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Short Report

Identification of novel *BRCA* large genomic rearrangements in Singapore Asian breast and ovarian patients with cancer

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Large genomic rearrangements have been reported to account for about 10–15% of *BRCA1* gene mutations. Approximately, 90 *BRCA* rearrangements have been described to date, all of which but one have been reported in Caucasian populations of predominantly Western European descent. Knowledge of *BRCA* genomic rearrangements in Asian populations is still largely unknown. In this study, we have investigated for the presence of *BRCA* rearrangements among Asian patients with early onset or familial history of breast or ovarian cancer. Using multiplex ligation-dependent probe amplification (MLPA), we have analyzed 100 Singapore patients who previously tested negative for deleterious *BRCA* mutations by the conventional polymerase chain reaction-based mutation detection methods. Three novel *BRCA* rearrangements were detected, two of which were characterized. The patients with the rearrangements, a *BRCA1* exon 13 duplication, a *BRCA1* exon 13–15 deletion and a *BRCA2* exon 4–11 duplication, comprise 3% of those previously tested negative for *BRCA* mutations. Of the *BRCA1* and *BRCA2* pathogenic mutations identified in our studies on Asian high-risk breast and ovarian patients with cancer to date, these rearrangements constitute 2/19 and 1/2 of the *BRCA1* and *BRCA2* pathogenic mutations, respectively. Given the increasing number of rearrangements reported in recent years and their contribution to the *BRCA* mutation spectrum, the presence of *BRCA* large exon rearrangements in Asian populations should be investigated where clinical, diagnostic service is recommended.

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Key words: Asian population – *BRCA1* – *BRCA2* – breast/ovarian cancer – genomic rearrangements

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With the growing use of germline mutation testing in high-risk breast cancer clinics, an increasing number of women are being counseled on their breast cancer risk based on their *BRCA* mutation status (1). Based on these genetic tests, mutation carriers are counseled on the options of prophylactic surgery, chemoprevention or closer surveillance. With more than 1000 deleterious mutations in *BRCA1* (OMIM +113705) and *BRCA2* (OMIM +600185) genes described throughout a cumulative reading frame of over 15,000 nucleotides, a negative test result is regarded as non-informative, based on the possibility of a false-negative test. Depending on the technique of mutation detection used, a variation in false-negative rates has been shown even in well-established genetic screening centers (2, 3). The prevalence of large exon rearrangements in the *BRCA* genes in the screened population is of particular interest, as these are usually not detected by standard polymerase chain reaction (PCR)-based methods. While estimates of 10–28% (4) have been reported in previous studies, these have been exclusively carried out in Caucasian population, and their incidence in population groups of Eastern origin is yet to be determined extensively in a large sample cohort.

The aim of our study was to assess the prevalence of large exon rearrangement in the *BRCA* genes in a study cohort of 100 high risk Chinese, Malay and Indian patients, previously tested negative for *BRCA* mutations using standard PCR-based screening methodologies.

Material and methods

Patients

The study population consisted of 100 consecutive unrelated early-onset and familial breast and/or ovarian patients with cancer presenting at the Surgery and Oncology Clinics of the National University Hospital, Singapore. All had a personal history of histologically proven breast and/or ovarian cancer. Their personal and family history of breast and ovarian cancer is listed in Table 1. Selection was based on *a priori* risk of at least 10%, of harboring a deleterious *BRCA* mutation (5, 6). The mean age of diagnosis was 35.17 years (range 22–40 years) and 36.6 years (range 17–49 years) for breast cancer and ovarian cancer cases, respectively. Fifty-nine patients had early onset of breast (under 40 years of age) or ovarian cancer with no family history of cancers. All patients were Singaporean women who previously tested negative for deleterious *BRCA* mutations by conventional PCR-based mutation detection methods (7, 8). They belonged to the following ethnic groups: Chinese ($n = 87$), Malays ($n = 9$) and Indians ($n = 4$). Although early-onset disease was the most common indication for mutation testing, 20% of cases were classified as members of a hereditary breast and/or ovarian cancer (HBOC) families (9, 10). This study had institutional ethical committee approval and signed written informed consent was obtained from each participant before blood collection. The family histories on number and age of relatives, together with numbers of affected relatives and age of onset of cancers were determined by direct interview and were confirmed with the National Cancer Registry.

Molecular studies

DNA isolation

Peripheral blood lymphocytes were isolated from blood samples using Ficoll–Paque™ PLUS according to the manufacturer's instructions (GE Healthcare, Amersham, UK). Total genomic DNA was extracted from the lymphocytes using standard procedures (11).

