รายงานการวิจัย ฉบับสมบูรณ์

ทุนพัฒนานักวิจัย สำนักงานกองทุนสนับสนุนการวิจัย

สัญญาเลขที่ RSA/21/2540

วิเคราะห์การถ่ายทอดและการกลายพันธุ์ของยีนมะเร็งเต้านม *BRCA1* และ *BRCA2* ในครอบครัวไทย

Analysis of Breast Cancer Susceptibility Genes, ***BRCA1*** **and** ***BRCA2*** in Thai Familial Breast and Ovarian Cancers.

หัวหน้าโครงการ รองศาสตราจารย์ ดร. พิมพิชญา ปัทมสิริวัฒน์
ภาควิชาจุลทรรศนศาสตร์คลินิก
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มหาวิทยาลัยมหิดล

รวมระยะเวลาดำเนินการรวม 3 ปี (2541-2543)

สรุปผลการดำเนินงาน

โครงการวิจัย ทุนพัฒนานักวิจัย (RSA/21/2540) **เรื่อง วิเคราะห์การถ่ายทอดและการกลายพันธุ์ของยีนมะเร็งเต้านม *BRCA1* and *BRCA2* ในครอบครัวไทย**

1. การวิจัย การวิจัยได้ดำเนินการไปตามเป้าหมายทุกประการ มีการเปลี่ยนแปลงกลยุทธด้านวิธีการบ้างเพียงเล็กน้อยเพื่อความเหมาะสมในการดำเนินการ ผลงานได้รายงานมายัง ส.ก.ว. ตามกำหนดทุกปี ได้แก่

1.1 รายงานความก้าวหน้าของโครงการประจำปี (ครั้งที่ 1 ระยะเวลาดำเนินการ 1 มีนาคม 2541-31 ธันวาคม 2541)

1.2 รายงานความก้าวหน้าของโครงการประจำปี (ครั้งที่ 2 ระยะเวลาดำเนินการ 1 มกราคม 2542-31 ธันวาคม 2542)

1.3 Executive Summary ในเอกสารรวบรวมการเสนอความก้าวหน้าของผลงานโครงการ ทุนพัฒนานักวิจัย วันที่ 21-22 ธันวาคม 2543

1.4 รายงานฉบัยสมบูรณ์ (คือฉบับนี้)

2. Out Put ผลงานวิจัยที่ได้มีการเผยแพร่แล้ว หรือกำลังดำเนินการอยู่ มีดังนี้

2.1 Submit to "Int J Cancer" ใน Research article เรื่อง "Analysis of Breast Cancer Susceptibility Genes BRCA1 and BRCA 2 in Thai Familial and Isolated Early-onset Breast and Ovarian Cancer" ซึ่ง final manuscript อยู่ในรายงานฉบับสมบูรณ์นี้แล้ว

2.2 เผยแพร่ผลงานร่วมกับ The MAGIC Project, IARC-WHO: Mutational Profile of BRCA1/2 Mutations in Worldwide Populations ใน Proceedings of the American Association for Cancer Research 92nd Annual Meeting (March 24-28, 2001) New Orleans, LA vol. 42 (March 2001) (แนบเอกสารในรายงานฉบับสมบูรณ์นี้แล้ว)

2.3 เผยแพร่ในการประชุมวิชาการ The Fourth Princess Chulabhorn International Science Congress, Organized by CRI: Novel BRCA1 and BRCA2 Mutations in Familial Thai Breast and/or Ovarian Cancers (abstract P 139) ทั้ง abstract และเนื้อเรื่อง ได้แนบในรายงานฉบับสมบูรณ์นี้แล้ว)

2.4 เผยแพร่และตีพิมพ์ในหนังสือรายงานการสัมมนาวิชาการพันธุศาสตร์ครั้งที่ 11 ปี พ.ศ. 2542 พันธุศาสตร์ช่วยชาติแก้วิกฤติ (ไพศาล เหล่าสุวรรณ บรรณาธิการ) เรื่อง วิเคราะห์การกลายพันธุ์ของยีนมะเร็งเต้านม BRCA1 และ BRCA2 ในครอบครัวไทย หน้า 96-108 (แนบเอกสารในรายงานฉบับสมบูรณ์นี้แล้ว)

