

## Short Report

# Family history, BRCA mutations and breast cancer in Vietnamese women

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The purpose of this report is to estimate the proportions of familial and hereditary breast cancers among unselected cases of breast cancer in Vietnam. Two hundred and ninety-two unselected cases of incident breast cancer were recruited from the National Cancer Hospital, Hanoi, the largest cancer centre in Vietnam. Family histories were collected for 292 cases and a DNA sample was obtained for 259 cases. DNA samples were screened for mutations in the large exons of BRCA1 and BRCA2 using the protein truncation test and by allele-specific testing for 17 founder mutations which have been reported in other Asian populations. Complete gene sequencing was performed on two cases of familial breast cancer. Seven of 292 cases reported a relative with breast cancer and one patient reported a relative with ovarian cancer. A pathogenic BRCA mutation was detected in 2 of 259 cases; one BRCA1 carrier was diagnosed at age 51 and one BRCA2 carrier was diagnosed at age 42. Neither case reported a relative with breast or ovarian cancer. A family history of breast cancer is very uncommon among Vietnamese breast cancer patients. The frequency of pathogenic BRCA mutations in Vietnamese breast cancer patients is among the lowest reported worldwide.

### Conflict of interest

There are no conflicts of interest to declare.

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In 2010, breast cancer is an important cause of death in all countries. However, there are striking differences in the age-specific incidence rates of breast cancer between countries and between ethnic groups (1, 2). The reason for these differences is not clear, although variation in genetic factors may play a role. Rising breast cancer rates appear to coincide with increase in development and westernization. Vietnam has a population of 87 million and has one of the lowest age-standardized incidence rates for breast cancer reported worldwide. The incidence rate is 15–27 per 100,000 per year (3), compared to 82 per 100,000 per year in Ontario, Canada (4). It is not clear if the risk factors for breast cancer in Vietnamese women are the same as those for western women. It is also not clear

to what extent familial and genetic factors contribute to the burden of breast cancer in Vietnam. We conducted a hospital-based case-control study of genetic and familial risk factors for breast cancer in the largest cancer hospital in Vietnam.

### Methods

A total of 298 consecutive incident cases of breast cancer were recruited from the Vietnam National Cancer Hospital in Hanoi. This is the largest cancer hospital in Vietnam, and is the site of the National Cancer Institute. The study was approved by the Research Ethics Board of Queen's University, Kingston, Ontario and the Vietnam National Cancer Hospital, Hanoi.

Cases were recruited between September 2007 and March 2008. To qualify as an incident case, the date of surgery was 12 months or less prior to the date of recruitment. Cases were unselected for family history or age at diagnosis. The diagnosis of breast cancer was confirmed by the Central Pathology Laboratory at the Vietnam National Cancer Hospital. Of 300 cases recruited, two withdrew consent, leaving 298 cases for analysis. All 298 cases completed an epidemiology and family history questionnaires and 259 cases provided a DNA sample for BRCA testing.

The family history questionnaire was completed through an in-person, private interview with our local (Vietnamese-speaking) epidemiologist at the National Cancer Hospital. Each case was asked if she knew of any maternal or paternal relative with cancer (first, second and third degree), sites, and ages of diagnosis and death.

For 259 cases, a blood sample was obtained for DNA extraction. Blood samples were obtained at the National Cancer Hospital, Hanoi. The buffy coat was extracted locally and frozen. Buffy coats were stored at  $-70^{\circ}\text{C}$  before shipping in 'dry' liquid nitrogen canister to Women's College Hospital, Toronto, where DNA extraction was performed.

The 'Southeast Asian mutational panel' was designed for this study; it consists of 13 mutations in BRCA1 and 4 in BRCA2. These mutations have been reported previously among the Chinese (5–9), Indonesian (10), Thai (11), Malaysian (12), Korean (13) and Japanese (14, 15) populations. The DNA was tested for the 17 Asian founder mutations (Table 1) using allele-specific assays and the protein truncation test (PTT). Of the 17 mutations, 15 were in the large exons of BRCA1 (exon 11) and BRCA2 (exons 10 and 11) and were covered with the PTT (see below). Specific assays were designed for the other two mutations. To identify the BRCA1 4446C>T mutation, the amplification refractory mutation system assay (16) was used. A restriction enzyme digestion was used for the BRCA1 5589del8 mutation.

