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Genetic predisposition to breast and/or ovarian cancer – focus on the candidate *BARD1* gene

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Abstract

Germline mutations affecting the *BRCA1* and *BRCA2* genes explain 16-40% of breast and/or ovarian cancers aggregated in families. Besides the *BRCA1/2* genes and several genetic factors associated with hereditary syndromes which increase the risk of breast cancer, a considerable fraction of potential breast cancer predisposing factors (~50%) remains unknown. It is presumed that candidate genes, functionally related to the *BRCA1/2* genes, may account for some of the missing heritability. The *BARD1* gene, which encodes a protein indispensable for BRCA1-mediated tumor suppression function and adequate apoptosis regulation, serves as a candidate breast cancer susceptibility gene. Some initial reports indicated that *BARD1* is a plausible target for several pathogenic mutations associated with increased breast and/or ovarian cancer risk. Nonetheless, further mutational studies are necessary to determine the penetrance and role of the *BARD1* gene in cancer predisposition.

Key words: *BARD1*, breast and/or ovarian cancer, familial cancer predisposition, DSB repair genes

Introduction

Breast cancer is the most frequent cancer and the primary cause of malignancy-associated deaths among women worldwide. Ovarian cancer, which frequently co-occurs with breast cancer in familial setting, is the fifth most common cancer (constitutes ~5% of all registered cancer cases) and the fourth leading cause of death attributed to cancer (constitutes cause of ~6% of cancer-related deaths) among Polish women. The highest breast and ovarian cancers rates are observed in western, developed countries (http://onkologia.org.pl/) (Youlden et al., 2012).

There is a number of risk factors associated with breast cancer. Age (> 40), early menarche (age of < 12), and late menopause (age of > 54) substantially increase the risk of breast cancer. Among other factors increasing the risk of breast cancer are: longstanding hormonal replacement therapy, high body mass index (BMI), and regular alcohol intake. Breast feeding and early age at first pregnancy are counted as protective factors, whereas hormonal contraceptives decrease the ovarian cancer risk and slightly increase breast cancer risk. Familial aggregation of breast cancer cases, especially among first-grade relatives, are important factors of breast cancer risk, indicating that genetic factors are essential determinants of breast and/or ovarian cancer risk (Hankinson et al., 2004; Lux et al., 2006).

Unified paradigms for breast and ovarian cancer etiology are difficult to elucidate. The longstanding exposure to hormones, as well as the interplay of environmental and genetic factors, modulate the probability of developing these complex diseases (Hankinson et al., 2004; Permuth-Wey and Sellers, 2009). Overall heritability of breast and ovarian cancer was estimated based on twins studies (monozygotic and dizygotic) for approximately 30% and 20%, respectively (Lichtenstein et al., 2000). Familial breast cancer constitutes 5-10% of all breast cancer cases. In the middle 1990's, studies conducted in families with strong aggregation of breast and/or ovarian cases led to the identification of *BRCA1* (**br**east **ca**ncer **1**) and *BRCA2* (**br**east **ca**ncer **2**) genes (Miki et al., 1994; Wooster et al., 1995). Germline mutations in these genes account for 16-40% of familial breast cancers (Beggs and Hodgson, 2009; Ripperger et al., 2009). Additionally, about 5% of breast cancer cases aggregated in family is attributed to mutations in genes associated with various hereditary syndromes and genes conferring moderate risk. Finally, it was reported that common

SNPs cumulatively explain 14% of the familial breast cancer cases (Michailidou et al., 2013). Other genetic breast cancer susceptibility factors (~50%) are unknown. There is a supposition that a fraction of inherited breast and/or ovarian cases can be attributed to heterozygous mutations in candidate genetic factors [e.g. *BARD1* (**B**RCA1 **a**ssociated **R**ING **d**omain **1**)], which contribution to breast and/or ovarian cancer remains to be evaluated – Figure 1 (Wooster and Weber, 2003).

Therefore, further investigation of hereditary genetic alterations which predispose to breast and/or ovarian cancer could reveal a fraction of missing heritability of breast and/or ovarian cancer and consequently may uncover new models for inherited susceptibility evaluation and contribute to the development of targeted preventive strategies (Manolio et al., 2009).

