BRCA1 and BRCA2 Germline Mutations in Malaysian Women with Early-Onset Breast Cancer without a Family History

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Abstract

Background: In Asia, breast cancer is characterised by an early age of onset. In Malaysia, approximately 50% of cases occur in women under the age of 50 years. A proportion of these cases may be attributable, at least in part, to genetic components, but to date, the contribution of genetic components to breast cancer in many of Malaysia’s ethnic groups has not been well-characterised.

Methodology: Given that hereditary breast carcinoma is primarily due to germline mutations in one of two breast cancer susceptibility genes, BRCA1 and BRCA2, we have characterised the spectrum of BRCA mutations in a cohort of 37 individuals with early-onset disease (<40 years) and no reported family history. Mutational analysis of BRCA1 and BRCA2 was conducted by full sequencing of all exons and intron-exon junctions.

Conclusions: Here, we report a total of 14 BRCA1 and 17 BRCA2 sequence alterations, of which eight are novel (3 BRCA1 and 5 BRCA2). One deleterious BRCA1 mutation and 2 deleterious BRCA2 mutations, all of which are novel mutations, were identified in 3 of 37 individuals. This represents a prevalence of 2.7% and 5.4% respectively, which is consistent with other studies in other Asian ethnic groups (4–9%).

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Introduction

Carcinoma of the breast is the most prevalent female malignancy worldwide [1]. However, there is considerable geographical variation in disease rates whereby the highest age-standardised incidence rates (ASR) are found in developed regions in the West such as North America and Europe [1,2]. Incidence rates in developing areas and countries of the Far East, albeit on modified by other genetic and non-genetic factors [15].

In Malaysia, breast cancer constituted 31% of all newly diagnosed female cancer cases in 2003, making it the most common cancer amongst Malaysian women across all major ethnic groups [3]. The overall incidence rate in 2003 (ASR of 46.2 per 100,000) is comparable to that of Singapore and the Philippines, but are relatively higher than those reported in China, Japan, India and Thailand [2]. It is also noteworthy that approximately half of the cases reported in Malaysia occurred in women under the age of 50 years. The apparent lower mean age of onset compared to the Western population has also been reported in other Asian countries [1]. One possible explanation is the “Westernization” of these populations [4], i.e. delayed childbearing, lower parity, reduced breast-feeding, decreased exercise and dietary changes, has led to a ‘cohort effect’ where the younger cohort have increased breast cancer risk compared to their mothers and grandmothers, thus giving rise to an apparent lower mean age of onset. However, given that an individual with breast cancer is more likely to carry a susceptibility gene mutation the younger she is at the time of diagnosis [5–7], an alternative possibility is that the allelic frequency of high penetrance genes in the Asian population may be higher than that in Caucasian populations.

Hereditary breast carcinomas are predominantly a consequence of loss-of-function germline mutations in one of two major breast cancer susceptibility genes, BRCA1 and BRCA2 [8–10]. Epidemiological studies suggest that deleterious mutations in either of these genes confer a significant increase in lifetime risk of developing breast and, to a lesser extent, ovarian cancers [11–14]. It is argued that this estimated risk is nonetheless imprecise and may be further modified by other genetic and non-genetic factors [15].

Our current understanding of BRCA mutations and their contribution to hereditary breast cancer has arisen primarily from
studies conducted in Caucasian women. A number of studies which have been carried out in several Asian countries, however, indicate that BRCA1 mutations are equally prevalent or may possibly be higher within the Asian population [16]. The majority of studies to date, particularly those in South East Asia, have focused primarily on BRCA1, with few comprehensive reports on the BRCA2 gene. In women with early-onset breast carcinoma unselected for family history, the prevalence of BRCA1 mutations is estimated to be between four and nine percent [16–23]. Founder mutations that are unique to Asians have also been described [16]. Despite this emerging knowledge, little is known about the spectrum and frequency of BRCA1 mutations and their involvement in breast cancer incidence within the ethnically diverse Malaysian population of Malays, Chinese & Indians [24]. This report describes sequence variants of BRCA1 and BRCA2 that were identified in a hospital-based cohort of Malaysian women with early onset breast cancer (<40 years) with no reported family history of breast and/or ovarian cancers.

