well-identified BC susceptibility genes. By their mutation frequency and the magnitude of their impact in BC susceptibility, these genes can be divided into 'high-penetrance', 'moderate-penetrance', and 'low-penetrance' genes (Table 1). Mutations in the two major high-risk BC genes, *BRCA1* and *BRCA2*, occur rarely in the population, but confer a high risk of BC to the individual [11]. A moderate risk of BC is conferred by variants in genes functionally related to *BRCA1/2* in DNA repair pathways. These variants are rare, occurring in <1% of the population, and their contribution to the risk of BC is <5% [12]. Recently, a third class of low-penetrance susceptibility alleles has been identified. Due to their low penetrance, the real contribution of these common variant to MBC risk is not entirely clear.

Table 1: Classes of male breast cancer genetic susceptibility and comparison of their different features

	High penetrance	Moderate penetrance	Low penetrance
Genes	*BRCA2, BRCA1*	*CHEK2, PALB2*	2q35, 6q25.1 (*ESR1*), 10q21.2, 11q13.3, 12p11.22, 14q24 (*RAD51L1*) and 16q12.1 (*TOX3*)
Population frequency	<0.1%	MAF 1%	MAF >10%
Cancer risk (odds ratio)	>10.0	>2.0	0.76–1.57
Functional effect	Direct effect of mutation	Direct effect of variant	Direct effect of variant; linkage disequilibrium with causal variants
Strategy for identification	Resequencing of candidate genes	Resequencing of candidate genes	Case–control studies; genome-wide association study (GWAS)

high-penetrance BC genes

BRCA1 and *BRCA2* are the most important BC susceptibility genes in high-risk families. In MBC cases, *BRCA2* mutations are much more common than *BRCA1*. Mutations in *BRCA2* gene are estimated to be responsible for 60%–76% of MBCs occurring in high-risk BC families, whereas *BRCA1* mutations frequency ranges from 10% to 16% [13, 14]. In the frame of the first Italian multicenter study on MBC, we recently reported a frequency of *BRCA1/2* mutations of about 13%, in particular, *BRCA1* mutations were found in about 1% and *BRCA2* mutations in 12% of MBC cases [15]. Overall, *BRCA1* and *BRCA2* mutations are more prevalent in men with a positive first-degree FH compared with those without FH. All known*BRCA1/2* mutations are recorded in the Breast Information Core (BIC) database http://wvw.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/. To date, 1643 distinct germline *BRCA1* mutations and 2015 *BRCA2* mutations have been reported in BIC. The great majority of *BRCA1/2* mutations are truncating mutations; however, an elevated number of missense variants has been also identified. At present, there is no evidence for a correlation between the location of the mutation within *BRCA1* or *BRCA2* gene and risk of MBC. *BRCA1/2* polymorphic variants could be also associated to increased risk of BC [16–18]. Interestingly, we observed an association between the*BRCA2* N372H variant and risk of MBC in young men [19].

Specific *BRCA1* and *BRCA2* mutations show high frequency in specific countries or ethnic groups, particularly, in genetically isolated populations. These mutations are descendent from a single founder. Founder mutations may also explain variability in BC incidence rates among countries. For example three founder mutations, two in *BRCA1* (185delAG and 5382insC) and one in *BRCA2* (6174delT), have been observed at higher frequency (>2% in total) in the Ashkenazi Jewish male population than in the general US population [20, 21]. Generally, *BRCA1* mutations are quite rare in unselected MBC cases being more frequent in specific populations in which a founder effect is known to occur [22]. Notably, we showed a founder effect for the *BRCA1* 3347delAG mutation that was found in Italian MBC cases [15, 23, 24]. *BRCA1/2* large-scale rearrangements, including insertions, deletions, or duplications of more than 500 kb of DNA, have been also identified in both male and female BC patients [25–28]. Interestingly, large genomic rearrangements in *BRCA2* are more frequent in families with MBC [26, 29] and, on the other hand, we reported that both *BRCA1* and *BRCA2* rearrangements are infrequent in MBC cases unselected for FH [30].