Multiplex ligation-dependent probe amplification analysis

Multiplex ligation-dependent probe amplification (MLPA) analysis was performed using the test kits SALSA P002/P087 *BRCA1* and SALSA P045 *BRCA2* from MRC-Holland (Amsterdam, the Netherlands) according to the supplied manufacturer's protocols. The P002 test kit was used for the initial primary screen after which the P087 MLPA kit was used to reanalyze all suspected results obtained with the P002 probe mix for *BRCA1*. In summary, 100 ng of genomic DNA was denatured at 98°C and hybridized with the *BRCA1*-MLPA probe mix at 60°C for 16 h. This was followed by ligation of the annealed probe to the Ligase-65 mix at 54°C for 15 min. The appropriate PCR primers, dNTPs, SALSA PCR buffer and SALSA polymerase were added and the subsequent PCR reactions were performed for 33 cycles (30 s at 95°C, 30 s at 60°C, 60 s at 72°C) and one cycle at 72°C for 20 min. Each probe amplification product of 2 μl was then mixed with 0.2 μl of the GeneScan™-500 ROX™ Size Standard (Applied Biosystems, Warrington, UK), 10 μl of HiDi Formamide (Applied Biosystems) and analyzed using 3100 capillary sequencer (Applied Biosystems) with a 36-cm capillary array and POP-4™ polymer (Applied Biosystems).

Genescan software (Applied Biosystems) was used for fragment analysis and the results (peak sizes, height and areas) were exported to Excel files. All subsequent quantitative and statistical analyses of the data were then performed using the P002/P087 *BRCA1* and P045 *BRCA2*/CHEK2 Excel templates from the National Genetics Reference Laboratories, Manchester, UK (http://www.ngrl.org.uk). In short, the data from each ligation product were analyzed to produce dosage quotients (DQ) of the alleles with the expected values for normal, deleted and duplicated DQs being 1.0, 0.5 and 1.5, respectively. T-statistic was used to calculate the significance level or probability of deviation from the expected DQ by comparing the test sample to a series of five normal control subjects. The standard deviation (SD) of all the control ligation products was used to test the overall quality of the individual tests where the SDs should not exceed 0.1.

Fine characterization of the BRCA1 10 410-bp deletion

In order to determine the putative deletion, a series of nested PCRs were performed on the genomic DNA from the patient with the suspected rearrangement and from a control individual in

Table 1. Characteristics of the families studied

Patient	Breast/ovarian cancer (age at diagnosis)[a]	Family history[a,b]	
		Breast/ovarian cancer	Other cancers
Breast/ovarian cancer families ($n = 23$)			
N95	Br 56	Br 40 (M)	Lv 57 (F)
N154	Br 26	Ov 50 (PA)	
N223	Br 47	Br 30 (S)	
N276	BilatBr 45	Br 40 (PC), Br 30 (MC)	Co 60 (PU)
N280	Br 50	Br 45 (M)	
N393	Br 40	Ut 50 (S)	
N402	BilatBr3 7	Br 36 (N), Br 24 (N)	
N430	Br 55	Br 50 (S), Br 50 (N)	
N432	Br 52	Br 36 (D)	
N435	Br 52	Br 40 (S)	
N437	Br 40	Br 43 (S)	
N506	Br 39	Br (MA)	Lu 57 (F)
N526	BilatBr 39	Ut 27 (S)	
N538	Br 37	Br 34 (MGM)	
N543	Br 34	Br 40 (MA)	Npc (MGF)
N546	Br 44	Br 42 (S), lobBr 42 (S), Br 80 (M)	Head and neck 41 (MA), Lu 68 (MU), Npc 65 (MU)
N548	Br 38	Br 43 (PC),	Lv 64 (F), Cv (PA), Sto (PA)
N553	Br 35	Br 34 (MA),	Lu 48 (PA), Thr 46 (MGF)
N581	BilatBr 35	Ov 83 (MGM)	
N614	BilatBr 45	BilatBr 50 (MA), Br 50 (C)	Leu 7 (D)
N534	Ov 47	Br 35 (S)	
N588	Ov 55	Br 60 (S), Br 66 (S), Br 35 (N), Ov 40 (N)	Co 64 (B), Leu (F)
N629	Ov 50	Ov 62 (M), Ov (S)	
Non-breast/ovarian cancer families ($n = 18$)			
N150	Br 39		CSU
N152	Br 39		Npc 63 (F)
N361	Br 36		Lu (F)
N409	Br 36		Lv3 1 (F), Lv (PU)
N443	Br 35		Leu (M), Sto (PU)
N450	Br 35		Lv (F/PU)
N466	Br 39		Lv (F)
N480	Br 23		Npc 34 (MU)
N509	Br 39		Co (PU), Thr 50 (PGF)
N511	Br 34		Brn 53 (F)
N525	Br 32		Lu (F)
N539	Br 31		Npc (PGM)
N549	Br 38		Sto (MA)
N557	Br 38		GB 60 (PU), Lu 60 (PU), Leu 27 (PC)
N586	Br 34		Thy (MGM)
N524	Ov 44		Co (MGF)
N570	Ov 42		Co 50 (B)
N585	Ov 40		Sto (S), Lv (M)