Results

The Malaysian population is characterised by three major ethnic groups: the Malays (62%), Chinese (27%) and Indians (9%). Of the 3,738 new cases reported in 2003, 43.6% of patients were Chinese, while 39.7% were Malay and 11% were of Indian origin. Notably, the age-standardised incidence is higher among the Chinese and Indians in comparison to the Malays [3].

Patients for this study were recruited from one major breast cancer referral hospital in Malaysia, the University Malaya Medical Centre (UMMC), Kuala Lumpur. Due to its demographics, a larger proportion of patients i.e. 59.4% treated at this hospital were ethnically Chinese, compared to 25.8% Malays and 13.4% Indians. However, comparisons between the hospital and population data set show that collectively, breast cancer cases managed by UMMC over a ten-year period from 1996–2005 essentially mirror those that were reported to the national registry in 2003. Firstly, approximately 50% of cases in the population and those seen at the hospital occurred in women under the age of 50 years, 15% of whom were 40 years and below. Secondly, the most prevalent age group in both the population and hospital-based series was 40–49 years, whereby an average of 34% of all cases lies within this age bracket. Thirdly, discernable differences that are observed between the three ethnic groups are found in both the population and hospital data sets. In women below the age of 50 years, breast cancer incidence increased with age in all three ethnic groups. However, after the age of 50, incidence decreased in the Chinese and Malays but continued to rise within the Indian sub-population. A similar trend has been noted in an extensive study of trends of breast cancer incidence in women in Singapore, a country that is characterised by the same major ethnic groups, albeit in significantly different proportions [25].

Finally, it is clear that in both the population- and hospital-based series, disease onset in Malay women occur at a younger age in comparison to the Chinese and Indians. Taken together, this suggests that the hospital-based cohort is largely reflective of breast cancer cases within the Malaysian population.

A total of 37 proband patients diagnosed with early-onset breast cancer (<40 years) with a mean age of 33.7 years and no known family history of the disease were selected for this study. The ethnicity of this study group reflected the ethnicity of the hospital-based sample collection i.e. 21 Chinese (56.8%), 11 Malays (29.7%), 4 Indians (10.8%) and 1 individual of mixed race (2.7%). Within this cohort, we identified a total of 14 BRCA1 and 17 BRCA2 sequence variants, of which ten are novel (summarised in Tables 1 & 2).

BRCA1 Sequence Variants

Fourteen BRCA1 sequence variants, including three novel changes, were identified in this study population (Table 1). Only one clearly disease-associated mutation was found in this cohort. The splice site mutation (IVS5+2delT), which occurs within the proximal splice site of exon 3, was detected in one patient of Chinese descent.

Two BRCA1 missense variants of uncertain significance were identified in two out of 37 proband patients (5.4%). One variant at position 2405 (G>A) in exon 11 that led to a substitution of Ser for Arg residue at position 762 (R762S) was detected in an individual of Malay descent. Although its clinical significance is presently unknown, this substitution of this invariant Arg [27], which occurs outside any known functional domain, is unlikely to contribute to increased risk to breast cancer because it has been reported to occur in a cohort of cancer-free individuals [26].

The second BRCA1 variant of uncertain significance was identified in a Chinese patient. It involved an alteration at position 2685 (T>C) that resulted in a Tyr to His substitution at codon 856 (Y856H) in exon 11. This variant has been reported previously occurring at similar frequencies in breast cancer and cancer-free individuals of Chinese descent in Hong Kong [18] and Shanghai [26], suggesting that this variant is neutral or of little pathological significance.