3. การสร้างทีมงานในประเทศ และต่างประเทศ

3.1 โครงการนี้ มีความร่วมมือประสานงานร่วมกัน ทั้งภายในคณะเทคนิคการแพทย์ ต่างคณะ และความร่วมมือกับผู้เชี่ยวชาญจากต่างมหาวิทยาลัย สถาบัน มีการสร้างเครือข่ายศึกษามะเร็งเต้านม และมะเร็งรังไข่ที่ถ่ายทอดในครอบครัวไทย ซึ่งให้ความร่วมมือในการคัดเลือกผู้ป่วยเป้าหมาย และดูแลให้คำแนะนำต่อญาติผู้ป่วย (รายชื่อมีอยู่ในสำเนางานวิจัย ในหนังสือพันธุศาสตร์ช่วยชาติแก้วิกฤติ)

6. การเผยแพร่ให้ความรู้สู่สาธารณชน

นอกเหนือจากการเผยแพร่ในวงวิชาการดังกล่าวไว้ ในข้อ 2 (out put) แล้ว ยังได้เผยแพร่ความรู้ที่เข้าใจได้ง่าย ๆ ไม่ซับซ้อนให้ประชาชนทั่ว ๆ ไปดังนี้

6.1 บทความในหนังสือพิมพ์วัฏจักร ฉบับวันที่ 9 สิงหาคม พ.ศ.2543 เรื่อง "วิจัยพบการกลายพันธุ์ของยีนมะเร็งเต้านม"

6.2 เผยแพร่งานวิจัยมะเร็งเต้านม ทางสถานีโทรทัศน์ช่อง 7 สี ในรายการ "คนไทยวันนี้" วันศุกร์ที่ 22 กันยายน พ.ศ.2543 เวลา 19.30 น.

6.3 สัมภาษณ์ทางสถานีวิทยุรายการ "คลื่นอนาคต" (89.5 FM) ออกอากาศวันที่ 25 มีนาคม พ.ศ. 2543

6.4 สัมภาษณ์สดทางสถานีวิทยุรายการ "ร่วมด้วยช่วยกัน" AM Net work เมื่อเดือนเมษายน พ.ศ. 2543 และเทปเผยแพร่ซ้ำใน จ.ส. 100 ด้วย

7. ปัญหาอุปสรรค

7.1 อุปสรรคด้านงบประมาณ เนื่องจากความจำกัดเรื่องเงิน ทำให้การขยายงานให้ครอบคลุม จำนวนครอบครัวมากขึ้น และให้มีความลุ่มลึกของงานวิจัยมากยิ่งขึ้น จึงทำได้ลำบาก และจะขอความสนับสนุนจาก ส.ก.ว. ต่อไป

7.2 อุปสรรคด้านเวลาและภาระงานอื่น ๆ

คณะเทคนิคการแพทย์ มีการสอนนักศึกษาหลายระดับทั้ง ตรี โท เอก และจำนวนนักศึกษามีมาก ทำให้มีภาระการสอนตลอดทั้งปี ดิฉันชอบงานวิจัยแต่ก็เครียดเนื่องจากความรับผิดชอบเรื่องงานสอนก็ต้องให้ดีและวิจัยก็ต้องให้เสร็จตามที่ตั้งใจ ด้วยเหตุที่มีภาระมากดังกล่าว จึงไม่สามารถยื่นขอทุนได้อย่างต่อเนื่อง จำเป็นต้องมีการเว้น

ซึ่งเป็นเครือข่ายความร่วมมือที่เป็นอาสาสมัคร (ไม่สามารถจัดสรรค่าตอบแทนให้ได้)

3.2 สำหรับความร่วมมือกับต่างประเทศ มี 2 กลุ่มคือ

3.2.1 กลุ่มนักวิจัยจาก M.D. Anderson Cancer Center, Houston Texas ได้แก่ กลุ่มของ Professor Grady F. Saunders

3.2.2 กลุ่มนักวิจัยจาก International Agency for Research on Cancer (IARC) Lyon, France ได้แก่ Professor Gilbert Lenoir, Dr. David Goldgar และ Dr. Olga Sinilnikova เป็นต้น (ดังรายชื่อใน Manuscript ที่ submit ลง Int J Cancer ที่แนบมา)

4. การผลิตนักศึกษา

โครงการนี้ทำให้มีนักศึกษาที่ช่วยงานวิจัยซึ่งเป็นส่วนหนึ่งของปริญญา คือ นักศึกษาปริญญาโท 1 คน (จบปีการศึกษา 2543) และปริญญาเอก 1 คน (กำลัง เริ่มดำเนินการวิจัยใน Phase ต่อไป)

แทนนักวิจัย ตามที่กำหนดไว้ คือใช้เงินค่าตอบแทนไปเพียงร้อยละ 28.5 ของงบค่าตอบแทนที่ทุนพัฒนานักวิจัยมีเกณฑ์ไว้ให้เท่านั้น (ได้แนบรายงานการเงินในรายงานนี้แล้ว)