[a]Cancer type/site: Bilat, bilateral; Brn, brain; Br, breast; Co, colon; Cv, cervix; GB, gall bladder; Leu, Leukemia; Lu, Lung; Lv, liver; Npc, nasopharyngeal; Ov, ovary; Sto, stomach; Thr, throat; Thy, thymus; Ut, uterus; CSU, cancer site unknown.
[b]Affected relatives in parentheses: B, brother; C, cousin; D, daughter; F, father; M, mother; MA, maternal aunt; MC, maternal cousin; MGF, maternal grandfather; MGM, maternal grandmother; MU, maternal uncle; N, niece; PA, paternal aunt; PGF, paternal grandfather; PGM, paternal grandmother; PU, paternal uncle; S, sister.

order to isolate the site spanning, the exon 13–15 deletion. Long-range PCR (Expand Long Template PCR System; Roche Applied Sciences, Indianapolis, IN) was performed using specific primers, B1Ex12F and B1Ex16R (Table 2) to amplify a portion of exon 12, all of exons 13–15 and a portion of exon 16. The temperature profile was 94°C for 2 min, followed by 10 cycles of denaturation at 94°C for 15 s, annealing at 65°C for 30 s, and extension at 68°C for 10 min. The reaction parameters for the second round of amplification consisted of 20 cycles of 94°C for 15 s, 65°C for 30 s, 68°C for 10 min with a delay of 10 s per cycle. The long-range amplicon was purified and used as a template for the nested PCR with the internal primers B1int12F6 and

Table 2. Oligonucleotides primers used for rearrangement analysis of *BRCA1* and *BRCA2*

Primer	Nucleotide sequence	Nucleotide position[d]
BRCA1 exon 13–15 deletion analysis		
B1ex12F	5′-AGACTGCTCAG GGCTATCCTC-3′	37,743–37,763
B1ex16R	5′-CTTCTCCCTGCTCACACTTTC-3′	57,726–57 746
B1int12F6	5′-GGCAACCAT TGCTGTTCCTTC-3′	44,214–44,234
B1int15R2	5′-CCTTTGGACTCTTGTCTAACAG-3′	55,026–55,047
B1int15R4	5′-GGTTCAAGTG ATTCTGCTGC-3′	54,883–54,902
BRCA2 exon 4–11 duplication analysis		
B2ex11F3	5′-GACTTGACTTGTGTAAACGAAC-3′	22,841–22,862
B2int3R1	5′-TAATAAAGCAAATGGATGTGTAAC-3′	9334–9357
B2ex11F4[c]	5′-GATGAAAAAGAGCAAGGTACTAG-3′	24,743–24,765
B2int3R3[b]	5′-CACTACACACTTGTTAGAATGG-3′	8870–8891
B2int3F1[a]	5′-TCACACCAAAGAGCACGATTG-3′	8672–8692
BRCA1 exon 13 duplication analysis		
B1Ex13F	5′-GATAAAGCTCCAGCAGGAAATG-3′	46,182–46,203
B1Ex13R	5′-GGCTCCCATGCTGTTCTAAC-3′	46,240–46,259
Ex13dup1R	5′- AGATCATTAGCAAGGACCTGTG-3′	45,092–45,113
Ex13dup1F	5′-GATTATTTCCCCCCAGGCTA-3′	75,990–76,009

[a,b,c]Sequencing primers indicated in Fig. 2(b).
[d]Nucleotide positions numbered with reference to *BRCA1* and *BRCA2* genomic sequences, accession numbers L78833 and AY436640, respectively.

B1int15R2. The PCR products were subsequently sequenced with B1int12F6 and B1int15R4 (Fig. 1c).

Fine characterization of the BRCA2 exon 4–11a duplication

The tandem repeat of exon 4–11a was detected using a series of forward primers residing in intron 10 and exon 11 and reverse primers upstream of exon 4. Using Long Range PCR (Expand™ 20 kb Plus PCR System, Roche Applied Sciences), the PCR fragment that encompassed the 3′ breakpoint junction were obtained with the primers B2ex11F3 and B2int3R1 and subsequently sequencing with primers B2ex11F4 and B2int3R3. In addition, the 5′ breakpoint junction was also screened for any sequence changes by single-strand conformation polymorphism (SSCP) and sequenced using the primers B2int3F1 and B2int3R3 (Table 2).

Characterization of BRCA1 exon 13 duplication

To determine whether this duplication was the exon 13 founder mutation described in mutation carriers of Northern European descent, PCR was performed on the patient genomic DNA using the published primers (44). Primer pairs B1Ex13F/B1Ex13R and Ex13dup1R/Ex13dup1F both generate products that are 6 kbp and 800 bp, respectively; genomic DNA from a patient (gift from Dr Wera Hofmann, Berlin, Germany) positive for the exon 13 founder mutation was used as a control.

Sequence analysis

All purified PCR products that contained the wild-type sequence or the rearrangement breakpoints were sequenced using fluorescent-labeled primers. The sequence analysis was performed using ABI 3100 DNA automated sequencer with the ABI BigDye terminator cycle sequencing kit (Applied Biosystems).

