

***BRCA1* and *BRCA2* germline mutation analysis in the Indonesian population**

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Abstract Specific mutations in *BRCA1* and *BRCA2* genes have been identified in specific populations and ethnic groups. However, little is known about the contribution of *BRCA1* and *BRCA2* mutations to breast cancers in the Indonesian population. One hundred-twenty moderate to high risk breast cancer patients were tested using PCR-DGGE, and any aberrant band was sequenced. Multiplex ligation-dependent probe amplification (MLPA) was performed on all samples to detect large deletions in the two genes. Twenty-three different mutations were detected in 30 individuals, ten were deleterious mutations and 20 were “unclassified variants” with uncertain clinical consequences. Three of seven (c.2784_2875insT, p.Leu1415X and del exon

13–15) and two of four (p.Glu2183X and p.Gln2894X) deleterious mutations that were found in *BRCA1* and *BRCA2* respectively, are novel. Several novel, pathogenic *BRCA1* and *BRCA2* germline mutations are found in early onset Indonesian breast cancer patients, these may therefore be specific for the Indonesian population.

Keywords *BRCA1* · *BRCA2* · Hereditary breast cancer · Mutation analysis

Introduction

Breast cancer is the most common cancer in women. In 5% to 10% of breast cancer cases, the disease results from a hereditary predisposition [1, 2], which can to a large extent be attributed to mutations in either of two tumour suppressor genes, *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185) [3–5]. These genes are involved in DNA repair as well as transcriptional regulation [6, 7]. Women carrying pathogenic germline mutations in either of these genes tend to develop breast cancer at an early age [8, 9].

The *BRCA1* and *BRCA2* genes encode large proteins of 1,863 and 3,418 amino acids, respectively. Over 300 distinct mutations in *BRCA1* and *BRCA2* have been described [10, 11]. These mutations are widely scattered across both genes and most affect the structure and function of the gene. Nevertheless, a significant proportion (34% of *BRCA1* and 38% of *BRCA2* mutations) (http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic) are missense mutations that alter one amino acid, but do not truncate the protein and are rare sequence variants of unknown functional

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consequence. Moreover, a number of base substitutions do not alter the amino acid sequence or result in amino acid changes not associated with disease (polymorphisms) [12]. Hence the biggest challenge in interpreting the mutation analysis of *BRCA1* and *BRCA2* genes is to distinguish between harmless polymorphisms and deleterious mutations associated with increased cancer risk.

In addition, mutations specific for certain populations and ethnic groups have been identified in both genes. For example, specific *BRCA1* and *BRCA2* mutations were reported for Ashkenazi Jews [13]. Other common *BRCA1* mutations were especially found in Italian, Canadian, Belgian or Dutch breast cancer families [14–16]. In Indonesia, the contribution of the *BRCA1/BRCA2* mutations to the population incidence of early-onset breast cancer is largely unknown. In one pilot study, however, a new *BRCA2* mutation was identified [17] indicating that it was worthwhile to more extensively study the Indonesian population, which was the aim of this study. The accumulating knowledge about the prevalence and nature of *BRCA1* and *BRCA2* mutations in specific populations may facilitate the interpretation of genetic analysis with regard to breast cancer risk of individual patients.

Materials and methods

Patients

A total of 120 unrelated breast cancer patients and 16 of their family members from three Indonesian cities (Jakarta and Jogjakarta on the Java island, Denpasar on the Bali island) were analyzed. Breast cancer patients at moderate to high-risk of a hereditary predisposition were selected according to the following criteria: (A) Breast cancer before the age of 41 ($n = 102$); (B) Two cases of breast cancer in the same family before the age of 60 ($n = 9$); (C) Three or more cases of breast cancer in the same family ($n = 2$); (D) Bilateral breast cancer ($n = 7$). Subjects were asked to fill out questionnaires to evaluate their personal and family histories, and blood specimens were collected for determination of BRCA mutations. Informed consent was obtained from all the subjects in this study.

DNA extraction and PCR amplification

Genomic DNA was isolated by the saturated salt extraction procedure as described in [18]. All 22 coding exons of *BRCA1* and 26 coding exons of *BRCA2* were amplified using primer sequences developed by the

University of Groningen, The Netherlands [19]. Primers for DGGE were obtained from Ingeny (Goes, The Netherlands). Genomic DNA was amplified using 100–200 ng of template DNA, 10 pmol of the mixture of 40-mer primers, 30 mM of MgCl₂, 3 mM dNTPs (Invitrogen) and 0.7 unit of Platinum Taq (Invitrogen) in 9 μ l PCR reactions. In order to speed up the test, the PCR reaction was placed in 384 well plates using a pipetting robot (TECAN Miniprep 75). PCR conditions were performed as previously described [17].