Hereditary cancer syndromes

The majority of cancers are sporadic. Sporadic cancer is a complex and multifactorial disease that is acquired owing to environmental exposures, lifestyle or multiple genetic factors (variants) of very low risk effects. A fraction of some cancers (especially breast, ovarian, and colorectal cancers) occurs in the form of familial aggregations, i.e. is observed in closely related individuals more frequently than it could be expected based on the frequency of the cancer in general population. It is estimated that familial cancers constitute up to 15% of particular cancers. Predominantly, familial aggregation of cancer cases is attributed to a single loss-of-function mutation in a specific tumor suppressor gene associated with a particular cancer type. Among genes, which mutations underlie the most common familial cancer types are 1) DNA mismatch repair genes [e.g. *MSH2* (**M**ut**S** **h**omolog **2**), *MSH6* (**M**ut**S** **h**omolog **6**), *MLH1* (**M**ut**L** **h**omolog **1**)] associated with hereditary non-polyposis colorectal cancer (HNPCC), 2) the *APC* (**a**denomatous **p**olyposis **c**oli) gene predisposing to familial adenomatous polyposis (FAP), and 3) *BRCA1* and *BRCA2* associated with breast and/or ovarian cancer aggregation. The identification of these genes was essential for the understanding of pathomechanism of familial cancer syndromes and laid the foundation for familial cancer genetic diagnostics. Most of hereditary cancers constitute autosomal dominant disorders that display incomplete penetrance (Nagy et al., 2004). Inherited cancers are initiated by the transmission of a genetic mutation in the germline. However, it must be noted that the risk of the development of inherited cancer may also be modulated by lifestyle and environmental exposures as well as other genetic factors. Multiple cases of genetically associated hereditary cancers are often aggregated within the family and can be related to a particular inherited cancer syndrome (see Table 1). The probability of the inheritance of cancer predisposition within a family increases with the number of individuals affected by cancer (Ellis, 2011; Heald and Church, 2011). Inherited cancer susceptibility can be also associated with the presence of multiple primary cancers or simultaneous occurrence of nonmalignant disorders in affected individual (Nagy et al., 2004). The occurrence of several generations with numerous cases of the early-onset, bilateral or multisynchronous cancers within a family can be counted as the hallmarks of hereditary cancer syndromes (Heald and Church, 2011).

Hereditary breast and ovarian cancer

Genetic variants predisposing to breast cancer can be divided into three major groups according to the breast and/or ovarian cancer risk conferred by these variants and their frequency in the population (Fig. 2) (Foulkes, 2008; Ripperger et al., 2009). Importantly, a substantial fraction of existing breast and/or ovarian cancer susceptibility genes with various degree of penetrance still remains unidentified (Wooster and Weber, 2003; Karppinen et al., 2004; Beggs and Hodgson, 2009).

The first group of breast and/or ovarian cancer susceptibility variants encompasses rare, high risk heterozygous mutations occurring in genes associated with several rare hereditary syndromes (Foulkes, 2008; Beggs and Hodgson, 2009). The major genes associated with susceptibility to hereditary breast and ovarian cancer syndrome (HBOC) are *BRCA1* and *BRCA2*. Germline mutations in these genes are associated with the risk of 50-80% and 30-50% for breast and ovarian cancers, respectively. Germline mutations in *BRCA1* and *BRCA2* genes explain approximately 16-40% of breast and/or ovarian cancer cases aggregated in families (Beggs and Hodgson, 2009; Ripperger et al., 2009; Roy et al., 2012). Other cancer syndromes listed below explain less than 5% of familial breast cancer aggregation. The probability

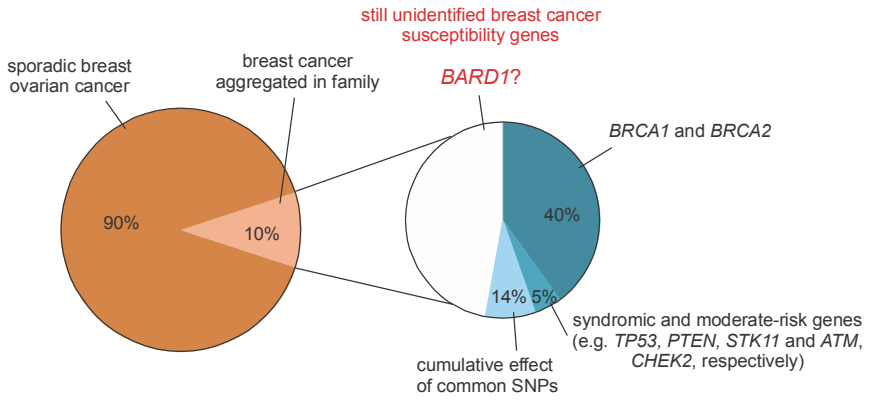

Fig. 1. Pie-chart schematically depicting the genes accounting for familial aggregations of breast cancer