It is now well established that, in women, *BRCA*-associated BCs tend to manifest specific genotype–phenotype correlations [31]. In particular, *BRCA1*-related BCs have distinct morphology and phenotype [32]. By contrast, *BRCA2*-related BCs are a heterogeneous group not fully characterized [33–35]. We recently investigated whether specific *BRCA*-associated phenotypes could be identified in MBC. We found that the majority of *BRCA1*-related MBCs are HER2 negative (HER2−),

grade 3 tumors and show high proliferative activity. Although based on a few cases, our results may suggest that *BRCA1*-related BCs in men represent a rare event characterized by a phenotype similar to that observed in women. On the other hand, *BRCA2*-associated MBCs display a characteristic phenotype not identified in women [35]. In particular, *BRCA2*-associated MBCs present with high tumor grade, absence of progesterone receptor (PR) expression and HER2-positive (HER2+) status [15]. Interestingly, it has been reported that the mean number of genetic aberrations in *BRCA2*-associated MBCs carriers is higher than in sporadic MBC [36] and specific gene copy number aberrations are associated with MBC cases [37], thus indicating that further genetic analyses on somatic alteration in MBC might provide insights into the biologic cancerous process.

moderate-penetrance BC genes

Direct interrogation of candidate genes involved in *BRCA1/2*-associated DNA damage repair pathways had led to the identification of other BC susceptibility genes, classified as moderate-penetrance genes. Variants found in this class of genes confer a smaller risk of BC than *BRCA1/2. CHEK2* 1100delC was the first moderate BC risk allele identified. The *CHEK2* 1100delC mutation has been shown to confer approximately a 10-fold increase of BC risk in men lacking *BRCA1/2* mutations and it was estimated to account for 9% of familial high-risk MBC cases [38]. However, this association is not so evident in MBC series unselected for FH [30, 39–41]. The contribution of the *CHEK2* 1100delC mutation to MBC predisposition varies by ethnic group and from country to country. Interestingly, a decreased frequency of the 1100delC allele in North to South orientation has been observed in Europe [30, 42–44].

The involvement of *BRCA2* in the Fanconi Anemia (FA) pathway promoted mutation screening of other FA genes functionally linked to *BRCA2*, such as *PALB2*, *BRIP1, RAD51C*, and, more recently, *XRCC2* [45]. Interestingly, *PALB2* mutations were found in families with both female and male BCs, suggesting that *PALB2* may be involved in MBC risk [46, 47]. Moreover, *PALB2* heterozygotes were 4-fold more likely to have a male relative with BC [48]. To date, five studies have reported on the frequency of *PALB2* mutations in MBC [49–53]. Overall, these studies indicate that *PALB2* may have a role as moderate-penetrance gene in MBC at a comparable extent as for FBC.

Recently, we investigated the role of *BRIP1* in MBC susceptibility, and we found no evidence that germline variants in *BRIP1* might contribute to MBC predisposition [54], thus suggesting that the contribution of *BRIP1* to BC predisposition in males is less consistent compared with other moderate BC susceptibility genes such as *CHEK2* and *PALB2*. Mutations in *RAD51C* were identified as BC susceptibility alleles, accounting for 1.3% of female patients from families with at least one case each of breast and ovarian cancer [55]. At present, there is no evidence that *RAD51C* mutations may contribute to MBC susceptibility [56]. A rare mutation in *XRCC2* was newly found by whole exome sequencing in an early onset MBC patient with a strong family history of BC [57], thus suggesting that *XRCC2* could be a MBC susceptibility gene.

low-penetrance BC alleles

A polygenic model, in which many genes that confer low risk individually act in combination to confer much larger risk in the population, has been suggested for susceptibility to BC and other common cancers [58]. BCs unaccounted for by currently known high- and moderate-penetrance BC susceptibility genes can be explained by this model. This hypothesis has recently been confirmed by multigroup collaborations working in genome-wide association studies (GWAS) carried out on very large series of BC cases and controls from different countries, in order to increase the power to detect small effects on the risk BCAC [59, 60]. GWAS have also identified associations between single-nucleotide polymorphisms (SNPs), mapping to more than 20 loci, and BC risk in women [60–66]. These SNPs act as common low-penetrance allele variants, each generally conferring a relative risk <1.40 [67, 68]. Overall, these SNPs are estimated to account for <4% of the familial risk of BC in women [60]. Many of the susceptibility alleles are in intronic portions of genes and often are noncoding regions. This might be explained by the observation that some of these loci are located in regions of linkage disequilibrium that cover different genes [67]. Furthermore, some of these SNPs could act as modulators of the risk conferred by mutations in the high-penetrance BC susceptibility genes *BRCA1* and *BRCA2* [69]. Also, a subtle regulatory effect of one allele in the prostate/breast cancer-associated 8q24 block, which acts as a *cis* enhancer of the *MYC* promoter, has been found [70]. Different haplotype blocks within 8q24 were specifically associated with the risk of different cancers, including prostate, colon, ovarian, kidney, thyroid, laryngeal carcinomas, and BC [71–73]. Intriguingly, many of the coding loci presently identified are in genes somatically mutated in diverse cancers, including BC. Germline variations in genes encoding for 'driver kinases' may also influence BC risk, thus suggesting that low-penetrance alleles might be a link between germline and somatic alterations in BC [74]. The relative risk associated with several of the loci identified to date shows BC subtype specificity in women, defined in particular by hormonal receptors status [67]. Notably, associations with most of the susceptibility loci are stronger for estrogen receptor-positive (ER+) rather than for ER-negative (ER−) BCs [75].