The other 15 mutations are covered by PTT which screens the largest exons of BRCA1 (exon 11) and BRCA2 (exons 10 and 11). Primer sequences used to amplify overlapping fragments for the PTT were obtained from the Breast Cancer Information Core. PTT was performed using the TNT<sup>TM</sup> rabbit reticulocyte lysate system (Promega Corporation, Madison WI), involving [<sup>35</sup>S] methionine/cysteine (New England Nuclear, Boston MA) for protein detection. Further information regarding both methods is available upon request. All variants identified by PTT and other

Table 1. Southeast Asian panel of recurrent BRCA1 and BRCA2 mutations

| Gene  | Exon | Mutation    | Origin     | References |
|-------|------|-------------|------------|------------|
| BRCA1 | 11   | 1081delG    | Chinese    | (8)        |
| BRCA1 | 11   | 1100delAT   | Chinese    | (6)        |
| BRCA1 | 11   | 2080delA    | Japan      | (14)       |
| BRCA1 | 11   | 2507delAG   | Japan      | (14)       |
| BRCA1 | 11   | 2552delC    | Korean     | (13)       |
| BRCA1 | 11   | 2845insA    | Malaysian  | (12)       |
| BRCA1 | 11   | 2919C>T     | Japan      | (14)       |
| BRCA1 | 11   | 3300delA    | Thailand   | (11)       |
| BRCA1 | 11   | 3478del5    | Chinese    | (7)        |
| BRCA1 | 11   | 3746insA    | Korean     | (13)       |
| BRCA1 | 11   | 4184del4    | Korean     | (13)       |
| BRCA1 | 13   | 4446C>T     | Chinese    | (9)        |
| BRCA1 | 24   | 5589del8    | Chinese    | (6)        |
| BRCA2 | 10   | 1627A>T     | Korean     | (13)       |
| BRCA2 | 11   | 3337C>T     | Chinese    | (5)        |
| BRCA2 | 11   | 5802delAATT | Japan      | (15)       |
| BRCA2 | 11   | 6775G>T     | Indonesian | (10)       |

screening methods were confirmed by direct DNA sequencing [BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing Kit, and 3130XL Genetic Analyzer (Applied BioSystems, Carlsbad, California)] according to the manufacturer's instructions.

In addition, we screened for the three common mutations, BRCA1 185delAG, 5382insC and BRCA2 6174delT (17, 18) that are most commonly seen in Ashkenazi Jews and women of Slavic ancestry but have also been reported on many occasions in other populations throughout the world.

Three patients were considered to have family histories suggestive of the familial breast ovarian cancer syndrome. For two of these, BRCA1 and BRCA2 were screened in their entirety using a combination of denaturing gradient gel electrophoresis (DGGE) and direct sequencing. For the third case, adequate DNA was not available to complete these assays.

## Results

### Family history

The mean age at diagnosis of the 292 breast cancer cases was 47 years (range 24–71 years); 64.7% of the cases were diagnosed below age 50. Seven of the 292 cases (2.4%) reported a first, second, or third degree relative with breast cancer. Of these, three patients (1% of the total) had a family history suggestive of the hereditary breast ovarian cancer (HBOC) syndrome ('familial breast cancer'): one patient, who was diagnosed at age 29, had a sister with breast cancer at 33; a second patient was diagnosed with breast cancer at 47 and reported a sister with bilateral breast cancer, the first of

which occurred at age 42; one patient reported a relative with ovarian cancer.

### BRCA testing

Two pathogenic mutations were found among 259 patients, one in BRCA1 (185insA) and one in BRCA2 (4706delAAAG). The BRCA1 mutation was identified using the primer set selected for detecting the BRCA1 185delAG mutation. The BRCA1 carrier was diagnosed with breast cancer at age 51 and had no family history of cancer. The BRCA2 carrier was diagnosed at age 42; she reported a brother with stomach cancer, and another with lung cancer. Neither of these cases knew of any relative with breast or ovarian cancer. Both mutations detected were previously reported among individuals of Caucasian European ancestry; however, neither carrier knew of any relatives of non-Vietnamese ancestry. None of the recurrent mutations reported among Southeast Asian populations, including two mutations recently described among Chinese (BRCA1 1081delG (8) and BRCA2 3337C>T) (5) were detected in our study sample.