6. การเผยแพร่ให้ความรู้สู่สาธารณชน

นอกเหนือจากการเผยแพร่ในวงวิชาการดังกล่าวไว้ ในข้อ 2 (out put) แล้ว ยังได้เผยแพร่ความรู้ที่เข้าใจได้ง่าย ๆ ไม่ซับซ้อนให้ประชาชนทั่ว ๆ ไปดังนี้

6.1 บทความในหนังสือพิมพ์วัฏจักร ฉบับวันที่ 9 สิงหาคม พ.ศ.2543 เรื่อง "วิจัยพบการกลายพันธุ์ของยีนมะเร็งเต้านม"

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6.4 สัมภาษณ์สดทางสถานีวิทยุรายการ "ร่วมด้วยช่วยกัน" AM Net work เมื่อเดือนเมษายน พ.ศ. 2543 และเทปเผยแพร่ซ้ำใน จ.ส. 100 ด้วย

7. ปัญหาอุปสรรค

7.1 อุปสรรคด้านงบประมาณ เนื่องจากความจำกัดเรื่องเงิน ทำให้การขยายงานให้ครอบคลุม จำนวนครอบครัวมากขึ้น และให้มีความลุ่มลึกของงานวิจัยมากยิ่งขึ้น จึงทำได้ลำบาก และจะขอความสนับสนุนจาก ส.ก.ว. ต่อไป

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คณะเทคนิคการแพทย์ มีการสอนนักศึกษาหลายระดับทั้ง ตรี โท เอก และจำนวนนักศึกษามีมาก ทำให้มีภาระการสอนตลอดทั้งปี ดิฉันชอบงานวิจัยแต่ก็เครียด

ANALYSIS OF BREAST CANCER SUSCEPTIBILITY GENES *BRCA1* AND *BRCA2* IN THAI FAMILIAL AND ISOLATED EARLY-ONSET BREAST AND OVARIAN CANCER

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ABSTRACT

Here we report the study on *BRCA1* and *BRCA2* mutations in 12 Thai breast and/or ovarian cancer families and 6 early-onset breast or breast/ovarian cancer cases without a family history of cancer. Five distinct rare alterations were identified in each gene: four introducing premature stop codons, one in-frame deletion, two missense changes, two intronic alterations and one silent rare variant. The *BRCA1* or *BRCA2* truncating mutations were detected in four of seven patients with familial or personal history of breast and ovarian cancer, in one of four isolated early onset breast cancer cases and in none of seven breast cancer site specific families. The *BRCA1* ,and *BRCA2* mutations yield in Thai patients is consistent with that reported from Europe and North America in similar groups of patients, being particularly high in individuals with personal or family history of breast and ovarian cancer. The *BRCA1* and *BRCA2* alterations found in this series are different from those identified in other Asian studies, and all but two have never been reported before. *BRCA1* 3300delA-ter1061 and Asp67Glu alterations were detected each in at least two families and thus could represent founder mutations in Thais.

P. Patmasiriwat

Introduction

Breast cancer is one of the important world health problems, not only in industrialised, but also in developing countries where its incidence has been gradually increased. In Thailand, this disease is the second leading cancer in women, following that of cervix uteri (Chindavijak and Martin, 1999). National estimation of age standardised incidence rate (ASR, world standard) of female breast cancer in this country shows an obvious increase; the estimated ASRs (per 100,000 women) being 7.0, 13.5 and 16.3 by the years 1985, 1990, and 1993, respectively (Cancer Statistics 1985; Vatanasapt et al., 1995; Chindavijak and Martin, 1999). Incidence rate of breast cancer is the highest in Bangkok (population 5.88 millions) with the ASR of up to 20.6, and it is the most frequent female cancer in this city followed by that of cervix uteri (ASR 18.6).

Ovarian cancer is the second most frequent gynaecological malignancy of Thai women, exceeded only by cancer of cervix uteri. However, the incidence rate of ovarian cancer is relatively low with ASR of 4.7 compared to 19.9 per 100,000 in Canada.

Family history is an important risk factor of breast and ovarian cancer. Approximately, five to ten percent of all breast and ovarian cancers are due to genetic predisposition with autosomal dominant transmission (Newman et al., 1988; Claus et al. 1991; Schildkraut et al. 1989). Two major breast and ovarian cancer susceptibility genes, *BRCA1* and *BRCA2*, have been identified (Miki et al., 1994; Wooster et al., 1995; Tavtigian et al., 1996). These genes are believed to be tumor suppressors whose function has not yet been completely elucidated. They are thought to be involved in DNA repair as well as transcription regulation (Scully et al., 2000; Zheng et al.,2000).