Table 1. Hereditary cancer syndromes

Syndrome	Gene	Chromosomal localization	Mode of inheritance	Associated cancer	References
Hereditary breast and ovarian cancer (HBOC)	*BRCA1* *BRCA2*	17q21 13q12.3	autosomal dominant	predominantly breast and ovarian	(Miki et al., 1994; Wooster et al., 1995; Nagy et al., 2004)
Hereditary site-specific breast cancer	*BRCA1* *BRCA2*	17q21 13q12.3	autosomal dominant	predominantly breast	
Hereditary site – specific ovarian cancer	*BRCA1* *BRCA2*	17q21 13q12.3	autosomal dominant	predominantly ovarian also: prostate, fallopian tube, stomach, pancreatic, laryngeal	
Li-Fraumeni syndrome	*TP53*	17p13.1	autosomal dominant	breast, brain, sarcomas, leukemias	(Nagy et al., 2004)
Hereditary nonpolyposis colorectal cancer (HNPCC)/ Lynch syndrome	*MSH2* *MSH6* *MLH1* *PMS1* *PMS2*	2p21 2p16 3p21.3 2q31.1 7p22.2	autosomal dominant	endometrial colorectal, stomach, ovarian, pancreas, brain	(Nagy et al., 2004)
Familial adenomatous polyposis (FAP)	*APC*	5q21-q22	autosomal dominant	colorectal, colon, gastric, pancreatic, adenomas	(Nagy et al., 2004)
Cowden syndrome	*PTEN*	10q23.3	autosomal dominant	breast, thyroid, endometrial	(Nagy et al., 2004)
Ataxia-telangiectasia	*ATM*	11q22-q23	autosomal recessive	lymphomas, leukemias, breast	(Savitsky et al., 1995; Khanna, 2000)
Hereditary diffuse gastric cancer syndrome	*CDH1*	16q22.1	autosomal dominant	gastric, breast	(Berx et al., 1995; Pinheiro et al., 2010)
Fanconi anemia	*BRIP1* *PALB2* *BRCA2*	17q22.2 16p12.2 13q12.3	autosomal recessive	breast, leukemia	(Mathew, 2006; Walsh and King, 2007)
Peutz-Jeghers syndrome	*STK11 (LKB1)*	19p13.3	autosomal dominant	colon, small intestine, stomach, breast, pancreatic	(Hemminki et al., 1998; Nagy et al., 2004)
Nijmegen-breakage syndrome	*NBN*	8q21	autosomal recessive	lymphoma, breast, colorectal	(Matsuura et al., 1998; Steffen et al., 2004)

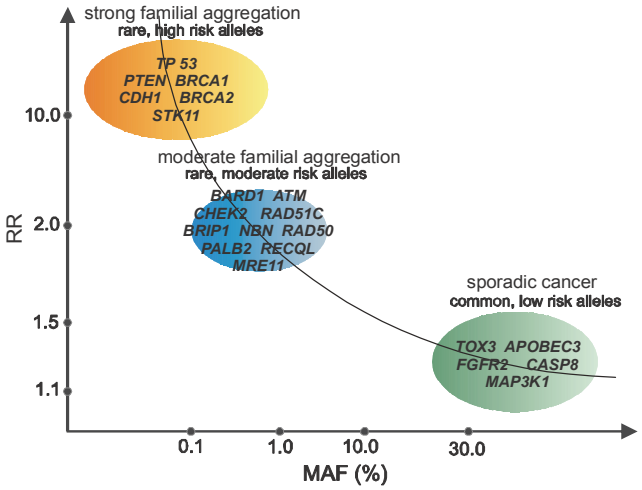

Fig. 2. The relation between the relative risk (RR) and the frequency (minor allele frequency, MAF) of genetic variants in breast and/or ovarian susceptibility genes

of the existence of genes with a population frequency and conferred risk comparable to *BRCA1* and *BRCA2* [e.g. predicted *BRCA3* (**br**east **ca**ncer **3**) gene] is very low (Narod and Foulkes, 2004; Foulkes, 2008). Li-Fraumeni syndrome is attributed to mutations in the *TP53* (**t**umor **p**rotein p**53**) gene encoding a protein "genome guardian" involved mainly in the control of cell cycle progression, repair of DNA damage, and apoptosis stimulation. Although less than 1% of early onset breast cancer cases clustered in families harbor germline mutations in *TP53*, women affected by Li-Fraumeni syndrome are at 49% risk of developing breast cancer by the age of 60 (Garber et al., 1991; Masciari et al., 2012). For women with germline mutations in the *CDH1* (**cadh**erin **1**) gene, associated with the hereditary diffuse gastric syndrome, or in the *STK11* (**s**erine/**t**hreonine **k**inase **11**) gene, associated with Peutz-Jeghers syndrome, the risk of developing breast cancer is approximately 30-40% (Hemminki et al., 1998; Pharoah et al., 2001; Lim et al., 2004). Recently, Tan and coworkers (Tan et al., 2012) have shown that pathogenic germline mutations in the promoter of the *PTEN* (**p**hosphatase and **ten**sin homolog) gene associated with Cowden syndrome can increase the lifetime risk of breast cancer up to 85%. Additionally, hereditary ovarian cancers can be attributed to variants in mismatch repair genes [*MLH1* (**mut**L **h**omolog **1**), *MSH2* (**mut**S **h**omolog **2**), *MSH6* (**mut**S **h**omolog **6**), and *PMS2* (**p**ost**me**iotic **s**egregation increased **2**)] associated with Lynch syndrome (Lynch et al., 2009).