Results

Of the 100 breast or ovarian patients with cancer analyzed, three novel *BRCA* large exon rearrangements were identified (Table 3) with patients harboring these rearrangements accounting for 3% of the previously mutation-negative probands (7, 8). The rearrangements involved the deletion of exons 13–15 of *BRCA1* in N588, an Indian ovarian patient with cancer with a strong family history of breast and ovarian cancer (Fig. 1a); an exon 4–11a duplication of *BRCA2* in a Chinese patient, N557, with early-onset breast cancer and a history of cancers in relatives on her paternal side (Fig. 2a); and an *BRCA1* exon 13 duplication in a Chinese patient, N419, with early-onset breast cancer (Fig. 3).

Fine characterization of the BRCA1 exon 13–15 genomic deletion in N588

Using primers located in introns 12 and 15, mutant and wild-type PCR products were amplified from the genomic DNA of N588 and a control individual, respectively. To determine the actual deletion breakpoints, we sequenced the mutant and wild-type bands separately using a series of internal primers.

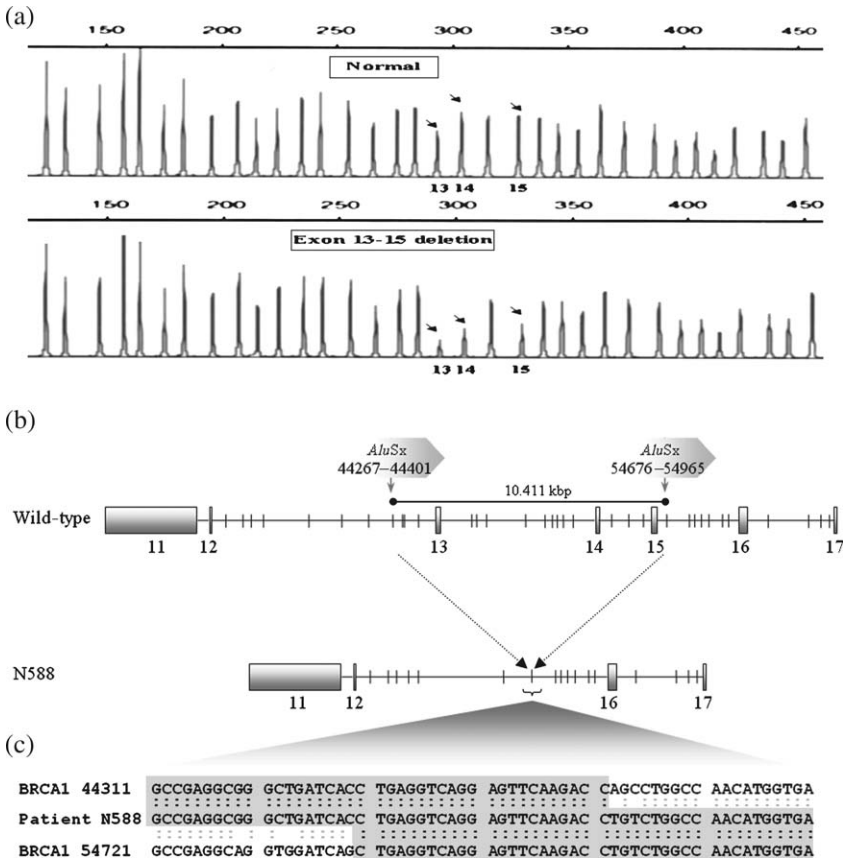

Fig. 1. Fine characterization of the *BRCA1* exon 13–15 genomic deletion. **(a)** Multiplex ligation-dependent probe amplification detection of the exon 13–15 deletion (arrows). **(b)** Schematic representation of the normal and mutant alleles indicating the *Alu*Sx-mediated (arrowed) deletion, exons 11–17 (filled boxes), introns (horizontal lines) and *Alu* elements (vertical lines). **(c)** Sequence analysis of the deletion breakpoints. The site of the crossover event was determine to lie within a 22-bp sequence when the polymerase chain reaction product sequence was aligned with the *Alu* sequences (shaded box) in both introns, resulting in a 10,410-bp deletion.

The 5′ breakpoint of 10,410-bp deletion occurred 1804-bp upstream of exon 13 in intron 12, and the 3′ breakpoint occurred 360-bp downstream of exon 15 in intron 15 (Fig. 1b). Sequence analysis indicated that 5′ and 3′ breakpoint of the deletion occurred within two *Alu*SX repeats oriented in the same forward direction, at locations 44,267–44,401 and 54,676–54,965, respectively (*BRCA1* accession number: L78833), and the site of the crossover event was determine to lie within a 22-bp sequence (Fig. 1c).