The majority of information concerning mutations in the *BRCA1* and *BRCA2* genes in breast and ovarian cancers comes from North America and Europe. However, few reports on this issue have been published from Asian countries. In the frame of the MAGIC project coordinated at IARC, we characterised the *BRCA1* and *BRCA2* mutations as well as polymorphic variants in the members of breast and/or ovarian cancer risk families and isolated cases of early-onset breast or breast/ovarian cancers in Thais. Most of the patients were from central region of the country including Bangkok.

P. Patmasiriwat

Materials and Methods

Patients

This study includes a total of 23 patients with breast and/or ovarian cancer. Seventeen patients were from 12 families having at least two affected cases of breast or ovarian cancer diagnosed at any age among first degree relatives. The other six out of 23 patients were isolated cases without family history of breast or ovarian cancer. Of these isolated cases four were breast cancer patients diagnosed before age 32 years and two had both breast and ovarian cancers. All the patients had given informed consent before providing their blood samples.

DNA extraction and mutation analysis

Peripheral blood leukocytes were separated from whole blood specimens by standard dextran sedimentation technique. Equal volume of whole blood and 1% dextran in normal saline were mixed gently and stand at room temperature for 40 minutes. The WBC enriched supernatant was separated and then incubated with proteinase K in the presence of 0.1M EDTA and 0.5% SDS for 12 hours at 55C°.

Genomic DNA was isolated by standard phenol-chloroform procedure described by Sambrook et al (1989). The purified genomic DNA samples were subsequently screened for *BRCA1* and *BRCA2* variants by PCR-based heteroduplex analysis according to Serova et al (1997). All 22 coding exons of *BRCA1* and 26 coding exons of *BRCA2* as well as the exon-intron junction regions were amplified using primer sequences described by Freidman et al (1994) and Tavtigian et al (1996), respectively. The PCR reaction includes 1xPCR buffer with 1.5 to 3 mM $MgCl_2$, 200mM dNTP, 20 pmol each primer, 0.05 µl ^{33}P-dATP (specific activity 2500 Ci/mmol, Amersham, Aylesbury UK), 1 unit platinum Taq polymerase (Gibco BRL) and 50-200 ng genomic DNA in a total of 20 µl reaction mixture.

The PCR products were denatured at 90C° for 5 minutes and cooled down to room temperature over at least 30 minutes to allow renaturation of the DNA strands. Homoduplex and heteroduplex were separated in non-denaturing 1xMDE polyacrylamide gel matrix (FMC Bioproduct, Rockland, ME) containing 7.5% glycerol at 300 to 600 V for 14 hours according to the manufacturer's protocol. After vacuum drying at 80C° for 45 minutes, the gel was autoradiographed using Kodak BioMax MR film for at least 4 hours at room temperature. PCR products exhibiting variant bands in *BRCA1* or *BRCA2* genes were subsequently reamplified and

DNA sequenced by modified manual Sanger's dideoxy chain termination method (Sanger et al., 1977) using USB PCR-product sequencing kit (Amersham).

Results

We analysed the *BRCA1* and *BRCA2* genes in 23 Thai patients affected with breast and/or ovarian cancer. Seventeen patients belonged to 12 breast or ovarian cancer families and six were early onset breast or breast/ovarian cancer cases manifesting no family history of these cancers. In four families, blood samples were obtained from two or more affected cases whereas blood samples from one affected member were collected in the other 8 families. Table 1 specifies the age of onset of cancer and the family history of the patients studied. Four out of five ovarian and breast/ovarian cancer families contained at least two first-degree relatives affected with ovarian cancer. The majority of the breast cancer specific families (5 of 7) presented two cases of breast cancer among first-degree relatives, two kindreds having three affecteds. Mean age of onset of familial breast cancer was 54 years.

Five distinct rare variants were identified in each gene: four introducing premature stop codons, one in-frame deletion, two missense changes, two intronic and one silent rare variant (Tables 1, 2). Only two out of ten variants identified, the *BRCA1* IVS20+78 and the *BRCA2* 2041delA, have been reported previously, each twice (Breast Cancer Information Core, http:// www. nhgri. nih. gov/ Intramural_ research/ Lab_ transfer/ Bic). The others are the novel mutations that have never been reported before.