Mutational analyses of candidate genes encoding proteins co-working with BRCA1 and BRCA2 in the same molecular pathways led to the identification of a second group of alterations that confer susceptibility to breast and/or ovarian cancer. This group comprises uncommon variants associated with moderate risk of breast and/or ovarian cancer (Beggs and Hodgson, 2009). It was reported that mutations in *BRIP1* (**BR**CA1 **i**nteracting **p**rotein C-terminal helicase **1**), *BARD1, RAD50, CHEK2* (**che**ckpoint **k**inase **2**), *NBN* (**nibrin**), *PALB2* (**p**artner **a**nd **l**ocalizer of **B**RCA**2**), and *ATM* (**a**taxia **t**elangiectasia **m**utated) are of intermediate penetrance and are associated with 2-4 fold increased risk of breast cancer. It is worth noting that very rare, bi-allelic mutations in breast cancer susceptibility genes *BRCA2, PALB2*, and *BRIP1* are associated with Fanconi's anemia. This suggests that some genes controlling DNA repair through homologous recombination and associated with this mostly recessive disorder may also contribute to the initiation of breast and/or ovarian cancer (Walsh and King, 2007; Foulkes, 2008; Beggs and Hodgson, 2009; van der Groep et al., 2011). Walsh and colleagues observed that mutations in Fanconi's anemia genes are involved in the development of hereditary ovarian cancer. The authors detected pathogenic heterozygous mutations in genes implicated in Fanconi's anemia pathway, [e.g. *BARD1, RAD50, NBN, PALB2, MRE11A* (**m**eiotic **re**combination **11** homolog **A**), *BRIP1,* and DNA repair protein gene *RAD51C* (**RAD51** homolog **C**)] in a group of patients with ovarian carcinoma not selected in terms of familial history of the disease (Walsh et al., 2011). Recently, Cybulski and coworkers have identified *RECQL* (**RecQ** helicase-**l**ike) as a new breast cancer susceptibility gene of moderate penetrance, with the use of the combination of a whole exome sequencing and a large-scale association study of recurrent mutations (Cybulski et al., 2015). The *RECQL,* similarly as other breast cancer susceptibility genes, is involved in DNA repair by resolving stalled DNA replication forks and thus preventing double-stranded DNA breaks.

The third group of breast and/or ovarian cancer predisposing variants comprises a common, low-penetrance polymorphisms, identified mainly in Genome Wide Association Studies (GWAS). Recently, 67 new and previously reported single nucleotide polymorphisms (SNPs) have been identified to be associated with a slightly increased breast cancer risk (odds ratio (OR) ~1,2) (Michailidou

et al., 2013). It was also presumed that the investigation of a copy number variation may uncover a substantial part of the still unidentified genetic *loci* related to the susceptibility to various complex diseases, including breast cancer. Until now, very few studies have assessed the association of CNV with breast cancer risk. Recently, a common large deletion in the *APOBEC3* (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3) gene cluster was correlated with an increased breast cancer risk ([OR] = 1.31 95% CI = 1.21 to 1.42 for one copy deletion) (Long et al., 2013).

In consonance with the polygenic model, the aggregation of breast and/or ovarian cases within a family, not attributed to mutations in *BRCA1* and *BRCA2*, can be caused by a combined effect of multiple genetic alterations in genes of low to moderate penetrance, presumably modified by environmental factors (Karppinen et al., 2004).

BRCA1 and BRCA2 – guardians of the genome integrity

The proteins encoded by *BRCA1* and *BRCA2* tumor suppressor genes work in concert to maintain the genome integrity through the interaction with a number of proteins, such as CHEK2, ATM, BARD1, NBN, RAD51, ATR (**a**taxia **t**elangiectasia and **R**AD3-related), p53, BRIP1, and PALB2. These genes act as guardians of the genome integrity and are involved in the pathways of DNA damage response (DDR), the regulation of transcription, cell cycle checkpoints, apoptosis, and ubiquitination (Narod and Foulkes, 2004; Roy et al., 2012).

Double strand breaks (DSB) constitute a threatening form of DNA damage, as unrepaired double strand lesions often lead to severe genomic rearrangements that contribute to cancer initiation. The main function of BRCA1 is to integrate the DSB repair mechanisms and checkpoint regulation that delay the cell cycle in order to provide time for DNA repair and to ensure that the genetic damage is not transmitted to the next generation whereas BRCA2 is responsible for the core mechanism of RAD51-mediated homologous recombination which was developed by mammals as one of the DDR systems (Hoeijmakers, 2001; Roy et al., 2012).

