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No evidence of involvement of germline *BACH1* mutations in Finnish breast and ovarian cancer families

S.-M. Karppinen, J. Vuosku, K. Heikkinen, M. Allinen, R. Winqvist*

Department of Clinical Genetics, Oulu University Hospital/University of Oulu, PO Box 24, FIN-90029 OYS, Finland

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Abstract

Recently BACH1, a novel putative DNA helicase mapping to chromosome 17q22, was reported to interact specifically with BRCA1, and was suggested to be a candidate gene for predisposition to breast and ovarian cancers. Here, we screened 214 breast and ovarian cancer patients from 151 Finnish families for germline *BACH1* mutations by utilising conformation-sensitive gel electrophoresis (CSGE) and genomic sequencing analysis. Four sequence alterations were observed in the exon regions of *BACH1*, three of which have been previously reported and were classified as polymorphisms. In 1 patient, a novel heterozygous 3101C > T variant was observed resulting in a proline to leucine substitution at codon 1034 (Pro1034Leu). This amino acid change occurs in the BRCA1 binding domain of the BACH1 protein. Although the 3101C > T transition was also found in one of the 304 control individuals with an unknown cancer status, it still remains possible that this alteration could represent a rare disease-related allele in the population. Functional assays are needed to resolve the biological significance of this novel BACH1 missense variant. Altogether, the available data suggest that germline mutations in *BACH1* are extremely rare.

Keywords: Hereditary breast and ovarian cancer; Germline *BACH1* mutations

1. Introduction

Breast and ovarian cancer are among the most frequent cancers in women in Western countries and it has been estimated that approximately 5–10% of the cases result from a hereditary disease predisposition [1,2]. The discovery of two major susceptibility genes, *BRCA1* and *BRCA2* [3,4], has considerably improved our understanding of the heritable background to breast and ovarian cancer. However, a significant proportion of the familial cases cannot yet be explained by mutations in any known gene [2,5–7]. For instance in the Finnish population, mutations in *BRCA1* or *BRCA2* have been observed in only approximately 20% of the studied breast cancer families [6,8–10]. At present, analysis of genetic linkage in high-risk families, evaluation of low-penetrance genes as modifiers of *BRCA1* and *BRCA2* function, and population-based case-control studies of suitable candidate genes are among the multiple different approaches being used to identify new cancer susceptibility genes [11,12].

BRCA1 and BRCA2 have been ascribed functions in processes related to the detection or repair of DNA damage. In addition, many of the proteins interacting with these molecules appear to share similar properties [11,13,14], thus making the corresponding genes plausible targets for germline mutations associated with susceptibility to cancer. Recently, based on its interaction with BRCA1, Cantor and colleagues (2001) [15] isolated a putative 130 kDa DNA helicase named BACH1 (Brca1-Associated C-terminal Helicase). BACH1, also known as BRIP1 (BRCA1-interacting protein 1), is ubiquitously expressed and the pattern is similar to that reported for BRCA1 [15]. The N-terminal part of BACH1 shares a strong sequence homology to the catalytic and nucleotide-binding domains of known members of the DEAH-helicase family, while a large portion of its most C-terminal region appears unique [15]. In humans, the Bloom, Werner and Rothmund–Thomson genomic instability disorders that include predisposition (Asp-Glu-Ala-His amino acid motif) to various cancers have been found to

* Corresponding author. Tel.: +358-8-3153228; fax: +358-8-3153243.

E-mail address: robert.winqvist@oulu.fi (R. Winqvist).

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result from germline mutations in the *BLM*, *WRN* and *RECQL* helicase genes, respectively [16].

BACH1 interacts through its C-terminal residues (between 888 and 1063) with the two BRCT (BRCA1 C-Terminal) repeats of the BRCA1 protein [15]. The BRCT domain, first identified in BRCA1, is an evolutionarily conserved protein–protein interaction motif frequently found in proteins involved in DNA repair, homologous recombination, transcriptional activation and cell cycle control [17–23]. Many cancer-associated *BRCA1* mutations result in truncated protein products lacking one or both of the BRCT motifs (Breast Cancer Information Core, BIC). Therefore, it is possible that proteins that interact with the BRCT motifs of BRCA1 play important roles in BRCA1-dependent tumour suppression. However, unlike other BRCA1 BRCT-interacting proteins, BACH1 co-localises with BRCA1 in nuclear foci and the interplay of these two proteins has been shown to be required for efficient DNA double-strand break repair.