Denaturing Gradient Gel Electrophoresis and DNA sequencing

A 4–6 μ l aliquot of each PCR product with relatively large melting temperature differences were pooled as previously described [17] with some modifications for optimal results. The fragment pool was designed based on melting profiles and sequence. Electrophoresis was performed in 0.5 TAE buffer at 58°C, 120 V for 16 h for *BRCA1* gene, and 55°C, 100 V for 18 h for *BRCA2*. Gels were stained with ethidium bromide and photographed under a UV transilluminator. The aberrantly migrating samples were re-amplified using sequencing primers and sequencing was performed using Big Dye Cycle-sequencing kit according to the manufacturer's instructions. The reaction products were analyzed using an ABI 3100 DNA Sequencer (Applied Biosystems, Torrance, CA, USA) and sequence files were edited using the Bio Edit program. The classification of gene alterations was performed in accordance with the entries in the Breast Cancer Information Core (BIC, Bethesda, MD).

Multiplex ligation-dependent probe amplification (MLPA)

The principle of the MLPA technique has been described elsewhere [20]. The MLPA test for *BRCA1* (P02) and *BRCA2* (P45) mutations were obtained from MRC-Holland, Amsterdam, The Netherlands. The fragments were analyzed on an ABI model 310 capillary sequencer (Applied Biosystems, Torrance, CA, USA) using Genescan-TAMRA 500 size standards (Applied Biosystems). Fragment analysis was performed with Genescan software.

Results and discussions

We identified 120 incident Indonesian breast cancer cases diagnosed before the age of 41 years, or having family history of breast cancer, or harboring bilateral

breast cancer during September 1999–April 2005 (Jogjakarta) and during July 2004–April 2005 (Jakarta and Denpasar). In addition, 16 of their family members were analyzed.

The entire coding regions and exon-intron junctions of *BRCA1* and *BRCA2* were screened in these 136 persons of breast cancer patients and their families using PCR-DGGE (Fig. 1) followed by sequencing (Fig. 2) for samples with aberrant migrating bands. To optimize the screening, MLPA, a relatively new technique, was also performed in all samples (Fig. 3). Here, we report on 116/120 women (96.7%) for whom *BRCA1/2* analysis were completed. The remaining four patients (all from group A) had to be excluded due to the small amount of extracted DNA that did not allow complete screening of the *BRCA1* and *BRCA2* genes.

BRCA1 and BRCA2 pathogenic mutations

The analysis of 116 unrelated breast cancer patients with breast cancer revealed that nine patients (7.8%) carried pathogenic germline mutations especially the early onset patients: 3 within *BRCA1* (2.6%) and 6 within *BRCA2* (5.2%) which is comparable to previous studies [21]. We only found *BRCA1* and *BRCA2* mutations in groups A (“early onset”, $n = 7$ out of 98, 7.1%) and B (two cases of breast cancer in the same

family before the age of 60, $n = 2$ out of 9 (22.2%)) (Table 1). There were twice as many *BRCA2* mutations as *BRCA1* mutations. Although the absolute numbers are low and no firm conclusions can therefore be drawn, this is comparable to other Asian regions [22–24] but seems to discern the Indonesian population from non-Asian ethnic groups where the reverse trend is seen.

Seven pathogenic mutations were found in nine probands: three in *BRCA1* (c.2784_2785insT, pL1415X (c.4361_4362insT), del exon 13–15) and four in *BRCA2* (c.3040_3043delGCAA, p.Glu2183X (c.6775G>T), p.Leu824X (c.2699_2704delTAAATG), p.Gln2894X (c.9008C>T)). All these mutations were classified as pathogenic as they are predicted to result in protein truncation. The three pathogenic mutations found in *BRCA1* were not previously reported in the BIC database as well as two novel nonsense mutations (p.Glu2183X and p.Gln2894X) identified in *BRCA2*. The p.Glu2183X mutation was found in 2 related patients that had breast cancer above the age of 60.

