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Mutation screening of the *MERIT40* gene encoding a novel BRCA1 and RAP80 interacting protein in breast cancer families

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Abstract *MERIT40* is a recently identified BRCA1 and RAP80 interacting protein that is essential for protein–protein interactions of a BRCA1 complex also containing Abraxas, BRCC36 and BRCC45. It is a mediator of checkpoint functions and DNA damage signaling through a (de)ubiquitination cascade. Based on its interaction with BRCA1 and its role in genome integrity maintenance, *MERIT40* is a novel candidate gene for being involved in hereditary susceptibility to breast cancer. Here, we report to our knowledge the first comprehensive mutation screening of this gene in affected cases of breast cancer families. Only a number of sequence variants were found, four of which are novel. None of the observed variants appeared to be disease related, suggesting that germline

mutations in *MERIT40* are rare or absent in familial breast cancer patients.

Keywords *MERIT40* · Breast cancer susceptibility · Germline mutation

Introduction

BRCA1 and *BRCA2* are the two major susceptibility genes involved in hereditary predisposition to breast cancer. However, germline mutations in these tumor suppressors account for a maximum 20% of the familial breast cancer cases worldwide [1]. The involvement of further high penetrance breast cancer susceptibility genes is questionable [2], and it has been suggested that if they exist, they would account only for a very small fraction of familial disease risk [3]. Instead, most of the remaining cancer cases are expected to be explained by mutations in moderate and low penetrance cancer susceptibility genes together with environmental factors [2–5]. Genome-wide association studies and candidate gene approaches with direct mutation analysis of tumor suppressor genes have proven to be successful approaches to identify susceptibility genes for the remaining familial breast cancer cases. A candidate gene approach based on physical interaction of the protein product with BRCA1 or BRCA2 has led to the identification of several important breast cancer susceptibility genes, such as *PALB2* [6], *BRIP1* [7] and *BARD1* [8, 9].

MERIT40 (Mediator of RAP80 Interactions and Targeting 40 kDa, alias HSPC142/NBA/c19orf62) is a newly identified BRCA1 interacting protein [10–12] that is essential for RAP80 interaction with BRCA1 [13–15]. By using a systems-wide interactome mapping approach, *MERIT40* was previously shown to interact with another

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BRCA1 interacting protein, BRCC45 [16, 17]. MERIT40 also associates with Abraxas (alias ABRA1 or CCDC98) [15, 18, 19] and BRCC36 [20, 21]. Interaction with MERIT40 is essential to maintain BRCA1-RAP80 complex integrity, protein stability and DNA double-strand break targeting. Consequently, MERIT40 is required for G2 checkpoint execution and viability responses to ionizing radiation [10–12].

The DNA damage response through BRCA1-RAP80 complex signaling is achieved in part through a (de)ubiquitination cascade that emerges as a central event in DNA damage signaling [22–25]. MERIT40 appears to control the cell cycle checkpoint and resistance to DNA-damaging agents by mediating interactions between the core BRCA1-RAP80 complex and BRCC36, thus targeting lysine-63-ubiquitin (K63-Ub) deubiquitination activity to DNA damage sites [10–12]. The MERIT40 gene product consists of 329 residues and possesses a von Willebrand factor A (VWA) domain at its N-terminus that is homologous to the VWA domain of the S5A proteasome subunit. To date, MERIT40 appears to primarily play a potential scaffolding role for the BRCA1 complex, although other activities cannot be ruled out, such as proteasome-linked protein processing activities through the VWA domain. The central region (amino acids 93–320) of MERIT40 is evolutionarily conserved down to invertebrates and plants. Deletion of the last 30 residues prevents MERIT40 nuclear accumulation, formation of ionizing radiation-induced foci, as well as interaction with BRCA1, RAP80, Abraxas and BRCC36 [10].

Given the dependence of DNA damage signaling on MERIT40, and its association with BRCA1, we examined whether mutations in *MERIT40* occur in the context of hereditary susceptibility to breast cancer. Here, we report the result of a comprehensive mutation screening of the *MERIT40* gene in affected index cases of 125 Finnish breast cancer families. The present investigation revealed 7 sequence variants, 4 of which are not currently reported in public databases. None of the observed germline changes, however, appeared to be disease related. Furthermore, the performed mutation analysis did not indicate any dosage variation in the *MERIT40* gene.

