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To cite this version:

Pauline Escudie, Sylvia Monteil-Onteniente, Laurence Gladieff, Viviane Feillel, Rosine Guimbaud, et al.. A novel germline CHEK2 deletion truncating the kinase domain identified in a French family with high-risk of breast/ovarian cancer. Breast Cancer Research and Treatment, 2009, 120 (1), pp.267-270. 10.1007/s10549-009-0551-6 . hal-00535396

HAL Id: hal-00535396

https://hal.science/hal-00535396v1

Submitted on 11 Nov 2010

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Breast Cancer Res Treat (2010) 120:267–270
DOI 10.1007/s10549-009-0551-6

LETTER TO THE EDITOR

A novel germline CHEK2 deletion truncating the kinase domain identified in a French family with high-risk of breast/ovarian cancer

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Received: 4 September 2009 / Accepted: 9 September 2009 / Published online: 19 September 2009
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To the Editor,

Five to ten percent of breast and ovarian cancers are mainly due to hereditary mutations in the two susceptibility genes, BRCA1 and BRCA2. In addition to these high-penetrance genes, other genes have been reported to confer moderate risks to develop breast/ovarian cancer [1–4]. Among these low- or moderate-susceptibility alleles, the checkpoint kinase 2 (CHEK2) gene has been involved in inherited cancer susceptibility, first in Li-Fraumeni families [5], and then in families with breast and ovarian aggregation [1]. CHEK2 encodes for a cell-cycle checkpoint kinase involved in the cellular response to DNA, through cell cycle and cell death regulation. Several variants of this gene have been successively identified as SNP [6–8]; small [5] or large genomic deletions [9]. Despite its low frequency, the c.1100delC CHEK2 mutation has been the most extensively studied in populations. This mutation results in an approximatively two fold increase of breast cancer in women [1, 10] but there was some evidence of a higher prevalence of CHEK2 c.1100delC among cases with a first-degree relative affected with breast cancer [10] or in families with a case of male breast cancer [1, 11]. More recently, a meta-analysis of 16 different studies demonstrated that CHEK2 c.1100DelC heterozygosity increases the risk of breast cancer three to five-fold with a 37% risk of developing breast cancer before the age 70 years if positive [12]. Considering these results, to complete the screening of predisposition genes for our high risk breast/ovarian cancer patients, we investigated the presence of c.1100DelC mutation in our population of BRCA1/BRCA2 negative high breast/ovarian cancer risk patients.

Breast/ovarian cancer families were ascertained after genetic counselling at the Institute Claudius Regaud in Toulouse in the south of France. All the 392 affected index patients entering this study have been tested for BRCA1 and BRCA2 germline mutations and present neither mutation in the coding exons of the genes including flanking intron–exon boundaries nor large rearrangements. DNA from peripheral blood was isolated as described earlier [13]. Primers (5′-TTAATTTAAGCAAATTAAAT GTC -3′ for forward primer and 5′-GAATAACTCCTAA-ACTCCAGC-3′ for reverse primer) were used to amplify the CHEK2 exon 10 coding region carrying the c.1100 DelC mutation (Genbank accession number AF086904.1). Sequencing was performed using the Big DyeTerminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in an automated Sequencer ABI Prism 3100 (Applied Biosystems).

We investigated the presence of the c.1100DelC mutation on CHEK2 exon10 in 392 patients from high breast/

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ovarian cancer risk BRCA1/2 negative families. Genotyping these patients revealed the presence of c.1100DelC mutation in only one patient (0.25%) (Fig. 1a). This woman was diagnosed with an invasive lobular carcinoma when 40 years old. A proband sister was diagnosed with a breast cancer at age 45, a paternal aunt and cousin at age 39 (Fig. 1b). The c.1100DelC CHEK2 mutation frequency varies according to the population studied. The highest frequencies have been observed in Northern Europe (1–11%) probably caused by an common ancestral origin [14, 15] but this variant is infrequent to absent in populations from Southern Europe including the Basque country (0.93%) [16], as well as the Spanish [17] or Italian [18] populations. The extremely low frequency of this mutation in our population coming from the south west of France confirms its weak relevance for the practical clinic in populations from Southern Europe.