The *BRCA1* mutation 3300delA-ter1061 has been found in two apparently unrelated families: F7 and F15. Patient 17 (family F7) revealed 3300delA only, while patients 27 and 28 (daughter and mother, family F15) appeared to carry the *BRCA1* rare missense substitution Thr1051Ser (3271C>G) together with 3300delA mutation, both being likely present on the same allele and thus suggesting Thr1051Ser variant to be a polymorphism. Both families showed a predominance of ovarian cancer. Their pedigrees, heteroduplex analysis and sequencing results are shown on Figure 1. Another *BRCA1* truncating mutation, 744ins20-ter240, was identified in an isolated case of breast and ovarian cancer (patient 32). This patient had bilateral breast cancer diagnosed at age of 41 years, and ovarian cancer at age of 42 years.

Among two clearly deleterious *BRCA2* mutations, one (2041delA-ter613) was found in an early-onset breast cancer patient (patient 21) manifesting no family history of cancer. The *BRCA2* 6382delT-ter2069 was identified in a 35-year-old breast cancer patient (patient 20) belonging to a

multiple cancer site family (F10: breast, ovarian, uterine, cervix, liver cancer). Her elder sister also developed a breast cancer while her mother had cancer of cervix uteri, her paternal aunt and uncle had ovarian and liver cancer, respectively.

The other five rare variants found in this series are of unknown significance. The *BRCA1* 320T>G giving rise to the aminoacid change Asp67Glu located in a close proximity of a RING finger motif, is a novel missense variant observed in three unrelated Thai breast cancer patients two of which had family history of breast or ovarian cancer (patients 34, 35, 36). The *BRCA2* IVS20-26A>G intronic variant was detected in a breast cancer patient (patient 24) having her sister affected with breast and mother with uterus cancer. Unfortunately, no DNA of the affected relatives of the carriers of the *BRCA1* Asp67Glu or the *BRCA2* IVS20-26A>G has been available in order to check the segregation of these alterations with the disease. The *BRCA2* 5527del9 in-frame deletion was identified in mother and daughter (patients 3 and 4, F2), both affected with breast cancer. This mutation is expected to result in a loss of three aminoacids, Lys1767, Leu1768 and Asp1769, from the BRCA2 protein. Although it remains to be established whether these three genetic alterations represent deleterious mutations or neutral polymorphisms, they were not observed in a total of 100 chromosomes from Thai population. The *BRCA1* IVS20+78G>A is an intronic variant which was found in an affected mother (patient 1), but not in her affected daughter (patient 2). A *BRCA2* silent substitution 2565G>T (Leu779Leu) was detected in a familial advanced-age breast cancer patient (patient 25). Given genetic and functional aspects of these two latter variants, they are likely to represent rare polymorphisms.

A total of seven different *BRCA1* and *BRCA2* germline alterations likely to be disease-associated were identified in ten apparently unrelated breast and/or ovarian cancer families or isolated cases. We found alterations in four of five ovarian and breast/ovarian cancer families (three *BRCA1*: 3300delA F7, F15, Asp67Glu F17; one *BRCA2*: 6382delT F10), in three of seven breast cancer families (one *BRCA1*: Asp67Glu F18, two *BRCA2*: 5527del9 F2, IVS20-26A>G F12), in one of two breast/ovarian cancer patients (one *BRCA1*: 744ins20 patient 32), and in two of four early-onset breast cancer patients (one *BRCA1*: Asp67Glu patient 36, one *BRCA2*: 2041delA patient 21). When considering truncating mutations only, three *BRCA1* and one *BRCA2* mutations were identified among seven patients with familial or personal history of breast and ovarian cancer, one *BRCA2* mutation among four isolated early onset breast cancer cases and none in seven breast cancer specific families.

P. Patmasiriwat

Discussion

We studied the incidence and spectrum of the *BRCA1* and *BRCA2* mutations in 12 Thai breast and/or ovarian cancer families and 6 isolated early-onset breast and breast/ovarian cancer cases. Ten different rare variants (five in *BRCA1* and five in *BRCA2*) have been identified, seven of them being likely associated with disease. Four mutations (two *BRCA1* and two *BRCA2*) are predicted to result in premature Stop codon; one *BRCA1* missense variant provokes an amino acid substitution of Asp to Glu at residue 67 close to the last cystein of the RING finger motif and located in the proteolysis-resistant domain of BRCA1 (Brzovic et al, 1998); one *BRCA2* mutation causes a deletion of three amino acids from the BRCA2 protein; and one intronic alteration affects the potential branching site of intron 20 of the *BRCA2* gene. However it can not be ruled out that the three latter non-truncating alterations could be neutral polymorphisms. Three other variants detected are likely to be non-deleterious polymorphisms given the following pieces of evidence. Thr1051Ser is apparently present on the same *BRCA1* allele as the truncating mutation 3300delA-ter1061 in family F15. The *BRCA1* intronic single nucleotide change IVS20+78 G>A does not appear to segregate with disease, and the *BRCA2* G2565T does not result in an amino acid change.

