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Mutation Analysis of the *BRCA1* Gene in Malaysian Breast Cancer Patients

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ABSTRACT

Thirty patients with early onset breast cancer or familial breast cancer from Malaysia were analysed for germline mutation in the early onset breast cancer 1 gene (*BRCA1*). Direct sequencing of the entire coding region of *BRCA1* identified a frameshift mutation, c.5447-5448insC (insC5447) (codon 1776 of exon 21) in a patient aged 32 of the Malay ethnic origin, who had no family history of breast and/or ovarian cancer. Eight polymorphisms (2201C>T, 2430T>C, P871L, E1038G, K1183R, 4427T>C, S1613G and IVS8-57delT) were identified in the samples tested.

Keywords: *BRCA1*, Breast cancer, Ethnic, Mutation, Malaysian

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INTRODUCTION

Breast cancer is a common malignancy affecting women around the world, including Malaysia. In 1995, 9.6% of the admissions for cancer in the government hospitals in Malaysia was for breast cancer[1] and 20% of 317 women who died of breast cancer were below the age of 40[2]. Early onset breast cancer susceptibility gene (*BRCA1*) has been linked to 52% of families with breast cancer[3,4]. Germline mutations of *BRCA1* in families are estimated to increase the risk of developing breast cancer for the first time for *BRCA1* carriers at 73% by the age of 50 and 87% by 70 years[4]. There is also a 29% risk for breast cancer patients to develop ovarian cancer by 50 years and 44% by 70 years[4].

Germline *BRCA1* mutations have been identified in young-onset breast cancer patients without a strong family history of breast cancer. Greenman et al[5] reported *BRCA1* mutations in 18% patients (five out of 27) with families with one to three relatives with breast or ovarian cancer. Another study identified 15 mutations from 208 breast cancer patients below the age of 45 who had first degree breast cancer family history (affected mother and/or sister with breast cancer)[6]. These studies show that a large proportion of women with first degree breast cancer family history may not carry germline mutations. However, in studies with four or more first degree relatives with breast and/or ovarian cancer, a higher prevalence of mutations have been reported. Couch et al[7] identified that 45% of families carried *BRCA1* mutations and similar findings were reported in 30 Canadian families[8].

Other breast cancer predisposing genes associated with familial breast cancer are the breast cancer 2 gene (*BRCA2*) and Phosphatase and Tensin Homolog (*PTEN*). *BRCA2* was found to be linked to 32% in families with breast cancer. However, the risk of developing breast cancer associated with *BRCA2* is lower compared to *BRCA1*[4]. In addition, *BRCA2* mutations among male carriers confer a higher risk of male breast cancer compared to *BRCA1*[4]. *PTEN* mutations have been associated with Cowden syndrome, characterised by multiple hamartomatous lesions of the skin, mucous membranes, intestinal polyps and increased risk of breast and thyroid cancer. Although *PTEN* mutations have been found in sporadic and familial breast cancer cases, it is not a major determinant of non-*BRCA1/BRCA2* cases[9,10].

Currently, most reported *BRCA1* mutations are from those of Caucasian origin and the type of mutations in Asians could be different. Li et al[11] screened for *BRCA1* mutations using single strand conformational polymorphism (SSCP) in patients with familial breast cancer and identified a novel mutation. *BRCA1* mutation analysis in tumours of sporadic breast cancer cases unselected for age and early onset breast cancer cases in Chinese from Hong Kong identified 589delCT as a potential candidate founder *BRCA1* mutation among the Chinese[12]. In Singapore, the study of 43 breast cancer patients found 2846insA in two unrelated Malay families[13]. Japanese studies found *BRCA1* mutations to be unique to the country[14,15]. These reports also observed that the prevalence of *BRCA1* mutations among their patients was similar to those reported in United States. SSCP was used in all the studies reported above.