Fine characterization of the *BRCA2* exon 4–11a genomic duplication in N557

In order to delineate the orientation of the duplication, a series of PCR amplifications were performed employing different combinations of

Table 3. *BRCA* genomic rearrangements in Singapore patients with either early onset or family history of breast/ovarian cancer

				Family history[c]	
Patient[a]	Ethinicity	Age	Mutation name	Breast/ovarian cancer	Other cancers
BRCA1 exon rearrangements					
N588 (Ov)	Indian	55	L78833[b]: g.44352_54761del 10410 (del. ex. 13–15)	Br60 (S), Br66 (S), Br35 (N), Ov40 (N)	Co64 (B), Leu (F)
N419 (Br)	Chinese	40	Duplication involving exon 13. Breakpoints not determined	Nil	Nil
BRCA2 exon rearrangement					
N557 (Br)	Chinese	38	AY436640[b]: g.8730_24909dup16180	Nil	GB60 (PU), Lu60 (PU), Leu27 (PC)

[a]Cancer type/site: Br, breast; Co, colon; GB, gall bladder; Leu, leukemia; Lu, lung; Ov, ovary.
[b]Accession number.
[c]Affected relatives in parentheses: B, brother; F, father; N, niece; PC, paternal cousin; PU, paternal uncle; S, sister.

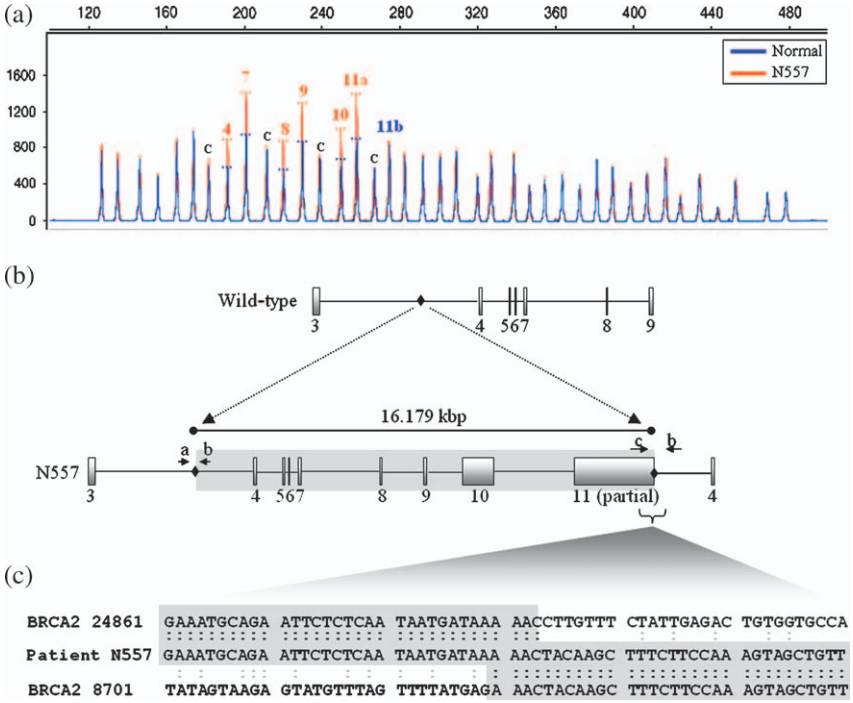

Fig. 2. Fine characterization of the *BRCA2* exon 4–11a genomic duplication. **(a)** Multiplex ligation-dependent probe amplification detection of the exon 4–11a duplication in the patient (blue), 'c' depicts control peaks. **(b)** Schematic representation of the normal and mutant alleles, showing the location of the 16,180-bp duplication (arrowed), exons (filled boxes) and introns (horizontal lines). **(c)** Sequence analysis of the duplication junction between the tandem repeats and alignment of the mutant and wild-type *BRCA2* sequences (shaded box). The site of the recombination event is thought to reside within a 4-bp sequence.

forward and reverse primers located in intron 3 and exon 11a. Using the forward and reverse primers B2ex11F4 and B2int3R3, located in exon 11 and intron 3, respectively, a PCR product was obtained with the patient genomic DNA. Sequencing and SSCP analysis of a PCR fragment containing the 5′ breakpoint junction using the forward and reverse primers, *a* and *b*, respectively (Table 2), located along the length of intron 3 showed no change in sequence. These results together with sequence analysis of the duplication junctions in the genomic DNA indicated that the duplication, 16 180 bp in length, was a tandem repeat of exons 4–11a at locations 8730–24,909 (Fig. 2b; *BRCA2* accession number: AY436640). A Basic Local Alignment Search Tool search of N577 sequences upstream and downstream of the breakpoints showed no similarity to any potentially recombining *Alu* sequences or recombination-associated motifs. However, it was noted that the breakpoint of the duplication in intron 3 occurred within a region of 425 bp, which had homology to a Line-1 (L1) repeat (genomic location 8466–8890).