More than half of breast/ovarian cancer families or isolated cases (4 of 7) appeared to carry *BRCA1* or *BRCA2* truncating mutations with the predominance of the *BRCA1* alterations. None of seven breast cancer specific families and one of four isolated early onset breast cancer cases were found positive for truncating mutations. The number of the *BRCA1* or *BRCA2* mutation carriers in our series might be actually higher supposing that some of the rare non-truncating variants identified could be deleterious and given that regulatory mutations and large rearrangements have not been looked for in this study.

The majority of the previously reported *BRCA1* and *BRCA2* mutations come from Europe, North America and Australia. More than 600 different germ-line *BRCA1* and *BRCA2* mutations have been identified so far, spread throughout the coding regions of both genes (Breast Cancer Information Core). There are few data on the *BRCA1* and *BRCA2* mutations in Asia. To our knowledge, this is the first report on *BRCA1* and *BRCA2* mutations in hereditary breast and ovarian cancers in Thailand. Our results on breast/ovarian cancer families and isolated patients appeared to be similar to the findings of Takano et al (1997) reporting seven *BRCA1* mutations in 19 ovarian and breast/ovarian cancer Japanese families. In Japan, China and Taiwan, 20 to 30% of breast cancer specific families were found to be attributable to *BRCA1* and *BRCA2* mutations (Inoue et al., 1995; Inoue et al., 1997; Katagiri et al., 1996; Li et al., 1999) and about 10% of early-onset

P. Patmasiriwat

breast cancers were due to *BRCA1* alterations (Tang et al., 1999; Sng et al., 2000). We identified no *BRCA1* and only one *BRCA2* truncating mutation among 11 breast cancer families and early-onset patients studied. However, the number of these patients was small and the families selection criteria were not stringent (at least two breast cancer cases diagnosed at any age among first degree relatives). On the other hand, some non-truncating, but likely functionally significant variants detected in the coding and intronic sequences of *BRCA1* and *BRCA2* might be causative of breast cancer in these patients.

On the whole, the *BRCA1* and *BRCA2* mutations yield in Thai patients in this study is consistent with that reported from Europe, North America and Asia in similar groups of patients, being particularly high in individuals with personal or family history of breast and ovarian cancer and less elevated in breast cancer families or isolated cases (Couch et al, 1997; Serova et al, 1997; Stoppa-Lyonnet et al, 1997; Hakansson et al, 1997, Takano et al, 1997; Inoue et al, 1995; Inoue et al, 1997; Katagiri et al, 1998; Li et al, 1999; Tang et al, 1999; Sng et al, 2000).

The *BRCA1* and *BRCA2* mutations found in our series are different from those reported in the other Asian studies, and nearly all of them are novel ones. *BRCA1* 3300delA and Asp67Glu alterations were detected each in at least two families and thus probably represent founder mutations in Thais. It is essential to estimate the contribution of these specific mutations in hereditary breast and ovarian cancer through the screening of a larger series of patients.

Further studies of cancer related mutations in Thai familial breast and ovarian cancers in different regions of Thailand and neighbour countries within SEA will lead to better understanding of genetic risk factors of these female cancers in this region. These studies will contribute to the assessment of the necessity of the preventive programme for mutation carriers as part of the National public health policy

Acknowledgement

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P. Patmasiriwat

Table 1: Characteristics of Thai breast and ovarian cancer patients studied

	Family No.	Patient ID	Cancer type	Age of onset	Other cancer cases in family			Gene	Exon / intron	Sequence variant and predicted effect
					BR	OV	Other			
Ovarian cancer specific families	F8	18	OV	48	no	1	uterus			
	F15	27	OV	42	no	2	nasopharynx	*BRCA1* *BRCA1*	11 11	3300delA-ter1061 C3271G-Thr1051Ser
	F15	28	OV	64	no	2		*BRCA1* *BRCA1*	11 11	3300delA-ter1061 C3271G-Thr1051Ser
Breast/ ovarian cancer families	F7	17	OV	50	1	1	cervix	*BRCA1*	11	3300delA-ter1061
	F10	20	BR	35	1	1	uterus, cervix, liver	*BRCA2*	11	6382delT-ter2069
	F17	34	BR	27	1	2		*BRCA1*	5	T320G-Asp67Glu
Isolated breast/ ovarian cancer cases		32	BR/OV	42/42	no	no		*BRCA1*	10	744ins20-ter240
		15	BR/OV	44/54	no	no				
Breast cancer specific families	F1	1	BR	60	1	no		*BRCA1*	IVS20	IVS20+78 G>A
	F1	2	BR	34	1	no				
	F2	3	BR	50	1	no		*BRCA2*	11	5527del9-del1767-1769
	F2	4	BR	?	1	no		*BRCA2*	11	5527del9-del1767-1769
	F3	5	BR	45	1	no				
	F4	7	BR	48	2	no				
	F4	8	BR	40	2	no				
	F4	10	BR	48	2	no				
	F12	24	BR	57	1	no	uterus	*BRCA2*	IVS20	IVS20-26 A>G
	F14	25	BR	72	1	no				
	F18	35	BR	54	2	no		*BRCA1*	5	T320G-Asp67Glu
Isolated breast cancer cases		36	BR	25	no	no	multiple myeloma	*BRCA1*	5	T320G-Asp67Glu
		21	BR	31	no	no		*BRCA2*	10	2041delA-ter613
		16	BR	31	no	no				
		19	BR	29	no	no	lung			

