



SHORT REPORT

## Somatic mutations in the *BRCA1* gene in Chinese sporadic breast and ovarian cancer

Ui-Soon Khoo<sup>\*1</sup>, Hilmi Ozcelik<sup>2,3</sup>, Annie NY Cheung<sup>1</sup>, Louis WC Chow<sup>4</sup>, Hextan YS Ngan<sup>5</sup>, Susan J Done<sup>2,3</sup>, ACT Liang<sup>1</sup>, Vivian WY Chan<sup>2</sup>, Gordon KH Au<sup>6</sup>, Wing-Fung Ng<sup>7</sup>, Cycles SP Poon<sup>8</sup>, Yuet-Foon Leung<sup>9</sup>, Florence Loong<sup>1</sup>, Philip Ip<sup>1</sup>, Gavin SW Chan<sup>1</sup>, Irene L Andrulis<sup>2,3,10</sup>, Jing Lu<sup>5</sup> and Faith CS Ho<sup>11</sup>

<sup>1</sup>Department of Pathology, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Hong Kong; <sup>2</sup>Department of Pathology and Laboratory Medicine and Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto M5G 1X5, Ontario, Canada; <sup>3</sup>Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto M5G 1X5, Ontario, Canada; <sup>4</sup>Department of Surgery, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Hong Kong; <sup>5</sup>Department of Obstetrics and Gynecology, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Hong Kong; <sup>6</sup>Department of Radiation Therapy and Oncology, Queen Mary Hospital, The University of Hong Kong, Pokfulam Road, Hong Kong; <sup>7</sup>Department of Pathology, Ruttonjee Hospital, Hong Kong; <sup>8</sup>Department of Pathology, Pamela Youde Nethersole Hospital, Hong Kong; <sup>9</sup>Pathology Institute, Jockey Club Polyclinic, Sai Ying Pun, Hong Kong; <sup>10</sup>Division of Preventive Oncology, CCO, Toronto, M5G 2L7, Canada; <sup>11</sup>Department of Pathology and Immunology, Monash University, Melbourne, Australia

**Inherited mutations in the *BRCA1* gene confer increased susceptibility to breast and ovarian cancer. Its role in sporadic carcinogenesis is not well defined. Somatic mutations in breast cancers have not been reported and to date there are only three reports of somatic mutations in sporadic ovarian cancers. To investigate the contribution of *BRCA1* mutations to sporadic breast and ovarian cancer in the Chinese population, we analysed 62 samples from Chinese women using the protein truncation test. There were 40 cases of breast cancer under age 50 and 22 cases of ovarian cancer, all unselected for family history. There was no age selection for the ovarian cancers. We found two somatic *BRCA1* mutations in exon 11, one in a breast cancer and the other in an ovarian cancer, both of which result in truncated proteins. Our results indicate that somatic *BRCA1* mutations, like somatic mutations in the *BRCA2* gene, though very rare, can be found in both breast and ovarian cancers and support a tumor suppressor function for *BRCA1* in sporadic tumors.**

**Keywords:** *BRCA1*; somatic mutations; breast cancer; ovarian cancer; Chinese

The observed high incidence of loss of heterozygosity in the *BRCA1* region in breast and ovarian tumors suggested the presence of somatic mutations in this gene. After the *BRCA1* gene was cloned, Futreal *et al.* (1994) actively attempted to demonstrate this but failed to find any somatic mutations in the 36 breast and 12 ovarian carcinomas they studied which showed LOH at the *BRCA1* locus. The first evidence for somatic *BRCA1* mutations came from Merajver *et al.* (1995), who described such mutations in four of their 47 ovarian cases studied. A further somatic mutation in

an ovarian cancer was described by Hosking *et al.* (1995) and a few more have been reported recently by Berchuck *et al.* (1998). No somatic *BRCA1* mutation has ever been reported from breast cancer.

Molecular studies of *BRCA1* mutations have generally been focused on Caucasian high-risk families (Szabo and King, 1997). The Japanese are the only Asian population in the literature so far. Both Katagiri *et al.* (1996) and Matsushima *et al.* (1996) have reported a lower incidence of 3.8 and 5% in their breast and ovarian cancer population respectively. To investigate the contribution of *BRCA1* mutations in the Chinese population of Hong Kong, we analysed a series of 40 breast and 22 ovarian cancer tumor samples from Chinese women, all cases being unselected for family history.