Exon 13 duplication in N419

A peak increase was detected for exon 13 in N419 after MLPA analysis. The same results were obtained twice when two different aliquots of the sample were analyzed with two different MLPA kits (P002 and P087). Genomic DNA of N419 when analyzed with the specific exon 13 primers showed that this duplication was different from the founder mutation, *BRCA1* ins6kbEx13, previously reported in Western populations with Celtic ancestry (12). Further experiments using different combinations of primers to detect for

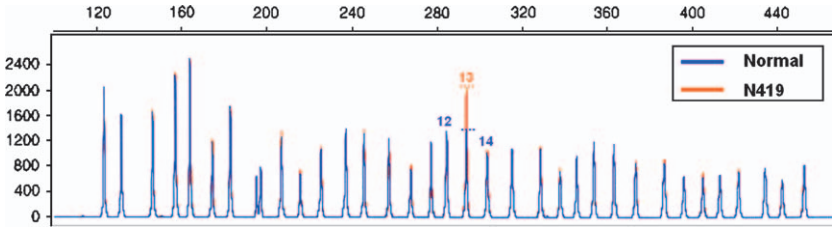

Fig. 3. Multiplex ligation-dependent probe amplification (MLPA) detection of the *BRCA1* exon 13 duplication in N419. Electropherograms of a normal control (blue) and (in red) patient N419 are superimposed. These results were obtained twice the sample was analyzed with the two different MLPA kits (P002 and P087). The area between the bars indicates increased intensity for the exon 13 probe.

duplications and triplications in various orientations also failed to amplify any PCR product.

Discussion

Large exon rearrangements, first described in *BRCA1* (13) and later in *BRCA2* (14), have been investigated for nearly a decade. Before their identification, over a 100-point deletions, and small insertions and deletions had already been described in both of these genes (15). The discrepancy in numbers can be partly explained by the relative difficulty in identifying genetic rearrangements that cannot be detected using conventional PCR-based methodologies in heterozygous cases, because of amplification of the wild-type allele. Unless other specific techniques are used, this would therefore result in a false-negative mutation test. The clinical significance of such missed mutations in genetic screening services would then be proportional to the prevalence of genetic rearrangements in the population studied. To date, over 75 and 14 such rearrangements have been described in *BRCA1* and *BRCA2*, respectively, with a prevalence generally ranging from about 5% to 15% of all mutations detected for *BRCA1* (Table 4). With the breakpoint of most of the reported rearrangements occurring within the *Alu* sequences, the contrast in rearrangement incidence between both *BRCA* genes is probably because of the difference in the number of *Alu* sequences present in the intronic sequences of both genes (16). In populations with a reported founder effect, the prevalence of genomic rearrangements has been shown to account for up to 40% of all *BRCA1* mutations (4, 17). As all prevalence studies reported to date have consisted of primarily European descendents (Table 4), the aim of our study was to assess the prevalence of genomic rearrangements in a study population consisting of Asian familial and early-onset breast and ovarian cancers.

Our inclusion criteria, PCR-based screening methods and mutation prevalence estimates in Chinese, Indian and Malay subjects have been described earlier. Essentially, in a study population of 195 consisting of probands with an *a priori* risk of 10% of harboring mutations in either *BRCA1* or *BRCA2*, 21 deleterious mutation carriers were found. Of the remaining non-carriers, 100 had consented to further genetic analysis for large exon rearrangements.

Initial genomic rearrangement prevalence studies used Southern blot analysis, a method of limited usefulness in routine screening because of low throughput and the need for large amounts of DNA (18). However, in recent years, the anticipation of frequently recurring genomic rearrangements in high-risk individuals of European descent has necessitated the deployment of newer and more rapid screening techniques such as real-time PCR, bar code analysis on combed DNA, quantitative multiplex PCR of short fluorescent fragments and in a recent large population-based study, mutation-specific multiplex PCR assay (2, 19, 20, 21). Given the preliminary nature of our study, we deployed MLPA, a relatively low-cost robust dosage analysis assay with proven efficacy and high throughput in the detection of genomic rearrangements (22).

In this study, we identified three novel *BRCA* rearrangements in 3% of our 100 mutation-negative Singapore patients and characterized the precise breakpoints for two of these genomic rearrangements. Of the *BRCA1* and *BRCA2* pathogenic mutations detected in our studies to date, these rearrangements constitute 2/19 and 1/2 of the *BRCA1* and *BRCA2* pathogenic mutations, respectively. The first, a *BRCA1* exon 13–15 deletion encompassing 10,410 bp was found in patient N588, a 55-year-old Indian ovarian cancer woman who belonged to HBOC family. This deletion appears to have been mediated by *Alu*SX repeats oriented in the same direction as *BRCA1* transcript. Characterization of the deletion breakpoints indicates that a recombination event between the two highly homologous *Alu*Sx repeats located within introns 12 and 15 is likely responsible for the occurrence of the deletion, and the site of the crossover event lies within a 22-bp sequence of perfect identity. This deletion of exons 13–15 is predicted to result in a truncated protein of 1437 amino acids with the loss of the carboxy terminus as well as several SQ (clusters of serine and threonine) sequences.