One of seven pathogenic mutations found in *BRCA1* and *BRCA2* showed a significant clinical impact on the patient (Table 2). Patient AE with a one nucleotide insertion (Thymine) between nucleotide 2784 and 2785 (c.2784_2785insT) in exon 11 of *BRCA1* suffered from bilateral breast cancer at a relatively early age (25 years). The insertion leads to frameshift and creates a premature stop codon in exon 11. The mutation takes place in the sequence within *BRCA1* encoding for aminoacids 758–1064 which interact with RAD51 protein that is required for homologous recombination (HR) repair of double strand breaks (DSBs) [25], which is one of the most important functions of the *BRCA1* protein. This patient presented in a late stage (stage III for both breasts) and only survived for 9 weeks after treatment. Her mother did not carry this mutation. Although her father may be carrier, the mutation is probably de novo as there was no family history of breast or other cancers.

The second pathogenic mutation with a significant clinical manifestation was a cytosine for thymine substitution on nucleotide 9008 of *BRCA2* leading to a premature stop codon in position 2894, c9008C>T (p.Gln2894X). Patient W presented at age 37 in a late stage and survived for only 107 weeks after initial treatment. She had no family history of breast or other cancers. This mutation lies within exon 21 of *BRCA2* which is the proposed site for interaction with the DSS1 protein that seems to have a fundamental role in enabling the *BRCA2*-RAD51 complex to associate with sites of DNA damage [26].

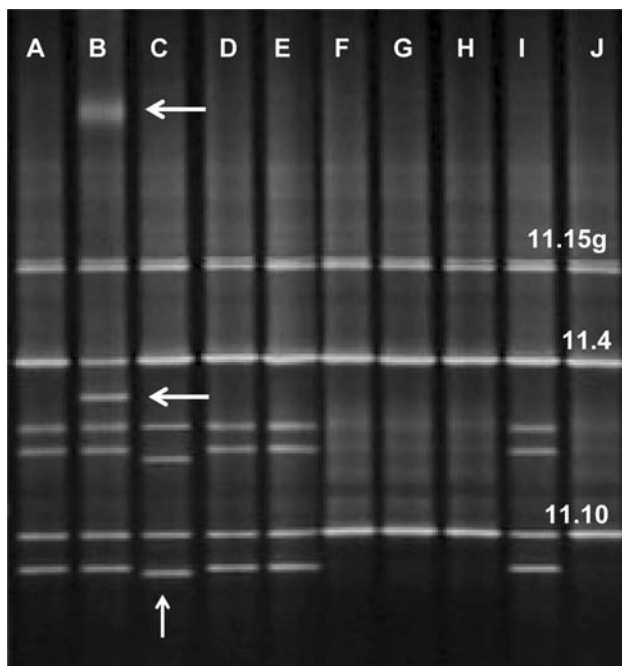


Fig. 1 DGGE analysis of fragments 11.15 g, 11.4 and 11.10 of the *BRCA2* gene in ten unrelated breast cancer patients. The arrows show altered band mobility compare to other patients

Fig. 2 Sequence electropherogram of a normal individual showing (A) wild-type *BRCA2* exon 11 sequence and (B) of breast cancer patient (B-3-5) showing c.2699_2704delTAAATG mutation