Eleven additional BRCA1 sequence variants were identified in this study group (Table 1). These include two novel changes in exon 11 that were detected in two Chinese patients. These variants are considered benign and may be polymorphisms as the single base alterations do not lead to amino acid substitutions (L625L and P977P) and are unlikely to be potential splice site variants. A further nine variants that were identified have been reported previously in BIC and are classified as neutral polymorphisms. Two alterations, a synonymous alteration in exon 3 (K398K) and a missense substitution in exon 11 leading to an Asp for Asn substitution at codon 693 (D693N), are rare and were only identified once each (1/37, 2.7%) in two Chinese patients. In contrast, seven other single nucleotide polymorphisms were found in 75.7% (28/37) of individuals across all three ethnic groups in this patient population. These correspond to three silent substitutions in exon 11 (S694S and L771L) and exon 13 (S1436S), and four missense changes in exon 11 (P871L, R1092G, K1138R) and exon 16 (S1613G). This block of seven common polymorphisms always occurred concurrently i.e. a patient that carries one variant was always found to carry the other six variants. Similarly, five of these polymorphic sites in exon 11 have been noted to co-occur in other Asian [18,22], as well as Caucasian populations [8,28].

BRCA2 Sequence Variants

A total of 17 BRCA2 sequence variants, five of which have not been previously reported, were identified in this patient population (Table 2). These include two distinct frameshift mutations that are predicted to encode truncated proteins and three missense variants of uncertain clinical significance. The two deleterious frameshift mutations were detected in two patients (5.4%) of Chinese descent. The first is a 2-bp deletion at nucleotide 2862 (2862 delCT) while the second involves the deletion of adenine at position 6901 (6901 delA). These two alterations are predicted to result in premature STOP codons at positions 879 (STOP 879) and 2228 (STOP 2228), respectively.

The three BRCA2 variants of uncertain significance identified in this study correspond to substitutions in exons 10 and 11 (Table 2) and none are likely to be clinically relevant. One is a novel variant which encodes a Thr to Pro substitution at codon 1723. This variant was detected in an index case who also carries the novel
**Table 1. Sequence variants identified in the BRCA1 gene.**

| Clinical relevance | Type | Exon | Nucleotide change (HGVS) | Nucleotide change | AA Change | Novel/Reported (BIC) | Frequency, n = 37 | Ethnicity |
|--------------------|------|------|--------------------------|------------------|-----------|---------------------|-----------------|-----------|
| 1 Deleterious      | IVS  | 3    | IVS5+3delT               | IVS5+3delT       | NA        | Novel               | 1/37, 2.7%      | Chinese   |
| 2 VUS              | MS   | 11   | c.2266 A>T               | 2405 A>T         | R762S     | Reported, [26]      | 1/37, 2.7%      | Malay     |
| 3 VUS              | MS   | 11   | c.2566 T>C               | 2685 T>C         | Y856H     | Reported            | 1/37, 2.7%      | Malay     |
| 4 Benign*          | Syn  | 11   | c.1873 C>T               | 1992 C>T         | No change | Reported            | 1/37, 2.7%      | Chinese   |
| 5 Benign*          | Syn  | 11   | c.2931 A>G               | 3050 A>G         | No change | Reported            | 1/37, 2.7%      | Chinese   |
| 6 Polymorphism     | Syn  | 3    | c.114 G>A                | 233 G>A          | No change | Reported            | 1/37, 2.7%      | Chinese   |
| 7 Polymorphism     | MS   | 11   | c.2077 G>A               | 2196 G>A         | D693N     | Reported            | 1/37, 2.7%      | Chinese   |
| 8 Polymorphism     | Syn  | 11   | c.2082 C>T               | 2201 C>T         | No change | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |
| 9 Polymorphism     | Syn  | 11   | c.2311 T>C               | 2430 T>C         | No change | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |
| 10 Polymorphism    | MS   | 11   | c.2612 C>T               | 2731 C>T         | P871L     | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |
| 11 Polymorphism    | MS   | 11   | c.3113 A>G               | 3232 A>G         | E1038G    | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |
| 12 Polymorphism    | MS   | 11   | c.3548 A>G               | 3667 A>G         | K1183R    | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |
| 13 Polymorphism    | Syn  | 13   | c.4308 T>C               | 4427 T>C         | No change | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |
| 14 Polymorphism    | MS   | 16   | c.4837 A>G               | 4956 A>G         | S1613G    | Reported            | 28/37, 75.7%    | Malay (10/11), Chinese (15/21), Indian (3/4) |