P. Patmasiriwat

Table 2. *BRCA1* genetic changes in Thai breast and ovarian cancer patients

Patient ID	*Exon/Intron*	*Nucleotide*		*Consequence*
		Position	*Change*	
34	exon 5	320	T>G	Asp67Glu
35	exon 5	320	T>G	Asp67Glu
36	exon 5	320	T>G	Asp67Glu
32	exon 10	744	ins20	Stop240
17	exon 11	3300	delA	Stop1061
27, 28	exon 11	3300	delA	Stop1061
	exon 11	3271	C>G	Thr1051Ser
1	intron 20	IVS20+78	G>A	unknown

Table 3. *BRCA2* genetic changes in Thai breast and ovarian cancer patients

Patient ID	*Exon/Intron*	*Nucleotide*		*Consequence*
		Position	*Change*	
21	exon 10	2041	delA	Stop613
25	exon 11	2565	G>T	unknown
3, 4	exon 11	5527	del9	del1767-1769
20	exon 11	6382	delT	Stop2069
24	intron 21	IVS20-26	A>G	unknown

P. Patmasiriwat

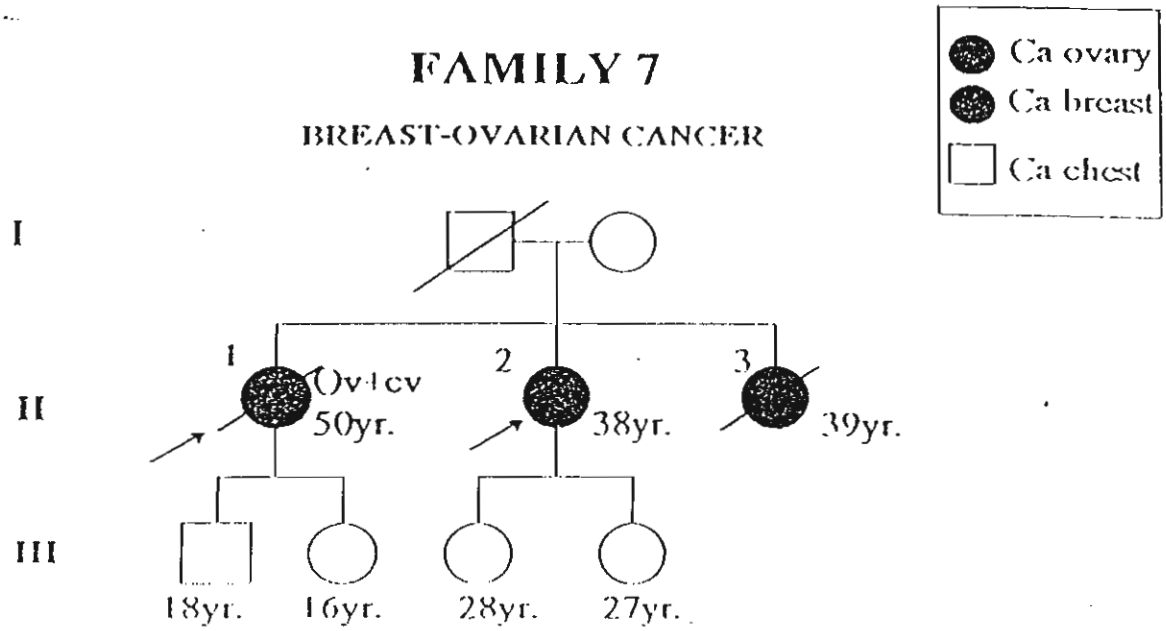
Figure 1. BRCA1 3300delA mutation in families 7 and 15.