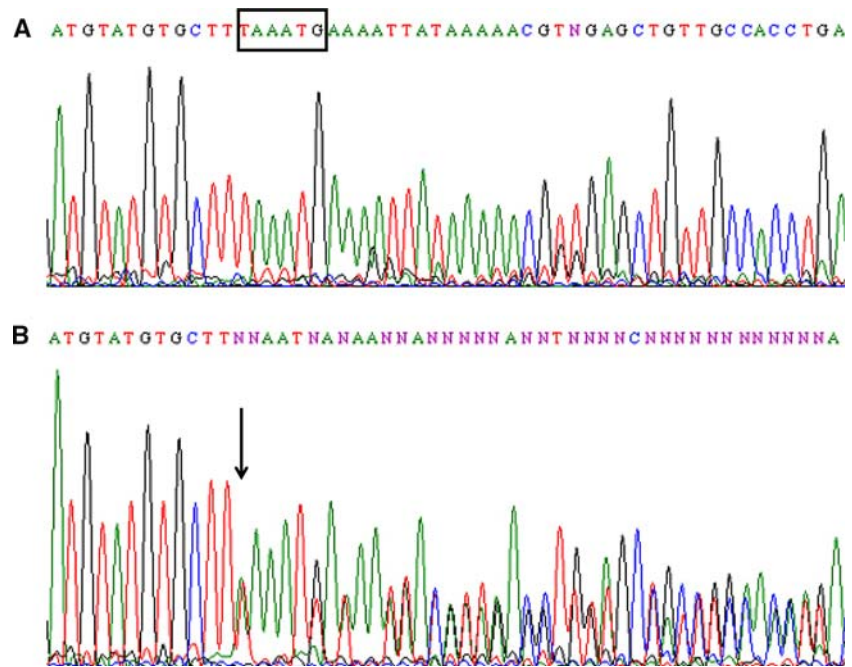
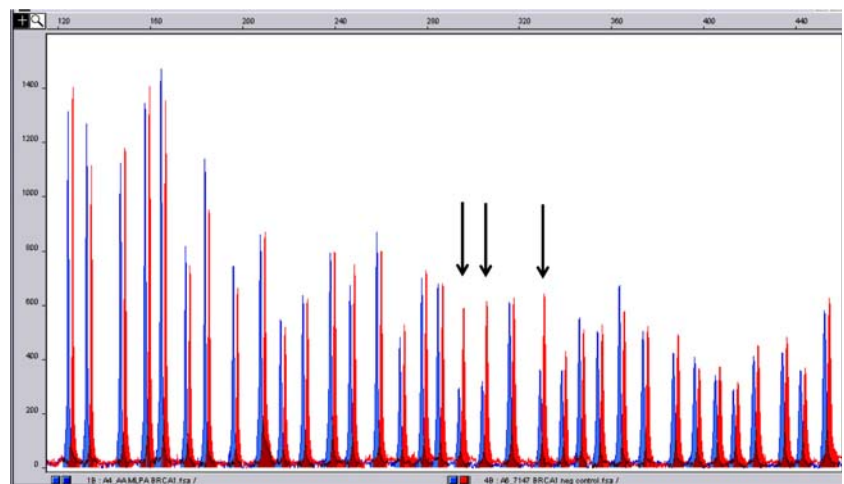


Fig. 3 MLPA analysis of *BRCA1* gene of patient sample (blue) compare to the normal control (red). X and Y axis represent peak size and peak height respectively. There are reduced peaks in the patient sample compared to the normal control in exons 13, 14 and 15 (arrows) indicating deletions



The c.2699_2704delTAAATG (p.Leu824X) in *BRCA2* that has been reported previously by us in the Indonesian population [17], was found in one other patient in the present study (Table 1). This mutation lies in exon 11 *BRCA2*, within the BRC repeats domain. The truncating mutation causes loss of three quarters of the protein leading to lack of interaction with the RAD51 protein. Different from *BRCA1*, the repair of DSBs by HR is the most important function of the *BRCA2* protein [27]. Patient B-III-5 was diagnosed with early stage breast cancer at age 30 with no family history of breast or other cancers. Her sister carried the same mutation, but with no present clinical manifestation as yet. Patient AZ who was diagnosed at

40 years of age, presented in late stage, only survived 46 weeks after initial treatment. This patient also harbored a mutation in exon 16 of *BRCA1*, a G to A substitution in nucleotide 5075 (c.5075G>A), which leads to amino acid change from Methionine to Isoleucine, (p.Met16521Ile) which has to date been reported 35 times in BIC as a UV mutation. As the c.2699_2704delTAAATG mutation was found in two unrelated patients, this mutation could be a good candidate as a founder mutation.

None of the families with more than 3 cases of breast cancer and families with bilateral breast cancer showed pathogenic mutations in the *BRCA1* and *BRCA2* genes. Family U had four first-degree relatives

Table 1 *BRCA1* or *BRCA2* germline mutations in Indonesian women with early onset breast cancer