Sixty-two rapidly frozen fresh tumor specimens were obtained from mastectomy or excision specimens of patients treated for breast carcinoma in Queen Mary Hospital, Tung Wah and Pamela Youde Nethersole Hospitals, Hong Kong; and from excision specimens of patients with ovarian carcinoma in Queen Mary Hospital. Breast cancer cases diagnosed under the age of 50 were selected for this study. Fifteen of the breast cancer patients were between ages 41–50, whereas the remaining 13 and 12 were between the ages 21–35 and 36–40, respectively. There was no age selection for the ovarian cancers which had ages ranging from 25–77 years. The genomic DNA and total RNA were extracted using standard procedures. Non-tumor tissue was also collected whenever available. Informed consent in accord with ethical guidelines was obtained from individuals donating blood specimens for analysis. Prior to extraction, a histological section was cut from all blocks to confirm the identity of tissues analysed.

The entire *BRCA1* coding sequence was analysed using the protein truncation test as described previously (Ozcelik *et al.*, 1996). Fresh frozen tumor DNA samples showing truncated products on PTT, as well as DNA extracted from additional tumor and non-tumour samples of these cases were analysed by

\*Correspondence: U-S Khoo  
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direct sequencing using Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Sciences) as described by the manufacturer.

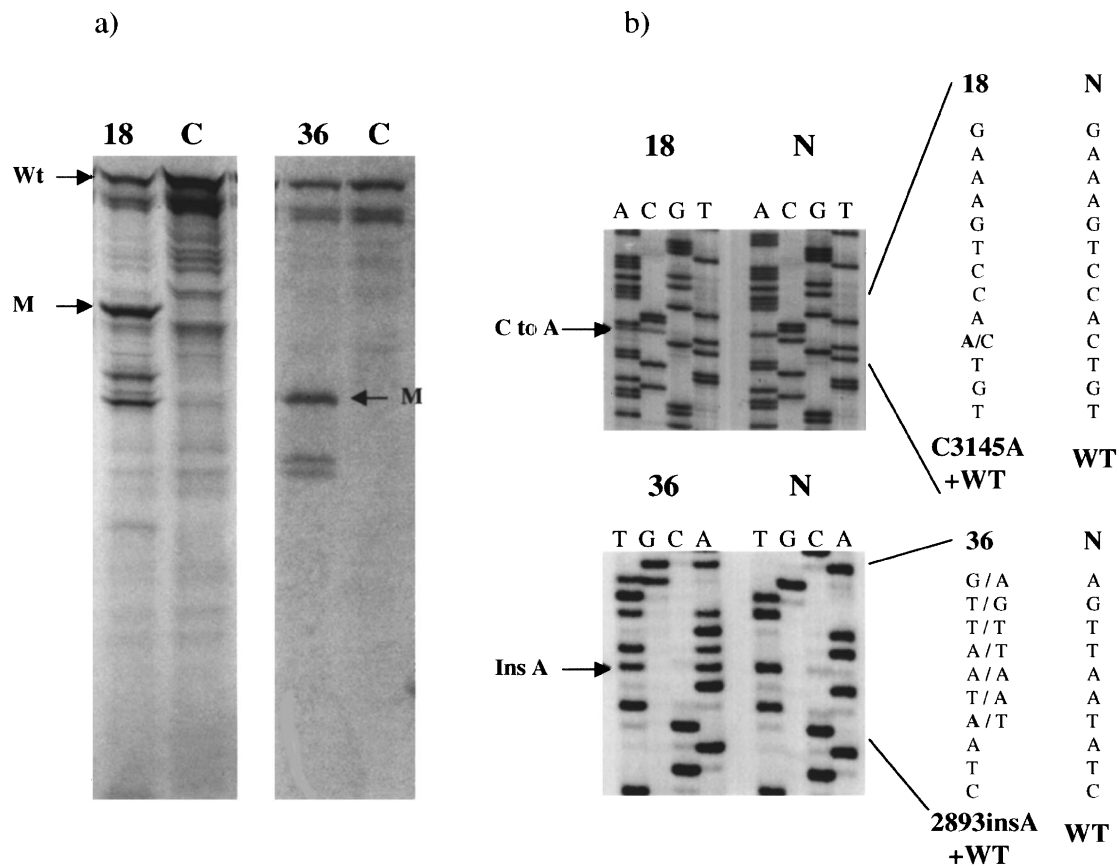
Mutations resulting in truncated protein products were identified in exon 11 of *BRCA1* in two cases (Figure 1). Case 36 was a grade III infiltrating ductal breast carcinoma, of no special histological type. The patient was aged 38 years, was lymph node negative, and the estrogen and progesterone receptor status of the carcinoma was negative. Case 18 was a poorly differentiated serous papillary adenocarcinoma of the ovary from a patient aged 66 years. Both patients were confirmed not to have any family history of breast or ovarian cancer. Forward and reverse sequence analysis revealed the 2893insA and C3145A mutations, in the breast and ovarian samples respectively (Table 1). Interestingly, the sequencing analysis of non-tumor and peripheral blood samples from the same individuals showed the absence of mutation in the germline, indicating that these are somatic mutations. In order to confirm this, as well as to exclude the possibility of sample mixing, DNA samples were further extracted from tumor and non-tumor sections of different tissue blocks from the same patients and re-analysed by sequencing. The tumor samples showed the presence of mutations, which were not found in the non-tumor samples. Moreover both forward and reverse sequence analysis of the tumor samples of both cases showed the