Mutations in *BRCA1* and *BRCA2*

According to the Breast Cancer Information Core (BIC) database (http://research.nhgri.nih.gov/bic/), approximately 3800 various genomic alterations in *BRCA1* and *BRCA2* genes have been detected so far (data of July, 2015). Missense and nonsense mutations, as well as small frameshift insertions/deletions and mutations affecting splice sites within introns (IVS), account for the most frequent alterations which occur in *BRCA1* and *BRCA2* genes (http:research.nhgri.nih.gov/bic/) (Thompson and Easton, 2004). Large genomic rearrangements in *BRCA1* and *BRCA2* have also been identified. It was reported that large mutations may account from 0% up to 36% of all mutations affecting *BRCA1* gene, across various populations. In *BRCA1* gene more than 80 various large mutations have been found, whereas in *BRCA2* gene much fewer large rearrangements have been reported (Hansen et al., 2009; Sluiter and van Rensburg, 2011).

It was estimated that mutations in *BRCA1* and *BRCA2* genes occur in about 1/400 individuals (Foulkes, 2008). In the majority of populations various mutations are located along the entire sequence of *BRCA1* and *BRCA2*. However, in some ethnic groups, owing to a founder effect, particular mutations in *BRCA1* and *BRCA2* occur with higher frequency. A founder effect can be defined as a loss of genetic variation which occurs due to interbreeding within a small group of individuals isolated from a larger group. As a consequence, relatively uncommon mutations become more frequent within such ethnic group (Ferla et al., 2007). For example in the Ashkenazi Jewish population, 1 in 40 individuals (Foulkes, 2008) is a carrier of 185delAG (c.68_69delAG) (Struewing et al., 1995) or 5382insC (c.5266dupC) (Roa et al., 1996) founder mutation in *BRCA1* or 6147delT (c.5946delT) (Neuhausen et al., 1996) founder mutation in *BRCA2*. In Iceland, a high frequency of founder 999del5 (c.771_775del5) *BRCA2* mutation was identified. This founder mutation was reported to cause the familial clustering of both female and male breast cancer cases. It was shown that 999del5 *BRCA2* mutation affects 40% of males with breast cancer from the Icelandic population (Thorlacius et al., 1996). Finally, in Poland, a high incidence of 5382insC, 300T > G (c.181T > G), and 4153delA (c.4034delA) *BRCA1* founder mutations has been identified (Sobczak et al., 1997; Gorski et al., 2000; Grzybowska et al., 2000; Gorski et al., 2004; Ratajska et al., 2008; Brozek et al., 2011). 3819del5 (c.3700_3704del5) and 185delAG mutations affecting the *BRCA1* gene were also reported to occur frequently in the Polish population (Gorski et al., 2000; Ratajska et al., 2008; Brozek et al., 2011).

BARD1 as a breast cancer susceptibility gene

Structure and functions of the *BARD1* gene and encoded protein

BARD1 gene is located at 2q34-35 and consists of 11 exons encoding protein of 777 amino acids. BARD1 was identified by yeast two-hybrid screening as a protein that associates with BRCA1 protein *in vivo*. BARD1 protein bears a striking structural resemblance to BRCA1 protein. Both proteins harbor a RING-finger motif and a nuclear export signal in the vicinity of their N-termini and two BRCA1 carboxy-terminal (BRCT) domains. BARD1 and BRCA1 proteins form a functional heterodimer through the binding of their RING-finger motifs. Apart from BRCT and RING domains, BARD1 contains three tandem ankyrin repeats (ANK) located in the central part of the protein. This structural motif is implicated in other protein-protein interaction (Wu et al., 1996). Neither BARD1 nor BRCA1 displays structural resemblance to BRCA2 (Irminger-Finger and Jefford, 2006).

Besides structural similarity, BARD1 and BRCA1 proteins share some common functions. Increased levels of these proteins are observed in spleen and testes, as well as in other proliferative tissues. Additionally, it was shown that the expression of *BRCA1* and *BARD1* in breast and ovaries is regulated hormonally and that the *in vitro* down-regulation of *BARD1* leads to the alteration of mammary epithelial cells phenotype (Irminger-Finger et al., 1998). Both *BARD1* and *BRCA1* deficiency is pathogenic for the cell. McCarthy and colleagues observed that *BARD1*$^{-/-}$ and *BRCA1*$^{-/-}$ as well as double *BARD1*$^{-/-}$*; BRCA1*$^{-/-}$ mice display phenotypic similarities. The deficiency of both *BARD1* and *BRCA1* leads to the deleterious genomic rearrangements and an early embryonic death which is attributed to the defective cell proliferation (McCarthy et al., 2003).