Common low-penetrance BC alleles associated with the risk of FBC were also investigated in MBC and SNPs at 2q35, 6q25.1 (*ESR1*), 10q21.2, 11q13.3, and 12p11.22 and 16q12.1 (*TOX3*) were confirmed to be significantly associated also with MBC risk [76, 77]. In the first GWAS on a large collaborative series of MBCs, we were able to identify a novel SNP in *RAD51B* at 14q24.1 that is significantly associated with MBC risk [77]. At present, whether variant alleles may be associated with specific clinical-pathologic features of MBCs is still unknown. Studies of well-defined MBC patient subgroups are needed in order to provide further insight into the role of low-penetrance alleles in MBC.

epigenetics

Epigenetic alterations (changes in gene activity that do not involve variations in the primary DNA sequence) play as crucial role as genetics in cancer development. Epigenetic events are responsive to environmental factors, are hereditable and

relatively stable, and regulate crucial biological processes such as X-chromosome inactivation, genomic imprinting, position effect variegation, reprogramming of the genome during differentiation and development, or RNA interference leading to post-transcriptional gene silencing. Defects in these processes have been found to be associated with many human disorders, including BC [78]. Two epigenetic mechanisms have emerged as the most critical players in transcriptional regulation: methylation of DNA and microRNA interference. These mechanisms are both involved in the initiation and progression of BC, as revealed by a large series of papers on the topic. At present, epigenetic alterations have been rarely studied in MBC.

DNA methylation

Hypermethylation of CpG islands consists of a covalent addition of a methyl group to a DNA sequence, usually a cytosine located in 5′ of guanosine. In more than 70% of genes, methylation occurs in the promoter and in the first exon regions, and it is thought to be especially relevant in reorganizing chromatin structure and in silencing important growth control pathways [79]. DNA methylation reaction is catalyzed by DNA methyl transferases (DNMTs), ubiquitously expressed in a tissue-specific way [80]. Two families of DNMTs have been identified in mammals: DNMT1, which functions in maintenance of DNA methylation during DNA replication, and DNMT3 (which include DNMT3a and DNMT3b), which is a de novo enzyme capable of methylating unmethylated or methylated DNA. DNMTs are overexpressed in various tumor types, but DNMT3b plays a predominant role in breast tumorigenesis [81]. More than 100 genes, in particular tumor suppressor genes, have been reported to be hypermethylated in female breast tumors or BC cell lines [79, 82, 83]. Many of the aberrantly methylated genes are players in cell-cycle regulation (*CCND2, p16INK4A, p14ARF, p15, RARbeta, RASSF1A*), apoptosis (*APC, HOXA5, BCL2, TWIST*), angiogenesis (*EFEMP1, THBS1*), DNA repair (*GSTP1, BRCA1, MGMT*), hormone signaling (*ESR1, ARH1, CYP1B1*), invasion, and metastasis (*CDH1, CDH3, CDH13, TIMP3*). Aberrant tumor suppressor gene methylation is a key factor in BC pathogenesis. Moreover, a progressive oncosuppressor inactivation can lead gradually from a less-aggressive, hormone-dependent phenotype to a highly invasive, hormone-independent one.