Interestingly, *BRCA1* maps to chromosome 17q21 and *BACH1* nearby to 17q22 [15]. The frequent documentation of allelic losses in 17q21–q22 and the failure to detect *BRCA1* mutations in the corresponding breast tumours indicate that this chromosomal region could harbour an additional breast cancer suceptibility gene besides *BRCA1* [24]. Loss of heterozygosity (LOH) in 17q is also a frequent event in ovarian cancer [25]. Therefore, based both on its suggested biological function and chromosomal location, *BACH1* would be a suitable loss of heterozygosity candidate target gene in breast and ovarian cancers.

Cantor and colleagues [15] screened 65 women with early-onset breast cancers for germline *BACH1* aberrations. Two distinct heterozygous missense mutations were detected and both of them resulted in amino acid changes (Pro47Ala and Met299Ile) in the helicase domain of the protein. As these alterations were absent among the studied healthy controls they were unlikely to represent polymorphisms. In addition, the Pro47Ala substitution occurred in a family with strong history of breast and ovarian cancers, and was found associated with BACH1 protein destabilisation. In a recent study by Ghimenti and colleagues (2002) [26], germline mutations in another BRCA1-associated gene, *BARD1,* were discovered. These mutations occurred in 10% of the studied *BRCA1* and *BRCA2* mutation-negative breast and breast-ovarian cancer families. Interestingly, evidence has been presented that both BARD1 and BACH1 exist in a three-protein nuclear complex together with BRCA1. The BACH1/BARD1 interaction appears to be indirect and relying upon the independent association of each protein with BRCA1 [15].

Viewed against this background, *BACH1* appears to be a suitable candidate gene that could reveal at least some of the still unexplained cases of genetic predisposition to breast or ovarian cancers. Therefore, in the present study we have screened 214 patients from 151 Finnish cancer families for germline *BACH1* alterations.

2. Patients and methods

Altogether 214 breast and ovarian cancer patients belonging to 151 families originating from Northern Finland were selected for screening for germline *BACH1* mutations (Table 1). Of the studied cancer families, 95 were associated with breast, 29 with breast-ovarian and 4 with ovarian cancer. All of these families met the criteria for either high (76 families) or moderate (52 families) genetic susceptibility to breast and/or ovarian cancer. The selection criteria used for these 128 cancer families were one or more of the following: (1) 2–3 or more cases of breast and/or ovarian cancer in first- or second-degree relatives, (2) early disease onset ($\leqslant$35 years), (3) bilateral breast cancer or (4) multiple primary tumours including breast or ovarian cancer in the same individual. In addition, 23 families showing single cases of breast or ovarian cancer along with multiple cases (2 or more) of other kinds of cancer in first- or second-degree relatives were also included in the study. The high-risk families mainly included 3 or more cancer cases and 11 of these families had previously been shown to be *BRCA1* or *BRCA2* mutation-positive [9]. All the other *BACH1* tested families were *BRCA1* and *BRCA2* mutation-negative. Furthermore, all of the high-risk families had also been screened for germline *CHK2* [27] and *TP53* [28] mutations. Informed consent to obtain pedigree data and a blood specimen for a

Table 1
Summary of the cancer families included in the screening for germline *BACH1* mutations

	Number of families	Number of studied cancer cases
Cancer families	151	214
Families with breast cancer	95	151
Families with breast and ovarian cancers	29	35
Families with ovarian cancer	4	5
Other multicancer families showing single cases of breast or ovarian cancer	23	23

Table 2
Sequence variation observed in the exon regions of *BACH1*

Exon	nt change	Effect on protein	Frequency		Status
			Cases	Controls	
19	2637G > A	None	10.3% (22/214)	ND	Known variant[a]
	2755C > T	Pro919Ser	50.0% (107/214)	ND	Known variant[a]
20	3101C > T	Pro1034Leu	0.47% (1/214)	0.33% (1/304)	Novel variant
20	3411C > T	None	57.5% (123/214)	ND	Known variant[a]

ND, not done; nt, nucleotide.
[a] Reported by Cantor and colleagues [15] and Luo and colleagues [30]

study on cancer susceptibility gene mutations was obtained from all patients. Control DNA samples from blood were derived from 304 anonymous individuals originating from the same geographical region as the cancer family material. Approval to perform the study was obtained from the Ethical Board of the Northern Ostrobotnia Health Care District and the Finnish Ministry of Social Affairs and Health.

DNA extraction from blood lymphocyte specimens was performed using the standard phenol-chloroform method or the Puregene D-50K purification kit (Gentra). The screening for *BACH1* mutations was done by conformation-sensitive gel electrophoresis (CSGE) [29] and detected alteration-positive samples were reamplified and purified with the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Sequencing analysis was performed with the Li-Cor IR^2 4200-S DNA Analysis system (Li-Cor Inc., Lincoln, USA) and using the SequiTherm EXCEL^{TM}II DNA Sequencing Kit-LC (Epicentre Technologies), following the protocol provided by Li-Cor. Oligonucleotides for CSGE and sequencing were designed by using the Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_ww.cgi) utilising the available sequence data (AC060798, AC005969 and AC002994) from the GenBank database (http://www.ncbi.nlm.nih.gov). Primer sequences and PCR conditions for CSGE and sequencing are available upon request.