However, this CHEK2 screening allowed us to identify a new mutation in another patient from a high breast/ovarian cancer risk family. This mutation consists in a 2 bp-deletion, c.1140-1141DelTC , on CHEK2 exon 10 (p.L380FsX 393) leading to a frameshift and the appearance of a stop codon at the residue 393 (Fig. 1a). The woman carrying this germline mutation has been diagnosed with ovarian cancer and breast cancer with clinical inflammatory signs and a dermal invasion at age 42, two paternal aunts were diagnosed with breast cancer at age 68 and 72 and a maternal aunt at age 53 (Fig. 1c). Tumor proband

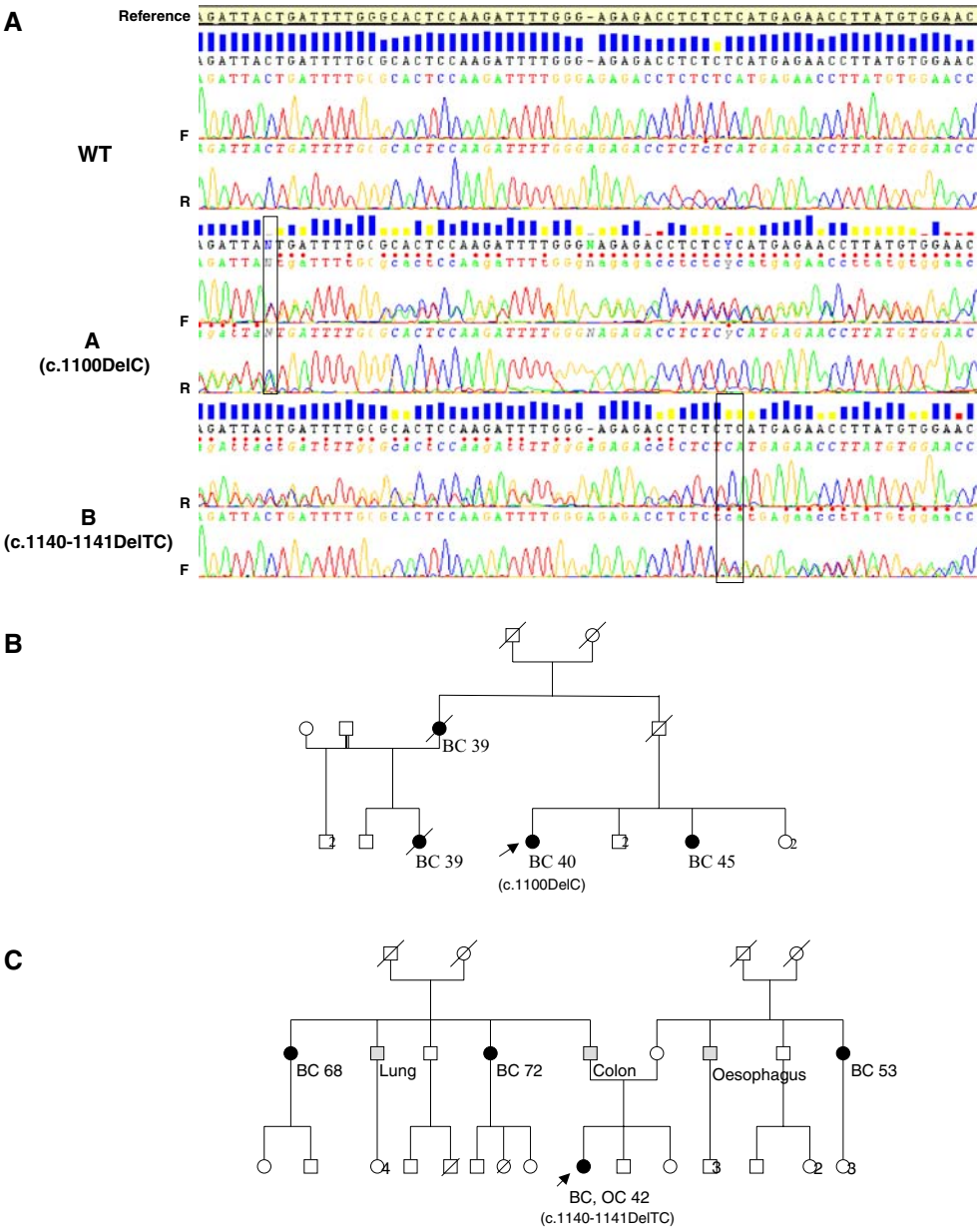

Fig. 1 Detection of the two truncating mutations, c.1100DelC and c.1140-1141DelTC, affecting the kinase domain of CHEK2. **a**: Forward (*F*) and reverse (*R*) sequencing analyses of the CHEK2 exon10 PCR product for patients carrying the c.1100DelC, the c.1140-1141DelTC mutations or wild-type (WT) sequences compared to the reference sequence (reference). *Black bars* surround the positions of the respective mutations. While the sequence of the WT patient is homozygous, the sequence of the different mutations becomes heterozygous at the corresponding mutation positions. **b** and **c**: Pedigrees of germline CHEK2 c.1100DelC (B) and c.1140-1141DelTC (C) mutations families. *Square symbols* indicate males, round symbols indicate females. Filled symbols indicate breast (*BC*) or ovarian (*OC*) or other cancers affected individuals. Ages of diagnosis are indicated below the individuals

characteristics were infiltrant ductal carcinoma, SBR grade 2, with positive hormone receptors. Inflammatory breast cancer is a rare clinically distinct and aggressive form of breast cancer preferentially hormone receptor-negative but with largely unknown genetic determinants. Molecular signature of this particular breast cancer type established up to date by different groups did not describe a differential regulation of CHEK2 in inflammatory breast carcinoma or a LOH in the tumor of the 22q12.1, location of the CHEK2 gene [19–21] in contrast to sporadic breast and ovarian tumors [22].