Further to the deletion, two *BRCA* duplications in two other patients were also detected. The first, a large duplication involving *BRCA2* was identified in a Chinese woman N557, presenting with early-onset breast cancer (38 years). Although this tandem duplication of exon 4–11 (partial) was in frame, there would be a predicted effect on the protein secondary structure and consequently, function. Sequence alignment of the sequences surrounding the breakpoints did not show any homology to any *Alu* sequences or recombination-association motifs that may have been involved in the rearrangement process suggesting a non-homologous recombination. A very short homologous sequence of 4 bp (AAAC) was identified at the 3′

Table 4. Summary of genomic rearrangements identified in *BRCA* genes including this study[a]

	BRCA1 exon	Country of study/ family ancestry	Rearrangement	Mutation size	Reference
1	Promoter	America	Deletion	13.8 kbp	(27)
2	Int2ψ*BRCA1*–Int2*BRCA1*	France	Deletion	36.9 kbp	(28)
		England			(29)
		Italy			(17)
		Germany			(30)
3	Exon 1–2	Italy	Deletion	ND	(17, 31)
		Germany		ND	(32)
		America/European		ND	(18)
		America		ND	(33)
4	Exon 1–2	American/German	Deletion	36.934 kbp	(3)
5	Exon 1–3	American/Norwegian	Deletion	23.395 kbp	(3)
6	Exon 1a–12	America/Irish	Deletion	88.55 kbp	(3)
7	Exon 1a–15	America/German	Deletion	>169.639 kbp	(3)
8	Exon 1–17	England	Deletion	ND	(34)
9	Exon 1–22	France	Deletion	161 kbp	(20, 35)
10	Exon 1–23	America/Irish	Deletion	86.853 kbp	(3)
11	Exon 1–24	Spain	Deletion	ND	(36)
12	Exon 3	Australia/NZ	Deletion	ND	(37)
13	Exon 3	America	Inverted duplication and deletion	1.029 kbp	(38)
14	Exon 3	America/English	Deletion, insertion	1.042 kbp	(3)
15	Exon 3–5	England	Duplication	ND	(34)
16	Exon 3–7	England	Deletion	ND	(39)
17	Exon 3–8	France	Duplication	17.2 kbp	(20, 40)
18	Exon 3–16	Denmark	Deletion	ND	(41)
19	Exon 5	Australia/NZ	Deletion	ND	(37)
20	Exon 5	Germany	Deletion	0.244 kbp	(30)
21	Exon 5	Italy	Deletion	ND	(31)
22	Exon 5–7	Germany	Deletion	5 kbp	(30)
23	Exon 5–7	Italy	Deletion	ND	(31)
24	Exon 5–8	Italy	Deletion	ND	(31)
25	Exon 8	the Netherlands	Deletion	1.458 kbp	(22)
26	Exon 8–9	America/African	Deletion	3.936 kbp	(3)
27	Exon 8–9	America/Northern European	Deletion	7.1 kbp	(18, 42)
28	Exon 8–13	France	Deletion	23.8 kbp	(20, 33)
		England		ND	(34)
		Spain		23.76 kbp	(36)
29	Exon 8–24	America/German	Deletion	65.520 kbp	(3)
30	Exon 9–12	England	Deletion	ND	(34)
31	Exon 9–19	Italy	Deletion	36.381 kbp	(17, 31)
32	Exon 11–15	Portugal	Deletion	ND ~23 kbp	(43)
		Spain			(36)
33	Exon 13	America	Duplication	6 kbp	(2, 3, 12, 18, 44)
		Australia			(12)
		Belgium			(12)
		Canada			(12)
		England			(12, 45, 46)
		Germany			(30, 47, 48)
		the Netherlands			(22)
		Sweden			(49)
34	Exon 13	the Netherlands	Deletion	3.835 kbp	(50)
		America			(2)
35	Exon 13	Singapore/Chinese	Duplication	8.46 kbp	(26)
36	Exon 13	Singapore/Chinese	Duplication	ND	This study[a]
37	Exon 13–15	America/French–German	Deletion	11.604 kbp	(51)
		Denmark			(41)
38	Exon 13–15	Singapore/Indian	Deletion	10.411 kbp	This study[a]
39	Exon 13–16	the Netherlands	Deletion	14 kbp	(50)
40	Exon 14	Spain	Deletion	4.95 kbp	(36)
41	Exon 14–19	Italy	Deletion	19.886 kbp	(52)
42	Exon 14–20	America	Deletion	26 kbp	(2, 3, 53)
43	Exon 15	France	Deletion	3 kbp	(33)