3. Results

In the studied breast and ovarian cancer patients, four germline sequence alterations within the protein-encoding region of the *BACH1* gene were detected (Table 2). A novel heterozygous missense-type variant in exon 20 was observed. This C > T transition at nucleotide 3101 results in substitution of proline to leucine at codon 1034 (Pro1034Leu). Interestingly, the amino acid change occurs in the BRCA1 binding domain of the BACH1 protein, therefore suggesting that the alteration could be disease-related. The 3101C > T substitution was initially observed in a 37 year old individual with ovarian cancer exhibiting a family history of gynaecological cancers (her sister had squamous cervical uterine carcinoma *in situ* at age 38, her mother had squamous cervical uterine carcinoma grade 2 at an unknown older age, and her maternal aunt had uterine cancer at age 28) (Fig. 1). Later, the same nucleotide change was also found in one of the 304 anonymous population controls of unknown cancer history. The three other alterations (2637G > A, 2755C > T and 3411C > T) have been previously reported by Cantor and colleagues [15] and Luo and colleagues [30], and were all classified as neutral polymorphisms. As the reported allele frequencies of these polymorphisms were mainly similar to those obtained in the present study, and corresponding allele frequencies were obtained for both the cancer cases and the healthy controls in the Swedish study [30], we only calculated the values for the cancer cases. Our study also revealed five previously unreported sequence variants located in the intron regions between exons 1 and 2, 5 and 6, as well as 14 and 15 (Table 3). Neither of the two heterozygous missense mutations (139C > G and 897G > A) discovered by Cantor and coworkers [15] were observed among the studied Finnish cancer families.

4. Discussion

In the current study, a novel germline *BACH1* 3101 C > T alteration was observed in an ovarian cancer

Table 3
Sequence variation observed in the intron regions of *BACH1*

Location	nt change	Frequency in the studied cancer cases (%)
IVS1 + 12	G > A	40.7 (87/214)
IVS5–28	G > A	0.47 (1/214)
IVS6–31	C > G	48.1 (103/214)
IVS14 + 7	G > A	0.93 (2/214)
IVS14 + 23	DelT	0.47 (1/214)

IVS, intervening space; nt, nucleotide.

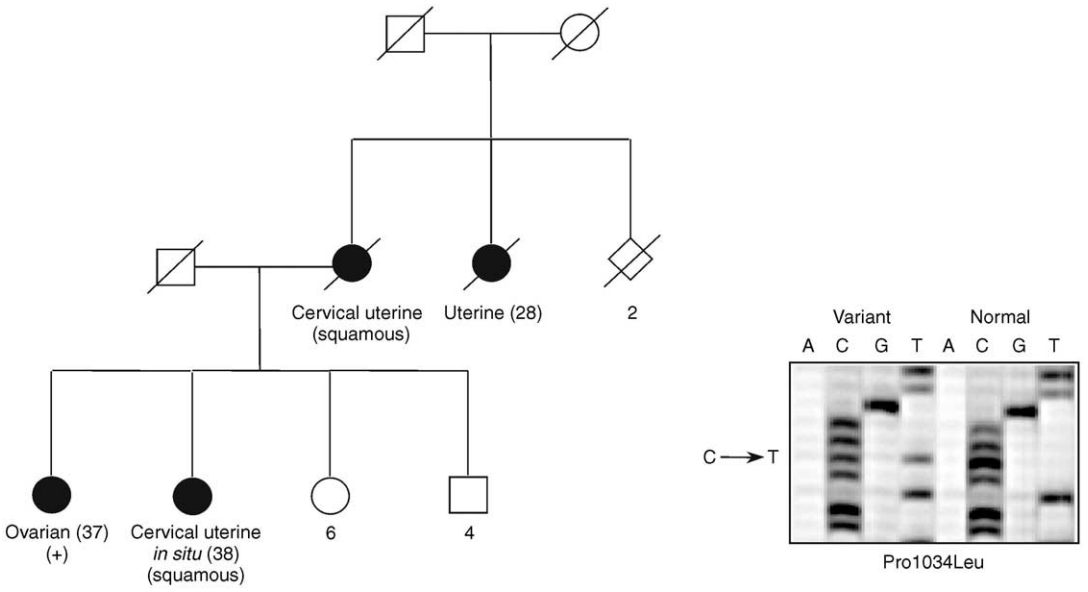

Fig. 1. Finnish cancer family exhibiting the novel *BACH1* 3101C > T missense variant. Filled/open symbols indicate cancer/non-cancer status. The age at diagnosis, when known, is marked after the malignancy and number of siblings is shown below the symbols. (+) = variant carrier.