This c.1140-1141DelTC mutation is located in the part of the CHEK2 gene sequence coding for the kinase domain of the protein. Indeed, the CHEK2 protein is composed of three different domains: an N-terminal serine-threonine rich domain (SQ–TQ) ranging from amino acid residue 20 to 75, a fork head-associated domain involved in CHEK2 binding to other phosphorylated proteins (residues 112–175) and a serine/threonine kinase domain (residues 225–490) which includes an activation loop. This kinase activity activated by ATM in response to DNA damage, is responsible of the phosphorylation of different proteins involved in cell cycle as CDC25 or p53, in DNA repair as BRCA1 [23]. It has been previously demonstrated that mutations located in the kinase domain of CHEK2 partially or completely abolish the kinase activity. Indeed, missense D438Y mutation reduces in vitro BRCA1 phosphorylation [5] while truncated proteins on residue 386 (c.1100DelC; T386Fs) and 475 (c.1422DelT, R475Fs) completely abolish in vitro CDC25C phosphorylation [6]. The germline mutation c.1140-1141DelTC detected in our work, induces a stop codon on residue 393, probably generating a truncated intermediary protein of the two previously described inactivated kinase mutants, T386Fs and R475Fs. In consequence, it can be reasonably postulated that this 2 bp-deletion is a complete loss-of-function mutation, generating an inactive CHEK2 kinase.

In conclusion, our study reveals the presence of CHEK2 exon 10 truncated mutations in two of 392 high-risk breast/ovarian cancer family probands (0.5%). Further investigations are necessary to determine the exact role of the loss-of-function c.1140-1141DelTC CHEK2 germline mutation in the risk of breast and ovarian cancers. However, our work strongly suggests that analyzing the involvement of CHEK2 in breast/ovarian cancer risk may not be restricted to the detection of the loss-of-function CHEK2 c.1100DelC mutation, in association or not with other missense variants, but would benefit from investigating all the loss-of-function mutations affecting the kinase domain of CHEK2, in concert or not with other susceptibility low-risk genes.

Acknowledgments This work was supported by the Groupe de Recherche de l'Institut Claudius Regaud. The authors want to thank Dr Maria-Isabel Tejada (Lisboa, Portugal) for providing us a c.1100DelC DNA control, Isabelle Gitlaw, Pauline Dejean and Chantal Darnau for their efficient technical assistance.

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