MS, missense; Syn, synonymous; FS, frameshift; VUS, variant of uncertain significance; BIC, Breast Information Core

**BRCA1 R762S variant** of uncertain significance. Based on multiple sequence alignment analysis, this Thr residue is not highly evolutionarily conserved, with alternative amino acids being found in dog, mouse and chick. Furthermore, the pairwise GMS of 38 for a Thr to Pro change is relatively low. Taken together, this variant is not likely to be disease causative. Two other missense variants of uncertain significance, C315S and M1149V were detected in two patients of Chinese and Malay descent, respectively. These two variants have been reported in BIC, primarily in individuals of Asian origin. Based on multiple sequence alignment analysis, Grantham score (GMS = 112) and the SIFT ([26]) prediction programme, the C315S missense variant because allelic frequency data from population studies are currently not available.

Nine previously reported common BRCA2 polymorphisms were also identified in this study group. These polymorphisms corresponded to sequence alterations in exons 10, 11 and 14. Four sequence variants involved changes that resulted in amino acid substitutions (N289H, H372N, N991D and G1771D) while five were synonymous changes (S455S, H743H, K1132K, V1269V and S2414S) that did not affect the protein sequence of BRCA2. These nine polymorphic sites were found to occur at varying frequencies. One variant, G1771D, was identified in one of 37 patients (2.7%). Four of these, namely N289H and S455S in exon 10 and H743H and N991D in exon 11, occurred concurrently in three patients (3/37, 8.1%). The V1269V alteration in exon 11 was detected in 21.6% (8/37) of the study group while the three remaining polymorphisms, H372N (exon 10), K1132K (exon 11) and S2414S (exon 14), occurred at relatively high frequencies. They were detected in more than 50% of the cohort, with 28 patients carrying at least one of the three variants. Of these, 12 patients (12/28, 42.9%) were found to carry all three polymorphisms.

**Conclusion**

Mutational analysis by direct sequencing of the entire coding region of the BRCA1 and BRCA2 genes on 37 breast cancer patients aged 40 years and under, with no reported family history of breast and/or ovarian cancers, identified a total of 14 BRCA1 and 17 BRCA2 sequence variants. These include ten novel mutations (4 BRCA1 and 6 BRCA2) that have not been previously reported. One pathogenic BRCA1 mutation and two novel
pathogenic BRCA2 mutations were detected in two of 37 index cases. This represents a prevalence of 2.7% and 5.4% respectively.

To date, only one other mutational analysis has been conducted on Malaysian breast cancer patients [24]. This study focused exclusively on the BRCA1 gene. Clearly, there remain gaps in our understanding of how germline mutations in BRCA genes contribute towards breast cancer incidence in Malaysia, but given the relatively low yield of deleterious mutations in BRCA1 and BRCA2 despite a thorough examination of both genes, it might be concluded that germline mutations at these sites are unlikely to contribute to the observed lower age of onset of breast cancer compared to Western series.

Materials and Methods

A total of 619 pathologically confirmed breast cancer patients were recruited from the University Malaya Medical Centre (UMMC) in Kuala Lumpur, Malaysia between January 2003 and December 2006. Family histories were obtained by interviewing patients. From these, 37 individuals with early onset disease (<40 years) without any first and/or second degree relatives affected with breast and/or ovarian cancers were selected for this study cohort. Prior approval for this study was obtained from the University Malaya Medical Centre ethics committee, and written informed consent was obtained from all participating subjects.

DNA from peripheral blood was extracted using standard techniques and the entire coding sequence of BRCA1 and BRCA2 and splice sites (20 bp upstream and 10 bp downstream) were analysed for sequence variants by PCR and direct nucleotide sequencing. Samples were subjected to electrophoresis using MegaBACE™ 500 (Amersham Biosciences, Germany) and sequencing traces were analysed using the Mutation Surveyor v2.61 (Softgenetics Inc., USA) programme.