presence of both mutant as well as wild type sequence. The presence of this wild type sequence in the tumor samples would most probably have been derived from non-malignant stroma included in the sections of both tumors. This was confirmed by pathological evaluation of the histological sections taken from the tumor blocks used for DNA extraction.

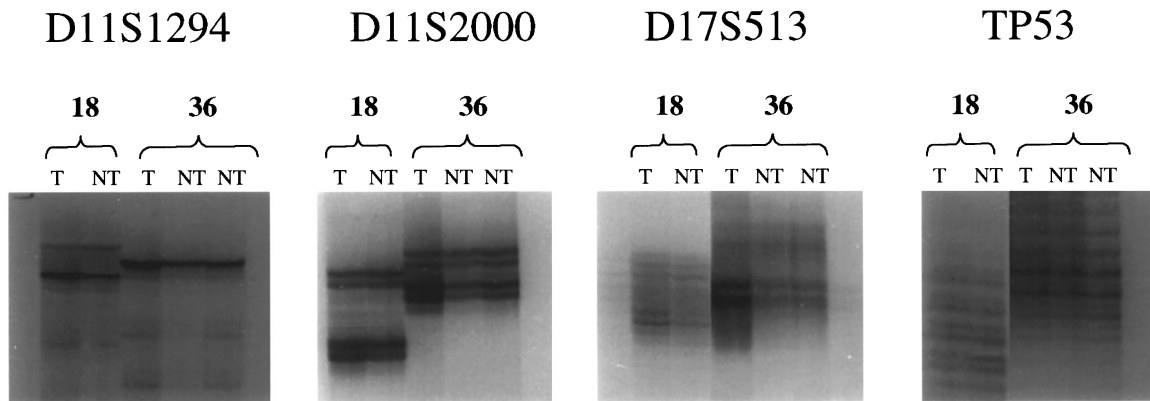
To confirm that the tumor and non-tumor samples of each case were derived from the same patient, tumor and non-tumor samples of both cases were also analysed with six different highly polymorphic micro-satellite markers on chromosomes 7, 11 and 17. Markers with high frequency of heterozygosity were chosen (67–90%) and it was thus expected that each individual would show a different pattern. DNA extracted from peripheral blood or tissue was subjected to PCR analysis in a 9600 Perkin Elmer Thermal Cycler using primers for six highly polymorphic di- and tetra-nucleotide repeat sequences (Research Genetics, Huntsville, AL, USA). Primer

**Table 1** Somatic *BRCA1* mutations in sporadic breast and ovarian cancer

Case no.	Age	Cancer type	Exon	Mutation	Stop codon
36	38	Breast	11	2893insA	937
18	66	Ovarian	11	C3145A	1009