BARD1 forms a heterodimeric complex with BRCA1 through the interaction of domains comprising RING finger motifs (Wu et al., 1996). A Heterodimeric state is preferred by BARD1 and BRCA1, because this interaction is thought to stabilize both proteins (Meza et al., 1999) and is required for the nuclear localization of the complex (Irminger-Finger, 2010). It was shown that BRCA1-BARD1 heterodimeric complex has the E3 ubiquitin ligase activity (Ruffner et al., 2001; Baer and Ludwig, 2002; Morris and Solomon, 2004). Although the individual BRCA1 and BARD1 ubiquitin ligase activity is very low, it is considerably enhanced after the heterodimerization of the proteins (Hashizume et al., 2001). It was reported that mutations associated with breast cancer located in the RING domain of BRCA1 disrupt the ubiquitin ligase activity of BARD1-BRCA1 complex and abolish BRCA1 involvement in the mechanisms responsible for the protection of cell from ϒ-radiation (Hashizume et al., 2001; Ruffner et al., 2001). It is also suggested that BRCA1-BARD1 E3 ubiquitin ligase is implicated in DNA repair and that BARD1 is essential for BRCA1 tumor suppression functions.

A number of BRCA1-BARD1 targets have been identified, including CDC25C (**c**yclin B and cell **d**ivision **c**ycle **25C**) (Shabbeer et al., 2013), ϒ-tubulin (Starita et al., 2004), and H2AX (Chen et al., 2002). BRCA1-BARD1 E3 ligase was reported to ubiquitinate the proteins that orchestrate G2/M cell cycle checkpoint, i.e. cyclin B and CDC25C, what leads to their degradation and loss of control over the cell cycle progression (Shabbeer et al., 2013). Additionally, BARD1-BRCA1 heterodimer can control centrosome duplication, mediating the destruction of ϒ-tubulin (Starita et al., 2004). H2AX can also be ubiquitinated by E3 ligase, what indicates that BRCA1-BARD1 heterodimer can be implicated in chromatin remodeling (Chen et al., 2002).

Ryser and colleagues also observed an interaction of BARD1 and BRCA2 in mitosis. As full length BARD1 associates with BRCA1 at spindle poles in early mitosis, BARD1 β isoform (without RING domain), frequently found in gynecological cancers, interacts with BRCA2 in late mitosis. Accordingly, BARD1 isoforms have different functions in mitosis and may functionally associate with BRCA1 and BRCA2 proteins, which are responsible for the control of early and late phase of mitosis, respectively (Ryser et al., 2009).

Besides BRCA1/2-mediated functions, independent cellular activities of BARD1 were also reported. Irminger-Finger and colleagues proposed a paradigm of the "dual mode of action" for BARD1 activity in the cell. The authors distinguished the survival mode, in which BARD1 associates with BRCA1 and is implicated in the DNA damage response, and the death mode in which the excess of BARD1 over BRCA1, performs pro-apoptotic functions independently of BRCA1. It was observed that the interaction of BARD1 and BRCA1 diminishes the apoptosis induction. The study indicates that the geno-

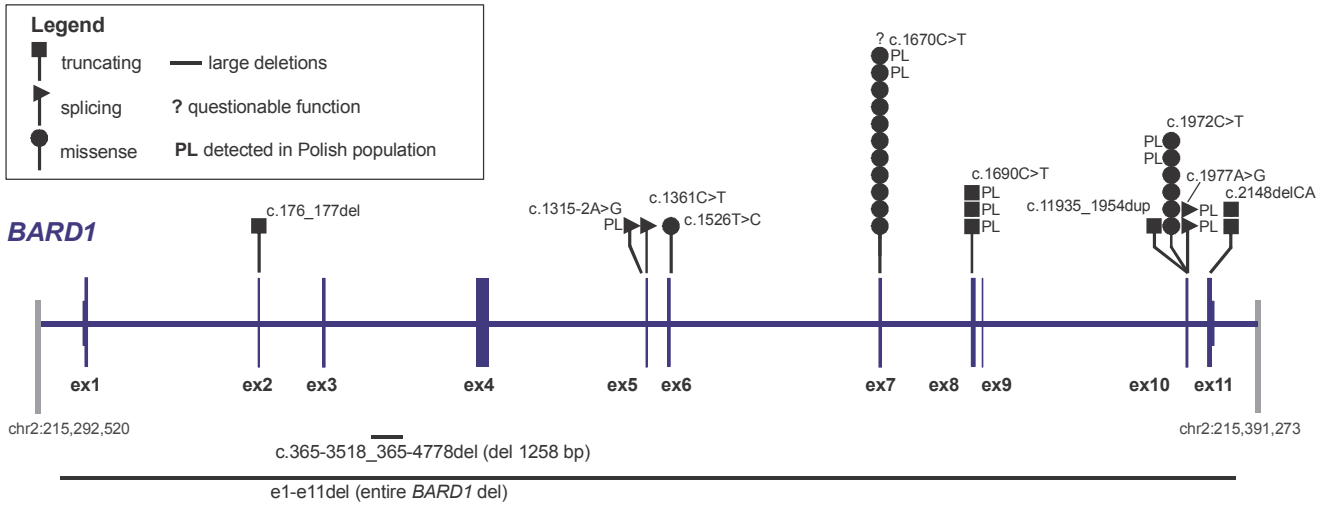

Fig. 3. Map of *BARD1* mutations identified in patients with breast and/or ovarian cancer. It does not show variants identified as neutral or of unknown significance, and common SNPs associated with cancers