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Author Contributions

Conceived and designed the experiments: ST GT. Performed the experiments: GT PK DL SL SS. Analyzed the data: SL. Contributed reagents/materials/analysis tools: NM SY CY. Wrote the paper: ST SL.

| Table 2. Sequence variants identified in the BRCA2 gene. |
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| No | Clinical relevance | Type | Exon | Nucleotide change (HGVS) | Nucleotide change | AA Change | Novel/Reported (BIC) | Frequency, n = 37 | Ethnicity |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | Deleterious | FS | 11 | c.2634 delCT | 2662 delCT | STOP 879 | Novel | 1/37, 2.7% | Chinese |
| 2 | Deleterious | FS | 11 | c.6673 delA | 6901 delA | STOP 2228 | Novel | 1/37, 2.7% | Chinese |
| 3 | VUS | MS | 10 | c.943 T>A | 1171 T>A | C31SS | Reported | 1/37, 2.7% | Chinese |
| 4 | VUS | MS | 11 | c.3445 A>G | 3673 A>G | M1149V | Reported | 1/37, 2.7% | Malay |
| 5 | VUS | MS | 11 | c.5167 A>C | 5395 A>C | T1723P | Novel | 1/37, 2.7% | Malay |
| 6 | Benign* | Syn | 10 | c.1275 A>G | 1503 A>G | No change (E425E) | Reported, [29] | 1/37, 2.7% | Chinese |
| 7 | Benign* | Syn | 11 | c.4578 A>G | 4806 A>G | No change (T1526T) | Novel | 1/37, 2.7% | Mixed |
| 8 | Benign* | Syn | 17 | c.7941 A>C | 8169 A>C | No change (L2647L) | Novel | 1/37, 2.7% | Malay |
| 9 | Polymorphism | MS | 10 | c.865 A>C | 1093 A>C | N289H | Reported | 3/37, 8.1% | Malay (1/11), Chinese (2/21) |
| 10 | Polymorphism | MS | 10 | c.1114 C>A | 1342 C>A | H372N | Reported | 19/37, 51.4% | Malay (5/11), Chinese (12/21), Indian (1/4), Mixed (1/1) |
| 11 | Polymorphism | Syn | 10 | c.1365 A>G | 1593 A>G | No change (S455S) | Reported | 3/37, 8.1% | Malay (1/11), Chinese (2/21) |
| 12 | Polymorphism | Syn | 11 | c.2229 T>C | 2457 T>C | No change (H743H) | Reported | 3/37, 8.1% | Malay (1/11), Chinese (2/21) |
| 13 | Polymorphism | MS | 11 | c.2971 A>G | 3199 A>G | N991D | Reported | 3/37, 8.1% | Malay (1/11), Chinese (2/21) |
| 14 | Polymorphism | Syn | 11 | c.3396 A>G | 3642 A>G | No change (K1132X) | Reported | 22/37, 59.5% | Malay (4/11), Chinese (16/21), Indian (1/4), Mixed (1/1) |
| 15 | Polymorphism | Syn | 11 | c.3807 T>C | 4035 T>C | No change (V1269V) | Reported | 8/37, 21.6% | Malay (3/11), Chinese (5/21) |
| 16 | Polymorphism | MS | 11 | c.5312 G>A | 5540 G>A | G1771D | Reported | 1/37, 2.7% | Chinese |
| 17 | Polymorphism | Syn | 14 | c.7242 A>G | 7470 A>G | No change (S2414S) | Reported | 22/37, 59.5% | Malay (5/11), Chinese (16/21), Mixed (1/1) |

*These sequence alterations are likely to be polymorphisms. They have been classified as benign as allelic frequency data from population studies are not currently available. MS, missense; Syn, synonymous; FS, frameshift; VUS, variant of uncertain significance; BIC, Breast Information Core

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