**Figure 1** Mutation detection of *BRCA1*. (a) Protein truncation test revealed truncated protein products within exon 11 of the *BRCA1* gene in tumor of cases 18 and 36 (M arrows). 'C' represents blood sample for case 18 and non-tumor tissue for case 36. (b). Sequencing of independent PCR products from tumor and non-tumor, done in both directions, revealed somatic mutations in *BRCA1* for both cases; C3145A in case 18 and 289insA mutation in case 36. The partial DNA sequence of the coding strand of tumor and normal samples as illustrated, show the presence of both mutant and wild type DNA in the tumor samples. The normal DNA found in the tumor samples would have been derived from the non-malignant stroma included in the sections of ovarian and breast cancer



**Figure 2** Microsatellite analysis to confirm same pattern of alleles within samples from patients 18 and 36. T=tumor, NT=non-tumor tissue. (See Table 2 for location of primer pairs and size of PCR products)

**Table 2** *BRCA1* primers for PTT

Name	Position 5'-3' cDNA	Exon	Purpose	PCR product length
<i>Genomic PCR</i>				
BR11F1	793–813 <sup>a</sup>	11	Fragment 1a	1333
BR11R1	2125–2103	11	Fragment 1a	1333
BR11F2	1921–1943 <sup>a</sup>	11	Fragment 1b	1463
BR11R2	3383–3360	11	Fragment 1b	1463
BR11F3	3061–3082 <sup>a</sup>	11	Fragment 1c	1123
BR11R3	4183–4161	11	Fragment 1c	1123
<i>RT-PCR</i>				
PTBRE2BF	100–123 <sup>a</sup>	2	Fragment A	880
PTBR11R4A	979–958	11	Fragment A	880
PTBR11F4	4011–4032 <sup>a</sup>	11	Fragment E	1332
BRCA19R	5342–5321 <sup>b</sup>	20	Fragment E	1332
PTBR16F	4834–4855 <sup>a</sup>	16	Fragment F	858
BRCA13R	5691–5669	24	Fragment F	858

<sup>a</sup>T7-primer with promoter and sequence for initiation of translation: 5'-GCTAATACGACTCACATATAGGAACAGACCACCATGG-3' *BRCA1* primer sequence at 3'-position. <sup>b</sup>5'-GAC CAC ATC TCC TCT GAC TTC A-3'

loci (chromosomal location), expected length and heterozygosity rate were as follows: D7S522 (*7q31*), 217–229 bp, 67%; D11S1294 (*11q22-23*), 294 bp, 83%; D11S2000 (*11q22-23*), 213 bp, 87%; D11S1818 (*11q22-23*), 142–158 bp, 70%; D17S513 (*17p13*), 183–203, 89%; TP53 (*17p13*), 103–135 bp, 90%. The results shown in Figure 2, indicate that within each individual both tumor and non-tumor samples share the same pattern of alleles. This evidence strengthens the assertion that tumor and non-tumor are from the same individual in each case.

To our knowledge this is the first report of *BRCA1* mutations in Chinese breast and ovarian cancer. In both cases, the absence of mutation in non-tumor tissue reflects the somatic nature of these mutations. The presence of the normal band in the PTT gel is most probably due to the invariable presence of some non-tumor DNA included in the DNA extracted from the tumor. Histological analysis of the sections taken from tumor blocks used for DNA extraction showed up approximately 30–50% stromal tissue entrapped within the tumor tissue. Unfortunately, exclusion of these non-tumor components from the tumor sections could not be further minimized without the application of the technique of micro-dissection. Loss of heterozygosity was

shown by Berchuck *et al.* (1998) to accompany both germline and somatic *BRCA1* mutations in sporadic ovarian tumors.

Here we report the first somatic mutation to be found in a sporadic breast cancer and the fourth report of somatic mutations in sporadic ovarian cancer. Both mutations we found are in exon 11, and give rise to abnormal truncated protein products. The reported low incidence of somatic *BRCA1* mutations could be related partly to the fact that very few studies have focused on the mutational analysis of *BRCA1* in sporadic tumors (Futreal *et al.*, 1994; Merajver *et al.*, 1995; Hosking *et al.*, 1995; Matsushima *et al.*, 1996; Takahashi *et al.*, 1995; Berchuck *et al.*, 1998). Most studies of *BRCA1* mutation have been directed at finding the mutations responsible for cases of familial breast cancer and have used peripheral blood samples.