Patients and methods

Cases and controls

Mutation screening of the *MERIT40* gene was performed on 125 breast and breast-ovarian cancer families originating from northern Finland. For statistical purposes, one index patient from each family was chosen according to the youngest age of breast cancer onset. Inclusion criteria for the 72 high-risk families were the following: (1) three or

more cases of breast cancer, potentially in combination with single ovarian cancers in first- or second-degree relatives, or (2) two cases of breast cancer in first- or second-degree relatives, of which at least one with early disease onset (<35 years), bilateral breast cancer or multiple primary tumors including breast or ovarian cancer in the same individual. The remaining 53 families were indicative of moderate disease susceptibility, presenting either two cases of breast cancer in first- or second-degree relatives or one case of breast cancer under the age of 35 or one breast cancer and other types of cancer in the same family. Altogether fifteen of the studied index cases had previously been tested positive for known breast cancer-associated germline mutations in *BRCA1* or *BRCA2* (eleven), *TP53* (one) and *PALB2* (three). All of the biological specimens and clinical information of the familial breast cancer cases investigated were collected at the Oulu University Hospital, with the informed consent of the patients. The used control samples ($N = 192$) derived from anonymous female cancer-free Finnish Red Cross blood donors (age ≥ 45 years) originating from the same geographical region as the studied cancer families. Approval to perform the study was obtained from the Ethical Board of the Northern Ostrobothnia Health Care District and the Finnish Ministry of Social Affairs and Health.

DNA isolation and mutation analysis

DNA from blood lymphocytes was extracted using the standard phenol–chloroform method or the Puregene D-50 K purification kit (Gentra). The entire coding region and exon–intron boundaries of the *MERIT40* gene were screened for germline mutations by conformation sensitive gel electrophoresis (CSGE). Samples with deviating band patterns were sequenced with the Li-Cor IR² 4200-S DNA Analysis system (Li-Cor Inc.) using the SequiTherm EXELTMII DNA Sequencing Kit-LC (Epicentre Technologies). Besides sequence variants, also possible aberrations in DNA dosage were monitored for. Oligonucleotides for CSGE and sequencing were designed by the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) based on sequence information obtained from publicly available databases. Oligonucleotide sequence information is shown in the Supplementary Table S1, and PCR conditions are available upon request.

Statistical and bioinformatical analysis

Carrier frequencies between patients and healthy controls were compared by using Pearson Chi-Square or Fisher's exact test (two-sided) (SPSS version 17.0 for Windows). All alterations were checked with NNSplice software for potential splicing effects (http://www.fruitfly.org/seq_

tools/splice.html). The missense alteration Lys274Arg was tested for possible pathogenicity by using PolyPhen software (<http://genetics.bwh.harvard.edu/pph>).

Results

Screening of index patients of breast cancer families for germline alterations in *MERIT40* revealed 2 intronic and 4 exonic variants, and a variant in the 3' UTR of the gene, but did not uncover any DNA dosage aberrations. Four of the observed sequence changes are not reported in the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/SNP/>). Details and occurrence of the observed nucleotide variants are shown in Table 1, and the positions of the exonic changes in the protein are depicted in Fig. 1. Only one alteration resulted in an amino acid change (Lys274Arg). However, according to the result of PolyPhen software analysis, the change is not predicted to affect protein function. c.*87G > A was observed in one family but not in the controls, whereas all of the other variants were found at similar frequencies in the control population. The nucleotide alterations were also assessed for possible effects on consensus splice sites. Of the observed changes, c.*87G > A might be implicated in introducing a new splice acceptor site (score 0.68, vs. 0.53 for the wild-type sequence), the effect of which would be unclear. None of the variants located to the last 30 amino acids of the protein that is implicated in nuclear localization and protein–protein interactions with various members of the BRCA1 complex.