toxic stress induces upregulation of *BARD1*. The increased accumulation of BARD1 has an impact on the stabilization of p53 through the association of BARD1 with p53 and a subsequent induction of apoptosis. Accordingly, the repression of BARD1 synthesis leads to an impaired apoptotic response to severe DNA damage (Irminger-Finger et al., 2001).

Mutations in the *BARD1* gene

The mutational analyses of *BARD1* in non-*BRCA* subjects with familial breast and/or ovarian cancer led to the identification of various *BARD1* sequence variants. These variants include deleterious and potentially deleterious mutations leading to premature termination of translation, disruption of protein structure/function, or alternative splicing (Karppinen et al., 2004; De Brakeleer et al., 2010; Sabatier et al., 2010; Ratajska et al., 2012; Castera et al., 2014; Cybulski et al., 2014; Pennington et al., 2014; Churpek et al., 2015; Couch et al., 2015; Klonowska et al., 2015) (summarized in Fig. 3). However, it has to be noted that some results of these studies are not consistent and inconclusive in terms of the functional significance of the mutation.

Some germline mutations, including missense, frameshift and silent alterations (Thai et al., 1998; Ghimenti et al., 2002; Ishitobi et al., 2003; Sauer and Andrulis, 2005; Huo et al., 2007) as well as a large heterozygous deletion (1258 bp) within intron 3 of the *BARD1* gene (Rouleau et al., 2012) were identified, however their impact on the protein structure and function is not elucidated.

The p.Cys557Ser (c.1670G > C) [rs28997576 in dbSNP database; http:ncbi.nlm.nih.gov/SNP] is the most commonly studied mutation in the *BARD1* gene. Sauer and coworkers (Sauer and Andrulis, 2005) showed that a defective growth suppression and impaired apoptotic functions are attributed to an ectopic expression of *BARD1* affected by the p.Cys557Ser mutation, indicating a possible deleterious effect of this variant. p.Cys557Ser was reported to slightly increase the breast cancer risk in Nordic (Finish, Icelandic, Danish, Swedish, and Norwegian) families with breast cancer aggregation (*BRCA1/2* positive – OR = 3.2; $p = 0.01$; 95% CI = 1.2-8.3; *BRCA1/2* negative – OR = 2.6; $p < 0.001$; 95% CI = 1.7-4.0) (Karppinen et al., 2004; Karppinen et al., 2006), however this finding was not confirmed by Vahteristo and colleagues (Vahteristo et al., 2006) who showed no association of the p.Cys557Ser with familial breast cancer susceptibility in Finland. Neither has the p.Cys557Ser mutation been associated with an increased risk of breast cancer in Australian and Polish populations (Gorringe et al., 2008; Jakubowska et al., 2008; Johnatty et al., 2009) whereas in Iceland, Stacey and colleagues (Stacey et al., 2006) showed a modest increase of the risk of breast cancer attributed to p.Cys557Ser and demonstrated that the risk of breast cancer among carriers of double mutations in *BARD1* p.Cys557Ser and *BRCA2* 999del5 is significantly increased (OR = 3.11; 95% CI = 1.16-8.4; p. = 0.046) (Stacey et al., 2006). However, in studies conducted in different European populations the role of p.Cys557Ser variant as a modifier of *BRCA1/2*-

associated cancer risk has not been confirmed (Jakubowska et al., 2008; Spurdle et al., 2011).

Sabatier and coworkers, identified a homozygous deletion of the entire *BARD1* gene by carrying out an analysis of an array-based comparative genomic hybridization (aCGH) profiles of breast cancer tumors from 330 patients with invasive breast adenocarcinoma. An additional aCGH analysis of DNA from a blood sample of the carrier of homozygous mutation revealed the presence of a heterozygous deletion of the entire *BARD1* gene. Interestingly, patients who harbor *BARD1* deletion (but are not affected by *BRCA1* mutations) displayed clinicopathological features which are specific for a phenotype associated with mutations occurring in *BRCA1* (Sabatier et al., 2010).