Recently, Kornegoor et al. [84], investigating methylation of selected tumor suppressor genes in 108 MBC cases, demonstrated for the first time the important role of epigenetic inactivation in MBC development, even if, compared with female, methylation occurred less often in male BCs. All male tumor cases except one showed methylation of at least one gene, with an average of six genes. Interestingly, genes frequently methylated in female (*MSH6, WT1, PAX5, PAX6*, and *CDH13*) were frequently methylated also in male BCs. On the contrary, these genes were not found methylated in normal breast tissues, confirming the important role of methylation in the development of MBC. Other genes, including *BRCA1, CDKN2A, VHL, ATM*, and *CHFR*, were rarely found methylated in this study, while *ESR1* and *GSTP1* methylation was identified to be correlated with high mitotic count, suggesting a role for these two single genes in aggressive male breast carcinogenesis [84]. Compared with FBC, methylation of *ESR1, BRCA1* and *BRCA2* was less common in MBC [84], evidencing important differences between male and female breast carcinogenesis with regards to gene inactivation by methylation. This was confirmed by the results of a recent study on a cohort of familial BC cases in which we demonstrated that *RASSF1A* promoter resulted more frequently methylated in familial MBCs than FBCs (76% versus 28%, $P = 0.0001$), and *RARβ* more frequently methylated in female than male BC (17% versus 8%, $P = 0.3$). Furthermore, *RASSF1A* and *RARβ* resulted more frequently overexpressed in familial FBC than MBC (*RASSF1A*: 83% versus 30%, $P = 0.0001$; *RARβ*: 55% versus 22%, $P = 0.012$). When we compared methylation data with clinical characteristics, we reported that in familial FBC, a lower *RASSF1A* expression and higher methylation was associated with a higher positivity of ER, consistently with the role of *RASSF1A* in downregulating ERα [85]. In familial MBC however, higher methylation and lower expression of *RASSF1A* resulted significantly associated with absence of ER expression, thus underlining a different regulation of the ER pathway in the two genders mediated by *RASSF1A* methylation [86].

miRNA

microRNA (miRNA) are small, highly conserved noncoding RNAs (18–24 nucleotides) that regulate gene expression by targeting RNA degradation or translational inhibition through interaction with the 3′ untranslated region (UTR) of the mRNA target [79], but also the open reading frame (ORF) and the 5′UTR, as evidenced by some authors [87–89]. As a single miRNA can target hundreds of mRNAs and a single mRNA can be targeted by several miRNAs, aberrant miRNA expression can be involved in the initiation of many diseases, including cancer [90, 91]. miRNAs are frequently located in cancer-associated genomic regions that are often subjected to rearrangements, breakpoint regions, loss of heterozygosity, deletions and amplifications in cancer cells. They can act as either oncogenes or tumor suppressors given their inhibition of tumor suppressive or oncogenic miRNAs, respectively [91]. miR27a, miR10b and miR21, promoting cell migration, invasion and cellular proliferation, are examples of 'oncomiR' involved in BC [10]. In particular some authors have defined miR10b as 'metastomiR' because of its capacity to induce the development of BC metastasis [92]. On the contrary, let-7, miR17–5p, miR27b, miR125a/b, miR200c, and miR206 act as tumor suppressors in BC as their overexpression causes inhibition of cell growth, migration and invasion [10].

miRNA regulation has been very poorly investigated in MBC. The first miRNA signature was identified by Fassan et al. [93]. It was composed of 43 miRNAs that were differentially expressed between male breast tumors and gynecomastia samples, considered a potentially benign counterpart of male breast glands because it represents a condition of increased but benign ductal epithelial proliferation. In particular, 17 miRNAs were upregulated and 26 miRNAs were downregulated in cancers, but the most promising miRNAs of this MBC signature were miR10b, miR126, miR125a-5p, and miR125b [93]. When the authors compared altered miRNA expression between male and female BC samples they demonstrated differences between

genders, since 17 miRNAs were identified as differentially expressed. We have also demonstrated a different miRNA regulation between male and female BC cases. By analyzing miR17, miR21, miR124a and let-7a expression in a cohort of familial BC that included male and female cases, we showed that regulation of miR17 and let-7 seemed highly involved in women compared with men [86]. Moreover, the role of miRNAs in MBC has been investigated by Lehmann's group [94]. They studied 319 miRNAs in 9 MBC specimens and in 15 male gynecomastia specimens. A fluorescence-labeled bead technology revealed 33 statistically significantly up-regulated miRNAs (including miR21, miR519d, miR183, miR197, and miR493–5p) and 21 statistically significantly downregulated miRNAs (including miR145 and miR497) in MBC compared with gynecomastia specimens. Some aberrant expressed miRNAs in MBC were shared with FBC, including miR21 that was not found to be differentially expressed in MBC in our and Fassan's work. miR21, a miRNA implicated as an oncomir in both female and male BC, can be considered an example of intracellular and extracellular biomarker miRNA, and can also be considered a potential therapeutic target [95]. The different types of cancer specimens used by the two authors might explain the different expression of miR21. Fassan et al. [93, 94] used a cohort with a prevalence of basal cytokeratines (CK5/6 and CK14) expression that were completely absent in Lehmann's series; however, these two studies alone are not sufficient to understand miRNA regulation in MBC.