The finding of novel somatic *BRCA1* mutations in 4.6% (1/22) of ovarian and 2.5% (1/40) of young breast cancers in Chinese women supports the hypothesis that *BRCA1* may play a role in the development of sporadic breast and ovarian cancers. Like that observed for somatic mutations in the *BRCA2* gene (Foster *et al.*, 1996; Lancaster *et al.*, 1996; Miki *et al.*, 1996; Weber *et al.*, 1996), somatic *BRCA1* mutations, though very rare, can thus be also found in both breast and ovarian cancers. These findings support a tumor suppressor function for *BRCA1* in sporadic tumors. It also suggests that it may be valid to study alternative mechanisms of *BRCA1* perturbations in sporadic tumors, including changes leading to low levels of expression of *BRCA1* (Thompson *et al.*, 1995; Ozelik *et al.*, 1998; Rice *et al.*, 1998).

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## References

- Berchuck A, Heron KA, Carney ME, Lancaster JM, Fraser EG, Vinson VL, Deffenbaugh AM, Miron A, Marks JR, Futreal PA and Frank TS. (1998). *Clin. Cancer Res.*, **4**, 2433–2437.
- Foster KA, Harrington P, Kerr J, Russell P, DiCioccio RA, Scott IV, Jacobs I, Chenevix-Trench G, Ponder BAJ and Gayther SA. (1996). *Cancer Res.*, **56**, 3622–3625.
- Futreal PA, Liu QY, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett LM, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Söderkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart JD, Marks J, Ballinger DG, Barrett JC, Skolnick MH, Kamb A and Wiseman R. (1994). *Science*, **266**, 120–122.
- Hosking L, Trowsdale J, Nicolai H, Solomon E, Foulkes W, Stamp G, Signer E and Jeffreys A. (1995). *Nature Genet.*, **9**, 343–344.
- Katagiri T, Emi M, Ito I, Kobayashi K, Yoshimoto M, Iwase T, Kasumi F, Miki Y, Skolnick MH and Nakamura Y. (1996). *Hum. Mutat.*, **7**, 334–339.
- Lancaster JM, Wooster R, Mangion J, Phelan CM, Cochran C, Gumbs C, Seal S, Barfoot R, Collins N, Bignell G, Patel S, Hamoudi R, Larsson C, Wiseman RW, Berchuck A, Iglehart JD, Marks JR, Ashworth A, Stratton MR and Futreal PA. (1996). *Nature Genet.*, **13**, 238–240.
- Matsushima M, Kobayashi K, Emi M, Saito H, Saito J, Suzumori K and Nakamura Y. (1996). *Hum. Mol. Genet.*, **4**, 1953–1956.
- Merajver SD, Pham TM, Caduff RF, Chen M, Poy EL, Cooney KA, Weber BL, Collins FS, Johnston C and Frank TS. (1995). *Nature Genet.*, **9**, 439–443.
- Miki Y, Katagiri T, Kasumi F, Yoshimoto T and Nakamura Y. (1996). *Nature Genet.*, **13**, 245–247.
- Ozcelik H, Antebi YJ, Cole DEC and Andrulis IL. (1996). *Hum. Genet.*, **98**, 310–312.
- Ozcelik H, To MD, Couture J, Bull SB and Andrulis IL. (1998). *Int. J. Cancer*, **77**, 1–6.
- Rice JC, Masseybrown KS and Futscher BW. (1998). **17**, 1807–1812.
- Szabo CI and King MC. (1997). *Am. J. Hum. Genet.*, **60**, 1013–1020.
- Takahashi H, Behbakht K, McGovern PE, Chiu HC, Couch FJ, Weber BL, Friedman LS, King MC, Furusato M, LiVolsi VA, Menzin AW, Liu PC, Benjamin I, Morgan MA, King SA, Rebane BA, Cardonick A, Mikuta JJ, Rubin SC and Boyd J. (1995). *Cancer Res.*, **55**, 2998–3002.
- Thompson ME, Jensen RA, Obermiller PS, Page DL and Holt JT. (1995). *Nature Genet.*, **9**, 444–450.
- Weber BHF, Brohm M, Stec I, Backe J and Caffier H. (1996). *Am. J. Hum. Genet.*, **59**, 962–964.