Malaysia is a country in South East Asia with the main ethnic groups being the Malays, Chinese and Indians. Information on *BRCA1* mutations in the Malay ethnic group, a major ethnic group in South East Asia is still lacking. In this study, we carried a detailed analysis of the *BRCA1* gene in patients in Malaysia regardless of their ethnicity. The entire coding region of *BRCA1* gene was analysed by direct sequencing, as this approach would cause the least bias in the type of mutation identified while theoretically being the most sensitive method. This is important as the information on the mutation spectra of *BRCA1* among breast cancer patients (particularly among those with early onset sporadic breast cancer) in this region is limited. We performed the comprehensive screening for *BRCA1* mutations in Malaysian patients with early onset breast cancer and those with two or more relatives with breast and/or ovarian cancers.

MATERIALS AND METHODS

We screened *BRCA1* mutations in two clinically selected groups for whom testing might be indicated. The first group comprised of histologically confirmed cases of breast cancer aged below 35 years (i.e. early onset) and the second group comprised of histologically confirmed cases of breast cancer with two or more first or second degree relatives with breast and/or ovarian cancers regardless of age (i.e. familial breast cancer). The family histories of these women are summarised in Table I.

Breast cancer patients from the three main ethnic groups seen in Kuala Lumpur Hospital between 1994 and 1998 in average were 45% Malays, 35% Chinese and 18% Indians. Individuals from the different ethnic groups in Malaysia were represented in our series of patients - i.e. 16 (53%) Malays, 9 (30%) Chinese and 5 (17%) Indian.

Patients identified were attending the Breast Clinic, Kuala Lumpur Hospital, Malaysia from 1994 to 1998 and blood was collected from consenting individuals. Polymerase chain reaction was carried out with primers[16,17] on leukocyte DNA for the entire coding region of the *BRCA1* gene in 37 separate reactions, followed by direct sequencing with the ABI PRISM™ dRhodamine Terminator Cycle Sequencing Ready Reaction Kit on ABI PRISM™ 310 Genetic Analyser. Sequences obtained were compared with the published sequence (GenBank Acc. No. L78833).

RESULTS

Between 1994 and 1996 approximately 10% of the patients diagnosed at the Kuala Lumpur Breast Clinic were at or below the age of 35 while 2 - 5% of patients had two or more relatives with breast and/or ovarian cancer. Among the patients aged 35 years or below, none of them had any family history of cancer and only two of the patients had bilateral breast cancer. Patients studied comprised of 23 patients who were aged 35 or below of which two of them had a family history of breast cancer, and seven patients aged between 40 to 70 with a history of breast and/or ovarian cancer. An insertion was found in exon 21 in nucleotide 5447 codon 1776 in a 32-year-old patient of the Malay origin (Fig. 1). This mutation is predicted to result in a premature stop codon at position 1829. The eight polymorphisms identified and the number of cases in which they were identified are listed in Table II. All polymorphisms identified in patients were from the three ethnic groups studied in exception of one.

Table I. Age and family history of patients.

Patient no.	Age (years)	Family history
1	40	Mother, sister and maternal aunt (breast cancer)
2	55	Sister and 3 maternal aunts (breast cancer); Father (stomach cancer)
3	57	2 sisters (breast cancer)
4	25	Paternal grandmother and 1 maternal aunt (breast cancer); 1 maternal aunt (ovarian cancer)
5	70	2 maternal aunt (breast cancer)
6	45	Mother (breast cancer); Sister (ovarian cancer)
7	50	2 sisters (breast cancer)
8	49	2 maternal aunts and 1 maternal aunt's daughter (breast cancer)

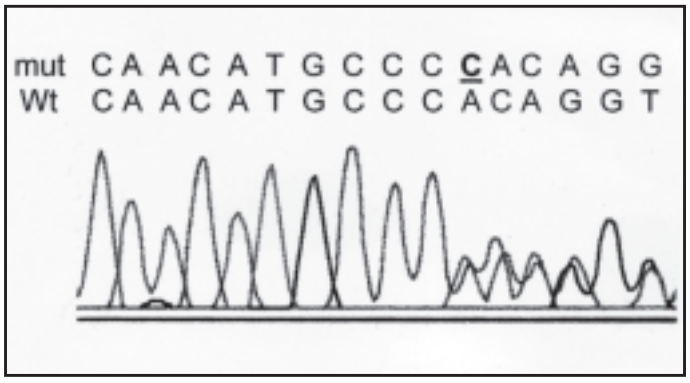

Fig. 1 Electropherogram of the frameshift mutation, c.5447-5448insC of exon 21. The mutant sequence (mut) with the insertion of C underlined and the wild type sequence (wt) are shown.