Table 4. Continued

	BRCA1 exon	Country of study/ family ancestry	Rearrangement	Mutation size	Reference
44	Exon 15–16	France	Deletion	6 kbp	(20)
45	Exon 16–20	Italy	Deletion	8.342 kbp	(31)
46	Exon 17	France	Deletion	1 kbp	(13)
		America/German			(3)
47	Exon 17	America/German	Deletion	2.680 kbp	(3)
48	Exon 17	Italy	Deletion	3 kbp	(31, 54)
		America			(3, 55)
		Germany			(30)
49	Exon 17	Germany	Deletion	5.105 kbp	(32)
50	Exon 17–19	American/European	Deletion	ND	(18)
51	Exon 17–19	the Netherlands	Triplication	8.352 kbp	(22)
52	Exon 17–20	America/Anglo-Saxon, Ash. Jewish descent	Deletion, insertion	11.5 kbp	(56)
53	Exon 17–23	the Netherlands	Deletion	22 kbp	(57)
54	Exon 18	France	Deletion and duplication	del 6 bp ins 12 bp	(58)
55	Exon 18–19	Italy	Deletion	4.826 kbp	(17)
56	Exon 18–19	England	Duplication	ND	(39)
57	Exon 18–19	America/African	Duplication	5.923 kbp	(3)
58	Exon 18–20	France	Duplication	8.6 kbp	(20)
59	Exon 19–20	Spain	Duplication	ND	(36)
60	Exon 20	America/French	Deletion	3.985 kbp	(3)
61	Exon 20	Canada	Deletion	4.0 kbp	(59)
62	Exon 20	Italy	Deletion	4.328 kbp	(17)
63	Exon 20	Greece	Deletion	3.2 kbp	(60)
64	Exon 20	England	Deletion	ND	(34)
65	Exon 20	Spain	Duplication	ND	(36)
66	Exon 20	Italy	Duplication	8.706 kbp	(31)
67	Exon 20–22	America/English	Deletion	11.357 kbp	(3)
68	Exon 20–22	the Netherlands	Deletion	11.395 kbp	(22)
69	Exon 21–22	America/Irish	Deletion	3.432 kbp	(56)
70	Exon 21–22	Spain	Amplication	ND	(36)
71	Exon 21–23	Dutch	Duplication	7.654 kbp	(22)
72	Exon 21–23	Australia/NZ	Deletion	ND	(37)
73	Exon 21–24	America/Irish	Deletion	19.245 kbp	(3)
74	Exon 21–24	England	Deletion	ND	(34)
75	Exon 22	the Netherlands	Deletion	510 bp	(50)
		Germany			(47)
		America			(2, 3)
76	Exon 23–24	Spain	Deletion	ND	(36)
77	Exon 23–24	Italy	Deletion	ND	(31)
	BRCA2 exon				
1	Exon 1–2	France[c]	Duplication	ND	(61)
2	Exon 1–2	England	Deletion	ND	(34)
3	Exon 1–2	Australia/NZ	Deletion	ND	(37)
4	Exon 1–2	America/English	Deletion, insertion	2.340 kbp	(3)
5	Exon 3	Sweden	Deletion	5.068 kbp	(14)
6	Exon 4–11a	Singapore/Chinese	Duplication	16.189 kbp	This study[a]
7	Exon 8–11a	Italy	Deletion	4.322 kbp	(62)
8	Exon 12–13s	Israel	Deletion	6.2 kbp	(63)
9	Exon 12–13	France[c]	Deletion	7.947 kbp	(61)
10	Exon 14–16	Australia/NZ	Deletion	ND	(37)
11	Exon 17–18	Italy	Deletion	10.838 kbp	(62)
12	Exon 19–20	America/Dutch, German	Duplication	9.700 kbp	(3)
13	Exon 20	Italy	Deletion	4.953 kbp	(62)
14	Exon 21	America/Hungarian	Deletion	1.518 kbp	(3)
15	Whole gene	France[c]	Deletion	298 kbp	(61)

[a]Indicates rearrangement identified in this study.
[b]TBEDS 2000 indicates *BRCA1* exon 13 Duplication Screening Group.
[c]Male breast cancer case.
ND, not determined.

breakpoint junction; however, its significance in the recombination event is unknown. While we did not find any potentially recombining *Alu* sequences or recombination-associated motifs within the DNA sequences flanking the breakpoints of the *BRCA2* exon 4–11 duplication, it was noted that the breakpoint of the duplication in intron 3 occurred within a region of 425 bp, which had homology to an L1 repeat. Besides *Alu* repeats, the other major mobile element in the human genome is the L1 element (23). These 6 kbp repeats are longer than the 300 bp *Alu* elements and consist of two open reading frames, of which one codes for a protein that has domains for reverse transcriptase as well as for an endonuclease that nicks the genome at the site of insertion (24, 25). Whether this L1 element contributes to the *BRCA2* exon 4–11 (partial) duplication event by generating an unequal crossing-over still remains to be elucidated.

The second duplication detected was an exon 13 duplication in *BRCA1* of a Chinese patient N419, with young-onset breast cancer and no family history of any cancer. When analyzed with the specific exon 13 primers, this duplication was found to be different from the founder mutation, ins6kbEx13, reported in Western populations with Northern English ancestry and the exon 13 duplication recently reported in a Singapore Chinese patient (26). The former and latter being duplications of exon 13 involving *Alu* repeats orientated in the same direction and in reverse sense to *BRCA1* transcription, respectively. Despite repeated analysis with different permutations of primers in various orientations, the exact breakpoints of this rearrangement could not be determined because of insufficient material and further work is in progress to characterize it.

In conclusion, our present data suggest that among Asian women with high-risk breast or ovarian cancerin our study, patients with rearrangements account for 3% of those previously tested negative for *BRCA* mutations. Given the increasing number of rearrangements being reported and their contribution to the *BRCA* mutation spectrum, we propose that rearrangement screening should also be included in both *BRCA1* and *BRCA2* mutational analysis in high-risk Asian populations.

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