Patient	Age ^a	gene	Exon	Mutation ^b	mutation type	Pathogenic mutation	BIC ^c	
AE	25	<i>BRCA1</i>	11	c.2784_2785insT	frameshift	+	no	
B10	31	<i>BRCA1</i>	13	p.Leu1415X	nonsense	+	no	
AA	40	<i>BRCA1</i>	13–15	- ^d	large rearrangement	+	no	
AB	34	<i>BRCA2</i>	11	c.3040_3043del4	frameshift	+	1	
B5	66	<i>BRCA2</i>	11	p.Glu2183X	nonsense	+	no	
B6	65	<i>BRCA2</i>	11	p.Glu2183X	nonsense	+	no	
B-III-5	30	<i>BRCA2</i>	11	p.Leu824X	nonsense	+	no	
AZ	40	<i>BRCA2</i>	11	p.Leu824X	nonsense	+	no	
W-II	37	<i>BRCA2</i>	21	p.Gln2894X	nonsense	+	no	
Q-II	40	<i>BRCA1</i>	2	c.101–10T>C	IVS	±	6	
P-III-19	19	<i>BRCA1</i>	9	p.Val191Ile	Missense	±	6	
J22	32	<i>BRCA1</i>	11	p.Leu1209Val	Missense	?	no	
AZ	40	<i>BRCA1</i>	16	p.Met1652Ile	Missense	±	35	
B1	24	<i>BRCA1</i>	20	c.5313–31A>G	IVS	?	no	
B7	31	<i>BRCA1</i>	24	p.Arg1835Gln	Missense	?	no	
216	33	<i>BRCA1</i>	24	p.Thr1852Ile	Missense	?	no	
P-III-19	19	<i>BRCA2</i>	5	p.Gln147Arg	Missense	±	6	
B3	24	<i>BRCA2</i>	10	p.Gln609Glu	Missense	?	no	
C-II-7	39	<i>BRCA2</i>	11	p.Met1149Val	Missense	±	5	
AO	28	<i>BRCA2</i>	11	p.Met1149Val	Missense	±	5	
AQ	44	<i>BRCA2</i>	11	p.Met1149Val	Missense	±	5	
BH	38	<i>BRCA2</i>	11	p.Met1149Val	Missense	±	5	
^a Age at time of diagnosis	172	36	<i>BRCA2</i>	11	p.Gln699Leu	Missense	?	no
^b Gen Bank Accession number, <i>BRCA1</i> : U14680, <i>BRCA2</i> : U43746	J32	29	<i>BRCA2</i>	11	p.Arg2108Cys	Missense	±	16
^c number of times reported in BIC	J6	33	<i>BRCA2</i>	11	p.Val950Ile	Missense	?	no
^d not determined, detected by MLPA	206	37	<i>BRCA2</i>	25	c.9485–16T>C	IVS	±	4
	BC	35	<i>BRCA2</i>	27	p.Ile3412Val	Missense	±	109
	166	33	<i>BRCA2</i>	27	p.Ile3412Val	Missense	±	109
	J24	35	<i>BRCA2</i>	27	p.Ile3412Val	Missense	±	109
	206	37	<i>BRCA2</i>	27	p.Lys3326X	nonsense	±	289

Table 2 Clinicopathological features of Indonesian breast cancer patients with deleterious *BRCA1* or *BRCA2* germline mutations

Patient	Age ^a	Gene with germline mutation	Mutation ^b	stage	Diagnosis	Menopausal status	family history of cancer	Survival status
AE	25	<i>BRCA1</i>	c.2784_2785insT	IIIB/IIIA	IDC, bilateral	pre	No	DOD 9 w
B10	31	<i>BRCA1</i>	p.Leu1415X	I	IDC	pre	No	DOD 57 w
AA	40	<i>BRCA1</i>	- ^c	IIIB	IDC N+	pre	No	AWD
AB	34	<i>BRCA2</i>	c.3040_3043del4	IIIB	IDC N+	pre	Sister, Int	DOD 17 w
B5	63	<i>BRCA2</i>	p.Glu2183X	IV	Tubular	post	Sister, Br	AWD
B6	65	<i>BRCA2</i>	p.Glu2183X	III	IDC	post	Brother, Br	AWD
B-III-5	30	<i>BRCA2</i>	p.Leu824X	I	IDC	pre	No	AWD
AZ	40	<i>BRCA2</i>	p.Leu824X	IV	IDC	pre	Sister, Cv	DOD 46 w
W-II	37	<i>BRCA2</i>	p.Gln2894X	IIIA	IDC	pre	No	DOD 107 w

^a Age at time of diagnosis^b Gen Bank Accession number, *BRCA1*: U14680, *BRCA2*: U43746^c not determined, detected by MLPA

IDC: invasive ductal carcinoma; DOD: dead of disease; bil: bilateral breast cancer; N+: with metastatic to lymph node; Int: intestine cancer; Br: breast cancer; Cv: cervical cancer

that were affected by breast cancer. Two of four members had bilateral breast cancer. In spite of this high familial breast cancer incidence, no *BRCA1/2* mutations were found.