DISCUSSION

In our study, 4.3% (1/23) of our breast cancer patients aged 35 years or below had *BRCA1* mutations. The detection of one frameshift mutation in this group

Table II. Distribution of BRCA1 polymorphisms among the Malays, Chinese and Indians.

Polymorphism	Number of patients with polymorphism/total tested	Amino acid change	Ethnic group with polymorphism
2201C>T	4/30	silent	Malay, Chinese
2430T>C	18/30	silent	Malay, Chinese, Indian
2731C>T	19/30	P871L	Malay, Chinese, Indian
3232A>G	14/30	E1038G	Malay, Chinese, Indian
3667A>G	19/30	K1183R	Malay, Chinese, Indian
4427T>C	18/30	silent	Malay, Chinese, Indian
4956A>G	20/30	S1613G	Malay, Chinese, Indian
IVS8-57delT	16/30	–	Malay, Chinese, Indian

could explain the moderate role of *BRCA1* in the pathogenesis of breast cancer in this age group. *BRCA1* mutational studies conducted among Caucasian patients from the same age group and without family history as in this study identified *BRCA1* mutations in 6 (5.7%) of the 105 patients[18] and 12 (6.2%) of the 193 patients[6]. These are quite similar as seen in our study.

The frameshift mutation, c.5447-5448insC (insC5447) (codon 1776 of exon 21) was found in a patient aged 32 with non-cancer family history. The patient had a grade 3 cancer with negative oestrogen receptor. Johnasson et al[19] found *BRCA1* related tumours to be oestrogen receptor negative. Another study which analysed tumours of patients aged below 40 years observed that younger patients are linked with adverse pathological features such as grade 3 tumour, oestrogen receptor negative and lymphatic vessel involvement[20]. In our patient there were histologically positive nodes. The same mutation identified in our patient had been identified in a Dutch Caucasian study among familial breast cancer patients[21]. This truncating mutation would disrupt the involvement of *BRCA1* in DNA binding and in protein-protein interactions[22].

Ethnicity differences and the small sample size among our patients may contribute to the low number of mutations identified in the study. The potential founder mutation, 589delCT reported among Chinese was not found in our series of nine Chinese patients[12]. In the Malay ethnic group, we studied 16 patients but did not find the 2846insA and 2885delA mutations found among Malay patients in Singapore[13].

Studies have shown that *BRCA1* mutations are not common among breast cancer patients with a modest history of breast cancer. A study of 169 cases of women with a history of one to 11 cases of breast cancer per family only reported 27 *BRCA1* mutations[7]. Malone et al[6] found BRCA1 mutations in 1.2% of patients (14 out of 312) with families having fewer than four cases of breast cancer only, with no history of ovarian cancer. In our study, it is possible that breast cancer among the four patients with at least three relatives was not due to *BRCA1* mutations. The lack of *BRCA1* mutations among the breast cancer patients with family history also may be due to mutations in the noncoding regions of *BRCA1* and the existence of other breast cancer susceptibility genes such as *BRCA2* and PTEN. The presence of mutations in the intron and noncoding regions of the gene would affect RNA transcription, splicing and stability. Swensen et al[23] identified a 14kb deletion that removed both of the *BRCA1* transcription start sites. In addition, mutational analysis using direct sequencing could miss large deletions. Puget et al[24] identified large germline rearrangements among American and French breast cancer families that did not indicate *BRCA1* mutation using classical detection techniques.

Polymorphisms previously found in Caucasians[16,25] and also identified in Chinese[12]: 2201C>T, 2430T>C, 2731C>T, 3232A>G, 4227T>C and 4956A>G were found in our series of patients. The IVS8-57delT polymorphism found in Hispanics was detected in the three ethnic groups among our patients[26]. Our finding of at least one heterozygous polymorphism in 20 of our 30 patients indicate that the deletion of the entire BRCA1 gene did not occur in these patients.

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