In addition, for two of the three 'familial cases', we sequenced the entire coding sequence of the BRCA1 and BRCA2 gene by a combination of DGGE and direct sequencing. No mutation was found.

### Discussion

Among 292 unselected breast cancer patients who attended the largest cancer centre in Vietnam, only three had a family history suggestive of familial breast or breast/ovarian cancer (1%). A total of only seven cases reported having any relative with breast cancer. Two of 259 cases for whom DNA was extracted carried a BRCA mutation (0.8%). Neither BRCA carriers had a family history of breast or ovarian cancer, nor were they diagnosed before age 40. These numbers are surprising low, given the association between familial cancer and early age of onset. In this study, 64.7% of cases were diagnosed before age 50 and 15.8% were diagnosed before age 40. In comparison, in Ontario, less than one-third of all breast cancer cases are diagnosed below age 50 and less than 5% are diagnosed below age 40. A family history of breast cancer is typically reported in 10–20% of all cases in North America (19). The prevalence varies with the definition of a positive family history, which in our study was loosely defined as having *any* first, second or third degree relative with breast or ovarian cancer.

The estimated frequency of BRCA1 and BRCA2 germline mutations in unselected breast cancer varies among different populations (1–10%) (reviewed in Ref. (20)). Pathogenic germline BRCA mutations account for up to 25% of families with hereditary breast cancer and 40% with HBOC (19). Since 1994, mutations in the BRCA1 and BRCA2 genes have been characterized in many populations in North America and Europe; more recently, the prevalence of mutations has been estimated for a number of Asian populations, including China, Japan, Korea, the Philippines, Thailand, and Malaysia (5, 8–11, 21–29). However, most cases were from high-risk clinics or were recruited on the basis of a positive family history or with early-onset disease (e.g. age <35 or 40). In our study of unselected cases in Vietnam, only 46 of 292 cases (16%) were diagnosed prior to age 40. Of these 46 cases, only one had a BRCA mutation.

There are a few other reports of BRCA mutations among East Asian breast cancer cases unselected for age or family history (21–24). In general, these studies of Asian populations report mutation frequencies that are lower than those of western populations, but the frequencies vary widely. A Japanese study of unselected breast cancer cases from Tokyo found BRCA mutations in only 0.8% of 1000 patients (21). Suter et al. (22) detected BRCA1 or BRCA2 mutation in 2.2% of 645 unselected Chinese breast cancer cases in Shanghai. A Korean study of sporadic breast cancer reported BRCA mutations in 3 of 97 cases (3.1%) (23). A study of 294 unselected, incident breast cancer cases in the Philippines reported a mutation positive proportion of 5.1% (24). To our knowledge, our prevalence rate is among the lowest reported at 0.8% (2/259) of unselected cases.

Our study has several limitations. We did not perform full sequencing of BRCA1 and BRCA2 on all cases, nor did we screen for large rearrangements using multiplex ligation-dependent probe amplification (MLPA). Nevertheless, we include all deleterious founder mutations reported among Han Chinese, and all founder mutations previously identified among other East and Southeast Asian populations. Recent data suggest that ethnic Vietnamese (Kinh) may be closer in genetic terms to other Southeast Asian neighbours (e.g. Cambodians and Dai ethnic minority) than they are to Han Chinese (30). A report of a HUGO Pan-Asian SNP Consortium (31) provides support for the South-to-North migration theory, which implies that East Asian populations (Chinese, Korean, and Japanese) are in fact derived from Southeast Asians. They conclude that, 'on the

basis of increased cultural, linguistic and genetic diversity, the origins of Southeast Asian populations (including Cambodia, Vietnam and Thailand) are thought to be more complex than the origins of those to the North'. It will be of interest to determine the prevalence of BRCA mutations in breast cancer patients from Cambodia and Laos.

In summary, it does not appear that familial factors or BRCA mutations contribute substantially to the burden of breast cancer in Vietnam, despite a typically early age of onset of disease. It will be of interest to see if these findings are replicated in other South Asian populations and try to identify, other non-genetic risk factors in this population.

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