Discussion

MERIT40 is involved in the integrity of a BRCA1 containing complex also including RAP80, Abraxas, BRCC36 and

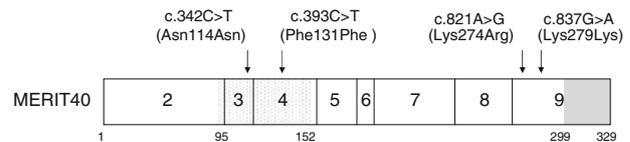


Fig. 1 Schematic diagram of the *MERIT40* protein and effect of the observed nucleotide changes in the coding region of the gene. Boxes represent exons. The only currently known functional domain, the von Willebrand factor A domain (aa 95–152) is dotted. Deletion of the last 30 amino acids of *MERIT40* prevents its nuclear accumulation and association with BRCA1, RAP80, Abraxas and BRCC36 (region shown in gray)

BRCC45 [10–12]. *MERIT40* is required for the recruitment of these interaction partners to DNA double-strand breaks upon ionizing radiation and for the participation through RAP80 and BRCC36 in a (de)ubiquitination signaling cascade. Interestingly, a potentially pathogenic amino acid deletion was recently reported in *RAP80* in two breast cancer patients [26]. This mutation abrogated ubiquitin binding and DNA damage response signaling for RAP80 and other members of the BRCA1 complex. These data suggest that *MERIT40*, similar to RAP80, may play a role in breast tumorigenesis via the interaction with BRCA1.

In our present study, the whole coding region and exon–intron boundaries of *MERIT40* were screened for germline mutations. Although several new alterations were found, they are unlikely to be pathogenic. Two nucleotide changes, however, may be functionally important. c.*87G > A was found in one index patient, but not in the control population, suggesting that it is either a rare polymorphism or it might exert a detrimental effect if it affected splicing or transcriptional stability of *MERIT40*. The only amino acid change observed, Lys274Arg, is also a novel variant, and though statistically its frequency was not significantly elevated compared to that of the controls, we cannot currently fully exclude the slight possibility that it may represent a low

Table 1 Observed sequence variations in the *MERIT40* gene

Location	Nucleotide change	Effect on protein	rs number	Frequency of heterozygotes, % (n/N)		P (OR; 95% CI)
				Familial cases	Controls	
Exon 3	c.342C > T	Asn114Asn	–	4.0 (5/125)	3.7 (7/192)	1.0 (1.1; 0.3–3.6)
Intron 3	c.344 + 41A > T	–	rs10420922	49.6 (62/125)	44.3 (85/192)	0.4 (1.2; 0.8–1.9)
Exon 4	c.393C > T	Phe131Phe	–	0.8 (1/125)	1.6 (3/192)	1.0 (0.5; 0.1–4.9)
Intron 8 ^a	c.787 – 6C > T	–	rs10406920	39.2 (49/125)	37.5 (72/192)	0.8 (1.1; 0.7–1.7)
Exon 9	c.821A > G	Lys274Arg	–	1.6 (2/125)	1 (2/192)	0.6 (1.5; 0.2–11.1)
Exon 9 ^a	c.837G > A	Lys279Lys	rs8170	39.2 (49/125)	37.5 (72/192)	0.8 (1.1; 0.7–1.7)
Exon 9 ^b	c.*87G > A	–	–	0.8 (1/125)	– (0/192)	0.4 (1.0; 0.98–1.01)

The following sequence information was used: NC_000019 (genomic DNA), NM_001033549.1 (mRNA), and NP_001028721 (protein)

OR odds ratio, CI confidence interval

^a rs10406920 and rs8170 were in linkage

^b 3'UTR change

penetrance susceptibility allele. Large-scale association studies would be needed to address this issue. A matter that could be of further interest is the observation that all of the amino acid changes seen in the current study are silent ones, except for Lys274 that is substituted in the most conservative fashion. This notion could reflect the functional essentiality of these amino acid residues. On the other hand, it might be speculated that Lys274 or Lys279 are potential post-translational modification sites and that a switch from Lys to Arg at position 274 in a heterozygous manner could subtly modulate MERIT40's role in DNA damage signaling. Consequently, a better understanding of MERIT40 functions, and particularly the role of its first 300 residues, would be helpful in evaluating the significance of genetic alterations in *MERIT40* regarding normal cellular functions and in human disease.

In conclusion, the present data suggest that mutations predisposing to breast cancer are either very rare or absent in the coding region of *MERIT40*. It would be interesting to know whether large genomic rearrangements, or mutations in transcriptional regulatory regions located further away from the coding region, could represent alternative and more typical ways of dysregulating MERIT40 function. On the other hand, it might also be that mutations in this gene are poorly tolerated, pointing to the essentiality of MERIT40 in the DNA damage response and other functions maintaining genomic integrity. To our knowledge, this is the first study exploring the possible involvement of the *MERIT40* gene in hereditary predisposition to breast cancer.

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