patient displaying a family history of gynaecological cancers (Fig. 1). Unfortunately, the lack of available DNA from any other members of this family made it impossible to test for cosegregation of this alteration with cancer. In addition, as there was no tumour tissue from this index patient, we could not test whether a possible LOH would affect the variant or the common *BACH1* allele. Therefore, in order to further resolve the nature of this novel 3101C > T missense variant, *in vitro* analyses of the ability of the altered BACH1 protein to bind BRCA1, and consequent effects on BRCA1-mediated DNA double-strand break repair function are warranted. In addition, the effect on BACH1 protein folding and stability needs to be investigated. However, as 3101C > T was also found in one of the 304 anonymous population controls of unknown cancer history, it is still possible that this alteration only represents a rare polymorphism unrelated to cancer predisposition. But as nothing is known about the cancer status of these population controls, we cannot completely rule out the possibility that this control case could have had a family history of cancer.

Correspondingly to our work, the recent Swedish *BACH1* study [30] was unable to detect any disease-related mutations in *BACH1*. In one of the 197 studied breast cancer families, however, they discovered a rather interesting rare missense variant (517C > T, Arg173Cys) affecting the putative nuclear localisation domain of the protein. As the same alteration also occurred at a frequency of 0.6% in both unselected breast cancer patients and healthy controls, it appears to be unrelated to cancer. Furthermore, the variant did not cosegregate with the breast cancer phenotype in the family concerned. As with the 3101C > T variant, functional and larger association studies will be needed to evaluate the putative cancer effect of the 517 C > T alteration in more detail.

Present knowledge of the genetic defects predisposing to breast and/or ovarian cancers indicate that only a very small fraction of the cases, perhaps 2–5%, can as yet be explained by germline mutations in known dominantly acting genes other than *BRCA1* and *BRCA2*. *TP53*, *CHK2* (also known as *CHEK2*), *ATM*, *BACH1*, *BARD1*, *PTEN*, *AR* (male breast cancer), *MLH1*, *MSH2* and *MSH6* are all among those less frequently involved genes [11,13–15,26–28,31–34]. Recently, however, the *CHEK2*-Breast Cancer Consortium [35] presented interesting findings indicating that the previously identified germline *CHK2* 1100delC truncating variant [31,36] could in fact be a commonly involved low-penetrance breast cancer susceptibility allele. It occurred in 5.1% of the patients from breast cancer families who tested negative for *BRCA1* or *BRCA2* mutations, but in only 1.1% of the healthy controls. They estimated that the presence of this germline variant known to abrogate the CHK2 protein's kinase activity results in an approximately 2-fold increase of breast cancer risk in females and a 10-fold increase of risk in males. It is possible that certain functionally-deficient variants of *BACH1* could also act as low-penetrance risk factors for cancer.

The failure to identify additional major dominantly-acting breast and/or ovarian cancer susceptibility genes besides *BRCA1* and *BRCA2*, despite much effort, may have a simple explanation—that there are no more such genes to be discovered. Instead, as was already indicated by the recent *CHK2* finding [35], it is possible that a considerable portion of the remaining hereditary breast

and/or ovarian cancer cases with an unknown genetic background will not rely on any single gene, but rather on the cooperative effect of defects in a larger number of low-penetrance genes, perhaps in combination with certain environmental factors. Based on observations from currently known high- and low-penetrance cancer susceptibility genes, suitable candidate genes are likely to be found especially among the many genes whose protein products act as either signal sensors, adaptors or effector kinases associated with DNA damage checkpoints, or that have other essential functions in DNA repair or the regulation of cell cycle progression [11,13,14,37].

In conclusion, although the proper interaction between BACH1 and BRCA1 proteins has been found to be crucial for BRCA1-mediated DNA double-strand break repair [15], it appears that cancer-related germline mutations in *BACH1* would be extremely rare. To date, only two such mutations, 139C > G and 897G > A, have been identified, the evidence being strongest for the former mutation that causes substitution of an evolutionary concerved amino acid in the nucleotide binding box of the protein [15]. Both germline mutations were discovered from early onset breast cancer cases, but the patient showing 139C > G also had a strong family history of breast and ovarian cancers. The novel 3101 C > T variant identified in the present study changes proline to leucine at codon 1034 and occurs in the BRCA1-interacting domain of the BACH1 protein. Functional studies of this germline alteration will be needed to resolve its potential importance in cancer.

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