CHEK2 1100delC mutation in Russian ovarian cancer patients

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Abstract

BRCA1 and *BRCA2* germ-line mutations occur in a significant number of unselected ovarian cancer (OC) patients, thus making a noticeable contribution to OC morbidity. It is of interest whether CHEK2, which is frequently regarded as a third breast cancer specific gene, is also relevant to ovarian cancer pathogenesis. In this report we analyzed the presence of CHEK2 1100delC founder mutation in 268 randomly recruited OC patients. The mutation was identified in 2 women with OC (0.8%) as compared to 1/448 (0.2%) healthy middle-aged and 0/373 elderly tumour-free women. Taken together this result and the negative findings of two other published reports on an association of CHEK2 with ovarian cancer indicate that there is no justification for intensive ovarian cancer screening in CHEK2 1100delC carriers.

Introduction

A close relationship between breast and ovarian cancer risks has been recognized by physicians for a considerable period of time. Not surprisingly, both "classical" breast cancer (BC) predisposing genes, *BRCA1* and *BRCA2*, turned out to be strongly associated not only with BC, but also with ovarian cancer [1, 2]. The third BC-associated gene, CHEK2, appeared to be relevant to ovarian cancer since it was observed that 4/99 (4.0%) carriers of the inactive 1100delC allele were present among the index cases from breast-ovarian cancer families as compared to only 18/1620 (1.1%) CHEK2 heterozygotes in control subjects [3]. While the role of CHEK2 in breast cancer predisposition has subsequently been proven [4, 5], there are only two published reports analyzing the relevance of 1100delC to ovarian cancer risk. Baysal et al. [6] found no CHEK2 inactivating alleles in 751 unselected ovarian cancer cases, but detected one 1100delC heterozygote (1.9%) among 52 familial OC patients; 521 control subjects contained 3 carriers (0.6%) of the defective allele. Szymanska-Pasternak et al. [7] also failed to detect truncating CHEK2 variants in 209 ovarian cancer patients, while 0.7% of 4000 control subjects had an inactive copy of this gene.

The distribution of the CHEK2 1100delC allele shows wide geographical variation; therefore only a few countries in the world are suitable for case-control studies on the pathological significance of the

CHEK2 1100delC variant. The CHEK2 1100delC allele was recently shown to be relevant to the incidence of breast cancer in Sankt Petersburg (Russia); therefore we reasoned that this population would be valuable in the evaluation of its frequency in ovarian cancer patients residing in the same city [8].

Table 1. Clinical characteristics of the ovarian carcinoma patients

Groups	Number	(%)
Age (years)		
≤40	34	12.7
41-60	160	59.7
≥61	74	27.6
Mean age: 53.1 years		
Age range: 21-89 years		
T status		
T=1	44	16.4
T>1	211	78.7
Non-informative	13	4.9
N status		
N=0	101	37.7
N=1	52	19.4
Nx	102	38.1
Non-informative	13	4.9
M status		
M=0	174	64.9
M=1	55	20.5
Mx	22	8.2
Non-informative	13	4.9
Tumour differentiation		
Grade 1	35	13.1
Grade 2	73	27.2
Grade 3	160	59.7
Histology		
Serous adenocarcinoma	235	87.7
Mucinous adenocarcinoma	8	3.0
Adenocarcinoma, unspecified	14	5.2
Other	10	3.7
Non-informative	1	0.4
Total	268	100.0

Materials and methods

The study included 268 ovarian cancer patients undergoing treatment in N.N. Petrov Institute of Oncology (Sankt Petersburg, Russia). The relevant characteristics of the cases are presented in Table 1. The healthy control population has already been described by Chekmariova et al. [8] and Buslov et al. [9]; it included 448 middle-aged female donors and 373 elderly tumour-free women. CHEK2 1100delC genotyping was performed by real-time allele-specific PCR. Primers were TTG GAG TGC CCA AAA TCA GT (specific for the wild-type allele), CTT GGA GTG CCC AAA ATC AT (specific for the mutated allele) and CTG ATC TAG CCT ACG TGT CT (common primer). The PCR mix included approximately 50 ng genomic DNA, 1 unit heat-activated Taq DNA polymerase, 1^{x} PCR buffer (pH 8.3), 1.5 mM $MgCl_2$, 200 μM dNTP, 0.5 μM each primer, and 0.5^{x} SYBR Green I in a final volume of 10 μl. PCR amplification and product detection were carried out using the iCycler iQ Real Time Detection System (Bio-Rad) for 50 cycles (95°C for 35 sec., 57°C for 30 sec., 72°C for 30 sec.) after an initial activation of the polymerase at 95°C for 8 min. For each batch of assays control wild-type and mutation-positive DNA samples were included. The specificity of the 120-bp PCR product was confirmed by DNA melting curve analysis. All mutation-positive cases as well as some randomly selected DNA specimens were also subjected to conventional allele-specific PCR (35 cycles in the same conditions) followed by restriction fragment detection by polyacrylamide gel electrophoresis.