BRCA1 and *BRCA2* unclassified variants

Sixteen (7 *BRCA1* and 9 *BRCA2*) rare mutations of so far unknown significance (“unclassified variants”,

UVs) were detected in 18 patients: 13 missense changes and 3 intronic variants. Of these 16 UVs, 7 were novel, whereas the other UVs have been previously reported in the BIC database (Table 1). From the 18 patients which carried UV mutations, two patients were detected in families from group D; one patient in a group B family and the other fifteen patients in families from group A.

Seven UV were found in the *BRCA1* gene, two mutations occurring in the intronic region between exons 1 and 2 (c.101–10T>C) and between exons 19 and 20 (c.5313–31A>G), and five missense mutations identified: p.Val191Ile (c.690G>A), p.Leu1209Val (c.3744T>G), p.Met1652Ile (c.5075G>A), p.Arg1835Gln (c.5623G>A) and p.Thr1852Ile (c.5674C>T).

Four out of seven *BRCA1* missense mutations; p.Leu1209Val (c.3744T>G), c.5313–31A>G, p.Arg1835Gln (c.5623G>A) and p.Thr1852Ile (c.5674C>T) were have not been described previously in the BIC. The p.Leu1209Val may not be a significant change as both Leucine and Valine belong to the same group of non polar amino acids. However, p.Arg1835Gln is possibly an important alteration since a positively charged Arginine is replaced by an uncharged Glutamine, which may have an effect on the structure and/or function of the protein. Another potentially important alteration concerns p.Thr1852Ile, where the hydrophilic amino acid Threonine is replaced by a hydrophobic Isoleucine. The sites of mutation of both p.Arg1835Gln and p.Thr1852Ile also have to be considered as they lie within the site for the activation domain of the *BRCA1* protein [28]. The intronic UV c.5313–31A>G also deserves further investigation as it may theoretically have an effect on splicing. However, according to splice site finder (<http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html>), the splicing sites in the wild type and mutant alleles are similar, so therefore we can suggest that the c.5313–31A>G has no effect on splicing.

Nine different UVs of the *BRCA2* gene were found in fourteen patients (Table 1), and three of them were novel; p.Gln609Glu (c.2053C>G), p.Gln699Leu (2324A>T) and p.Val950Ile (3076G>A). One truncating mutation near the C-terminal end of *BRCA2*, p.Lys3326X (c.10204A>T) is probably not pathogenic. Since the truncating mutation is at the very end of the protein, it is possible that protein functions are not affected. Most of the few entries in databanks describing nonsense mutations near the C terminus of *BRCA2* between codon 3308 and 3408 are described as UVs. Thus, the effect of this truncating mutation on cancer predisposition remains unclear.

The p.Val950Ile may not be a significant change as both Valine and Isoleucine belong to the same group of non polar, hydrophobic amino acids. However, p.Gln609Glu and p.Gln699Leu are potentially important alterations as for p.Gln609Glu, a non acidic, polar, hydrophilic Glutamine is replaced by a negatively charged Glutamic acid, whereas for p.Gln699Leu, an uncharged hydrophilic Glutamine is replaced by a hydrophobic Leucine. As it takes place within the BRC repeats of the *BRCA2* protein, the p.Gln699Leu alteration might affect protein structure and function.

To know more about the importance of amino acid substitutions for protein function, we compared the amino acid sequence of interest in seven other species, i.e. *Mus musculus*, *Rattus rattus*, *Bos taurus*, *Gallus gallus*, *Canis familiaris*, *Macaca mullata* and *Monodelphis domestica*. The missense mutation p.Leu1209Val lies in the conserved region of exon 11 of the *BRCA1* gene as the sequence is maintained in seven other species, whereas p.Arg1835Gln and p.Thr1852Ile are only conserved in four and three other species (comparison of p.Arg1835Gln and p.Thr1852Ile with *Bos taurus* sequence is not possible because the *BRCA1* gene is shorter). Therefore, even though the Leucine to Valine changes may not give any effect on amino acid charge, its conservation in evolution is suggestive of a functional role. Interestingly, p.Gln609Glu and p.Gln699Leu of *BRCA2* that result in a quite dramatic amino acid substitution that might lead to protein structure changes, are only conserved in four and five species respectively. As for the p.Val950Ile, the conservation in evolution is quite low. Although p.Gln609Glu is less conserved, we still believe that Glutamine to Glutamic acid substitution may have an effect on protein conformation as two adjacent acidic amino acids will be formed as the result of the substitution.