In conclusion, the differential promoter methylation and miRNA expression identified to date in female and male BC patients suggest a potential role for epigenetic alterations as diagnostic biomarkers.

ethical aspects in MBC management and care

The protection of human health and the right to access to appropriate medical treatments are fundamental targets of medicine. The primary mission of medical practice is the management of diseases that affect patients' health, regardless of patient gender or of disease type. As a consequence of science advancements, nowadays, the challenge of the community is to guarantee a personalized treatment that fits to the needs of individual patients and takes into account biological and physiological differences. Genetics and epigenetics represent an important support to the understanding of the molecular basis of several disease (i.e. BC) and to address the quality and effectiveness of treatments, based on the concept of personalized therapies [96]. MBC is a practical example of how personalized medicine is not always guaranteed to the patient, and how complex are the ethical issues arising from the interaction between patients, physicians and family members. At the present time, management and care that are used for MBC are based on those developed for women. Differently from FBC, there are limited statistical results available on the therapy and cure of MBC. Furthermore, the low incidence of MBC has not encouraged medical science in improving research and prevention. Nevertheless, in recent years, an increasing mortality rate in MBC was observed because of delay in diagnosis and absence in the social community of gender-specific information.

As in other multifactorial diseases, MBC requires a personalized and comprehensive approach to diagnosis and treatment, in which the knowledge of risk factors such as family history, specific-gender aspects, genetic susceptibility and predisposition, play a key role. Prevention and communication between doctor and patient are also crucial. Recent studies highlight the importance of the development of educational programs to let patient aware of the availability of preoperative and postoperative gender-specific information to relieve psychological problems associated with the BC diagnosis [97]. Furthermore, the absence of specific-gender and preventive treatments leave male patients alone and incapable to have and to give a feed back on their experiences. Because of this absence, a clear marginalization of male patients and disparity of treatment between similar subjects, in both prevention and cure of BC, was observed. At the same time, since the absence of specific-gender information on therapy and cure, several difficulties and concerns were observed also in the relationship between patient and doctor. For instance, these difficulties frequently occur in practicing genetic tests aimed to measure the susceptibility or predisposition to develop BC, where ethical issues involving the right to know and the right to ignore can be relevant in the management of the psychological condition of patients [98]. The absence of gender-specific statistical studies makes more complicated the diagnosis when low-penetrance BC genes are observed, preventing an appropriate and adequate genetic counseling and full information to the patient. Furthermore, this uncertainty and difficulties to diagnosis do not contribute to resolve the ethical issues about the extension of genetic testing to family members, which likely do not receive complete information about the utility of such instrument.

Overall, the above summarized problems highlight a clear contrast with the increasing demonstrations of the importance of personalized treatments in therapeutic performance, which take into account the specific differences (sex, age, subtype of pathology, etc.) between a patient and another. The problem of inappropriate medical advancement occurs for several other rare diseases in which medical research and translational medicine are conditioned by cost–benefit considerations, because of large investments required by medical research. Finally, the above questions suggest the necessity to address the management of MBC on a perspective of gender medicine (i.e. prevention screening and adequate adjuvant therapy) in order to guarantee to all individuals, regardless of their specific condition, a personalized cure and care.

conclusions

The identification of BC susceptibility genes, in particular *BRCA1* and *BRCA2*, has changed the management of BC patients with a FH of BC. Several models have been developed and are currently used to assess the pre-test probability of identifying *BRCA1/2* germline mutations in individuals at risk for hereditary cancer. Moreover, novel therapeutic strategies specific for *BRCA1* and *BRCA2* cancers are emerging, including cross-linking agents and PARP inhibitors [99]. Both genetic and epigenetic alterations are frequently associated to specific

biological and clinico-pathological tumor characteristics, allowing the identification of personalized therapies targeting specific molecular pathways. In particular, DNA methylation as well as miRNAs are currently emerging as interesting candidates for the development of therapeutic strategies against BC.

MBC, as well as other multifactorial and rare diseases, suffers from the absence of specific and comprehensive studies that may allow translation of research findings into a personalized management of the disease, particularly when dealing with issues involving complex gene-environmental interactions and implying large numbers of cases, as for studies of low-penetrance BC susceptibility. As demonstrated by the recent GWAS on MBC [77], the ongoing collaborative efforts facilitate research on this rare and peculiar disease and will eventually provide useful information for a more appropriate clinical management of MBC patients.

disclosure

The authors have declared no conflicts of interest.

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