Results

CHEK2 1100delC was identified in 2 (0.8%) out of 268 ovarian cancer patients. One of the 1100delC mutation positive patients, who was 67-years-old, presented with an early stage (T1N0M0) tumour that had serous adenocarcinoma histology (grade 2). The second patient was 66-years-old; the tumor was also a serous adenocarcinoma (grade 1), but the stage of disease was significantly more advanced (T3NxM1). Neither of the affected females reported a family history of breast or ovarian cancer. The *BRCA1* 5382insC mutation, which appears to be the most frequent mutation in unselected Russian ovarian cancer patients, was analyzed in 266 patients. From the 266 women,

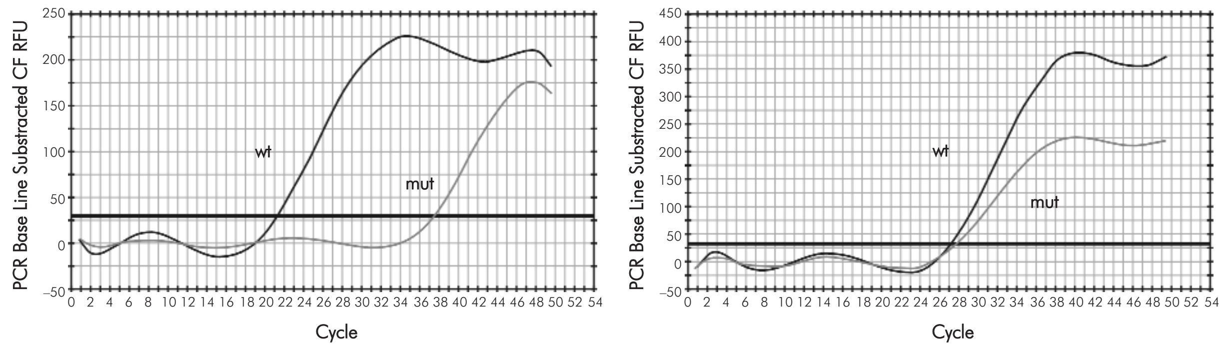

Fig. 1. Detection of CHEK2 1100delC mutation by real-time allele-specific PCR. When normal DNA is subjected to PCR, the amplification driven by the wild-type specific primer significantly exceeds that initiated by mutation-specific oligonucleotide (left). In the case of CHEK2 1100delC heterozygosity, both wild-type and mutation-specific curves cross their threshold of detection at approximately the same cycle (right)

22 (8.3%) *BRCA1* 5382insC heterozygotes were identified and there were no instances of a simultaneous occurrence of CHEK2 1100delC and *BRCA1* 5382insC alleles.

CHEK2 1100delC mutation was detected in 1/448 (0.2%) healthy middle-aged females and in none of 373 elderly tumour-free women [8]. None of the statistical tests demonstrated a significant difference, although a formal trend to an association was revealed when comparing the occurrence of CHEK2 1100delC variant in ovarian cancer patients against elderly controls (p=0.095).

Discussion

The most studied and well recognized breast/ovarian cancer genes, *BRCA1* and *BRCA2*, show a distinct distribution in breast and ovarian cancer patients. In breast cancer, the probability of finding a *BRCA1* or *BRCA2* mutation is increased when BC patients are selected for young age of disease onset and/or a family history and/or the presence of multiple primary tumours. In contrast, the selection of patients using these parameters is not justified in ovarian cancer patients: randomly recruited OC usually show a high frequency of *BRCA* mutations comparable to highly selected cases of BC. Furthermore, the trend of a family history or young age of disease onset is less evident in *BRCA*-associated ovarian cancer compared to breast cancers [10, 11].

While *BRCA* germ-line mutations contribute to a significant proportion of ovarian cancer cases [10, 11], it appears that the CHEK2 1100delC allele is not associated to any extent with ovarian cancer risk [6, 7, and the present study]. It is an interesting scientific question as to why *BRCA1* and *BRCA2*, being completely different genes [1, 12], predispose to both breast and ovarian tumourigenesis, whereas CHEK2 appears only to alter the risk of BC. It also remains unclear whether some rare cases of non-breast cancer can be somehow triggered by the CHEK2 1100delC allele, or its presence is absolutely neutral for the tumours developing outside mammary glands. Our data on the absence of the CHEK2 1100delC variant in elderly tumour-free women suggest that this mutation decreases the probability of achieving old age without cancer. However, although we observed a significant frequency of CHEK2 1100delC heterozygotes in Russian breast cancer patients (2.1%), the occurrence of this allele in the corresponding middle-aged control group is also very low (0.2%). Thus, it is unclear whether depletion of CHEK2 1100delC mutation carriers in middle-aged and elderly controls takes place as a result of an increased likelihood of there being a cancer-predisposing role of this allele, or its low frequency in the control groups is simply attributable to a chance.

Overall, the disease significance of CHEK2 1100delC is difficult to determine because of the low population frequency, uneven geographical distribution and low cancer penetrance. To resolve these issues will require much larger population-based studies requiring a significant number of cancer patients and tumour-free controls in order to clarify whether organs other than the breast are at risk in CHEK2 1100delC carriers. Taken together with published data [6, 7], the present study does not justify the need for intensive ovarian cancer screening in females bearing the CHEK2 1100delC allele.

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