Glycosylation moiety of an amino acid also plays a role in protein function. Amino acid substitutions involving Serine, Threonine and Asparagine, should also be checked for their O-GlcNac potential and threshold. Here we have a Threonine to Isoleucine substitution (p.Thr1852Ile) that after checking with YinOYang (<http://www.cbs.dtu.dk/services/YinOYang>) showed no significant threshold changes between the wildtype and the mutant allele.

The possible effect of amino acid changes in proteins can also be assessed using similarity scores (based on Grantham table [29]), in which a value above 100 for an amino acid substitution indicates a higher chance of impact on protein function. Among seven novel UVs in

Table 3 The amino acid properties of novel unclassified mutations in *BRCA1* and *BRCA2* within an Indonesian breast cancer population

Gene	Amino acid change	Change of charge	Change of amino acid group	Similarity score ^a	# species with conserved sequence
BRCA1	Leu to Val	None	No	32	7 ^{a,b,c,d,e,f,g}
BRCA1	Arg to Gln	Pos to no charge	Yes	43	4 ^{a,c,f,g}
BRCA1	Thr to Ile	polar to non polar	Yes	89	3 ^{a,c,g}
BRCA2	Gln to Glu	No charge to neg	Yes	29	4 ^{a,b,c,g}
BRCA2	Gln to Leu	Polar to non polar	Yes	113	5 ^{a,b,d,e,f}
BRCA2	Val to Ile	None	No	29	2 ^{f,g}

^a based on Grantham table [Grantham et al. [29], a score above 100 indicates significance changes

a = *Macaca mullata*, b = *Bos taurus*, c = *Canis familiaris*, d = *Rattus rattus*, e = *Mus musculus*, f = *Gallus gallus*, h = *Monodelphis domestica*

the *BRCA1* and *BRCA2* genes found in the present study, only p.Gln699Leu in *BRCA2* has a similarity score above 100, whereas p.Gln609Glu and p.Val905Ile in *BRCA2* have the lowest score (Table 3).

Overall, we propose that among the seven novel UVs, there are three mutations that are possibly pathogenic: p.Leu1209Val for its location in a conserved region, and p.Gln609Glu and the p.Gln699Leu because of two adjacent acidic amino acid being formed and a high similarity score, respectively.

When comparing the three different Indonesian regions, the percentages of breast cancer patients with pathogenic *BRCA1/2* mutations was significantly higher in Denpasar (Bali island) than in Jogjakarta and Jakarta (Java island) ((25% (3/12), 7.2% (6/83) and 0% (0/25) respectively ($P = 0.0255$, chi-square test)). The percentages of breast cancer patients with UV mutations in Jakarta, Jogjakarta, and Denpasar were 16% (4/25), 12% (10/83), and 25% (3/12), respectively (n.s.). Although the number of patients is too small to draw firm conclusions, these data may point to geographic differences within Indonesia.

It was initially suggested that the *BRCA1* and *BRCA2* genes would be responsible for most cases of inherited breast cancer, but more recent studies suggest that they would account for a far smaller proportion, with considerable variation among different populations [30]. We found that the incidence of mutations in these genes varies, depending on the diagnostic group. In this sense, mutations were present in (22/102) 21.6% of early onset patients (group A), 28.7% (2/7) in patients with bilateral breast cancer (group D) and (2/9) 22.2% of patients with two cases of breast cancer before the age of 60 (group B). The proportion of families affected by *BRCA1/2* mutations depends on the population analyzed and on the criteria used to select the patients. Family history of breast cancer was, however, absent or not suggestive of a hereditary predisposition in three-fourth of the

deleterious mutations carriers and in more than 90% of UV carriers. This suggests that *BRCA* screening policies based on family history only would miss a considerable proportion of mutation carriers.

In conclusion, a relatively high percentage of early onset Indonesian breast cancer patients carry a germline mutation in either *BRCA1* or *BRCA2*. Several novel, pathogenic *BRCA1* and *BRCA2* germline mutations have been found, as well as a variety of novel “unclassified variant” mutations that may therefore be specific for the Indonesian population. It is likely that some of the “unclassified variant” mutations may have a functional role in breast cancer development, which deserves to be explored further.

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