The mutational analysis of *BARD1* recently conducted in *BRCA1/2*-negative families with breast cancer aggregation has identified eleven intronic and fifteen exonic germline variants (one in-frame deletion, four silent, one frameshift and nine missense mutations). Three of these variants, i.e. p.Ile509Thr (c.1526T > C), p.Glu652fs (c.1935_1954dup) and p.Arg658Cys (c.1972C > T) have been shown to predispose to breast cancer and to co-segregate with the disease phenotype in the analyzed families. On the basis of *in silico* predictive analysis it was evaluated that the p.Ile509Thr missense variant, located within the ANK domain, causes the protein instability and improper protein folding. The p.Arg658Cys variant was found to have a "possible effect on function" based on protein modeling. Last but not least, de Brakeleer and colleagues have identified a novel protein truncating mutation, p.Glu652fs, which results in a loss of the entire second BRCT domain of BARD1, which may result in a defective DNA damage response (De Brakeleer et al., 2010).

Ratajska and colleagues conducted screening of germline variants of the *BARD1* gene in 109 of *BRCA1/2* -negative patients from families with the aggregation of breast and/or ovarian cancer via utilizing either denaturing high-performance liquid chromatography (DHPLC) or direct sequencing. Ten exonic and seven intronic variants, including five novel alterations were identified in this study. Three novel *BARD1* mutations [p.Gly439_Leu465del (c.1315-2A > G), p.Gln564X (c.1690C > T), p.Arg659Arg (c.1977A > G)] can be considered as possibly deleterious. The p.Gly439_Leu465del mutation is located in intron 4 within the consensus sequence of the splice acceptor site. The mutation causes skipping of exon 5 and disruption of two ANK repeats implicated in apoptosis and protein-protein association. *In silico* prediction suggests that this variant may cause an alteration of the BARD1 protein structure. Another nonsense, protein truncating mutation, p.Gln564X, which occurs in exon 8, leads to the loss of the BRCT domains. Finally, p.Arg659Arg *BARD1,* a presumably silent mutation located in exon 10 alters the exon splice enhancer motifs (ESE) and leads to exons 2-9 skipping (p.Cys53_Trp635delinsfsX12) (Ratajska et al., 2012).

The study performed recently on a large group (>800) of patients with breast and/or ovarian cancer indicated that large deletions are not common in *BARD1* and therefore may not contribute substantially to the breast cancer risk (Klonowska et al., 2015). The study also revealed that the p.Gln564X, p.Arg659Arg and p.Arg658Cys mutations are recurrent in the Polish population, what indicates their potential founder character (Klonowska et al., 2015). The founder character of these mutations is additionally supported by the fact that they were independently detected in other studies conducted in Polish population as well (Ratajska et al., 2012; Cybulski et al., 2014; Ratajska et al., 2015). The functional and *in silico* analyses suggested their possible deleterious character (Ratajska et al., 2012; Klonowska et al., 2015; Ratajska et al., 2015).

Recently, exome sequencing analyses focused on panels of breast cancer predisposing genes have also led to the identification of potentially deleterious *BARD1* mutations. Additionally, the study showed that *BARD1* is one of the most frequently mutated genes (after several moderate and highly penetrant genes, e.g. *PALB2*, *BRCA1* and *BRCA2)* in patients with breast and/or ovarian cancer (Walsh et al., 2011; Castera et al., 2014; Cybulski et al., 2014; Pennington et al., 2014; Churpek et al., 2015; Couch et al., 2015).

Conclusions

The genetic etiology of breast and/or ovarian cancer cases aggregated in families is only partially known. Apart from *BRCA1/2* and several other genes of moderate to high penetrance, a considerable fraction of breast cancer predisposing factors (~50%) is still unknown. It is presumed that DSB repair genes, encoding proteins that are involved in the same molecular pathway as BRCA1, may be candidate breast cancer susceptibility genes.

The findings that BARD1 is essential for BRCA1 tumor suppression functions and that it operates independently in the regultion of apoptosis, suggest that the *BARD1* gene may serve as a plausible target for mutations predisposing to breast and/or ovarian cancer. Although a number of mutational studies have already been conducted, a study on *BARD1* mutations in patients with breast and/or ovarian cancer is still in its infancy. Despite the fact that several potentially deleterious *BARD1* mutations have been identified, further studies should be carried out to evaluate their breast and/or ovarian cancer predisposing effect and to identify the unexplored mutations affecting the *BARD1* gene.

It is noteworthy that none of the studies conducted so far has provided a clear and statistically supported proof for the role of *BARD1* as a breast cancer susceptibility gene. Therefore, large-scale association studies of the selected *BARD1* mutations would be highly desirable to unequivocally confirm or reject the role that *BARD1* plays in breast and/or ovarian cancer susceptibility. Importantly, if breast cancer risk associated with *BARD1* mutations turns out to be considerably high, the inclusion of testing of the *BARD1* mutations into genetic diagnostics of breast cancer and other genetically associated cancers would be a far-reaching consequence.

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