1A: Pedigrees of families 7 and 15. Blackened circles indicate women affected with breast or ovarian cancer, grey circles and squares indicate individuals affected with other cancers. Br-breast cancer, Ov-ovarian cancer, Cx-cervical cancer, Na-nasopharyngeal cancer. Age at diagnosis or current age for non-affecteds are indicated. Screened individuals are II-1 in family 7 (patient 17) and II-3 and III-2 in family 15 (patients 28 and 27).

1B: Heteroduplex analysis of the BRCA1 exon 11 fragment (nucleotides 2897-3662).

1C: Sequencing analysis of this fragment in patient 17 revealing 3300delA mutation. Deleted nucleotide is marked with an asterisk.

P. Patmasiriwat



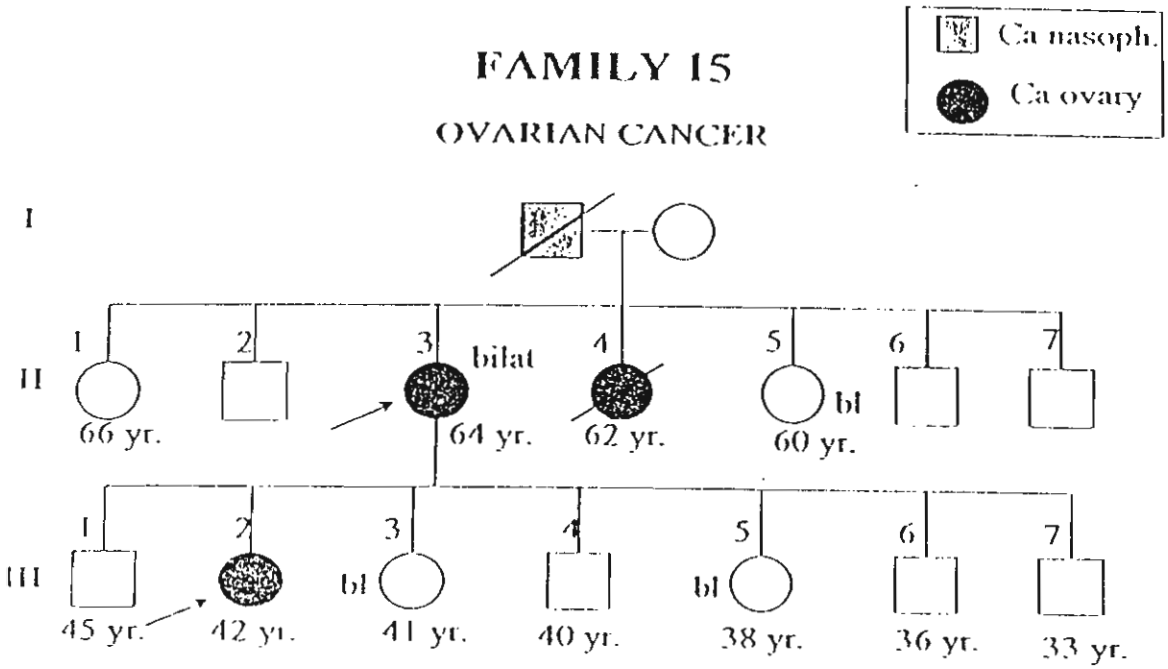


Figure 1A

17 18 19 20 21 24 25 27 28 32

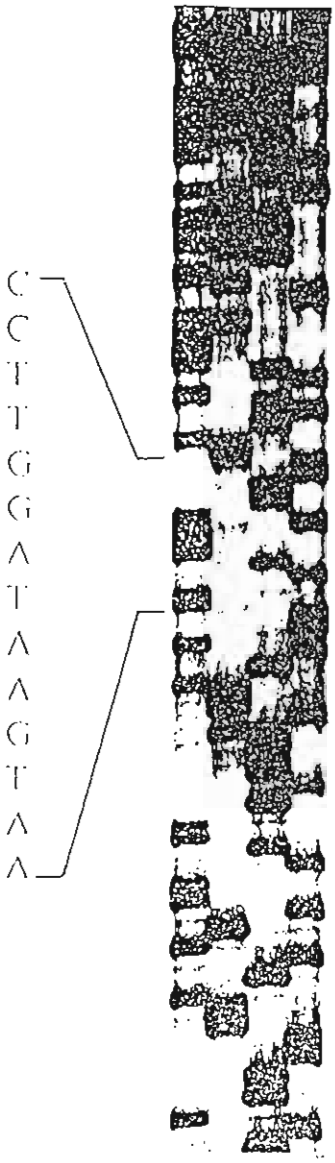
Patients' ID

Figure 1B

P. Patmasiriwat

Patient ID17
(*BRCA1*: 3300delA)

Control



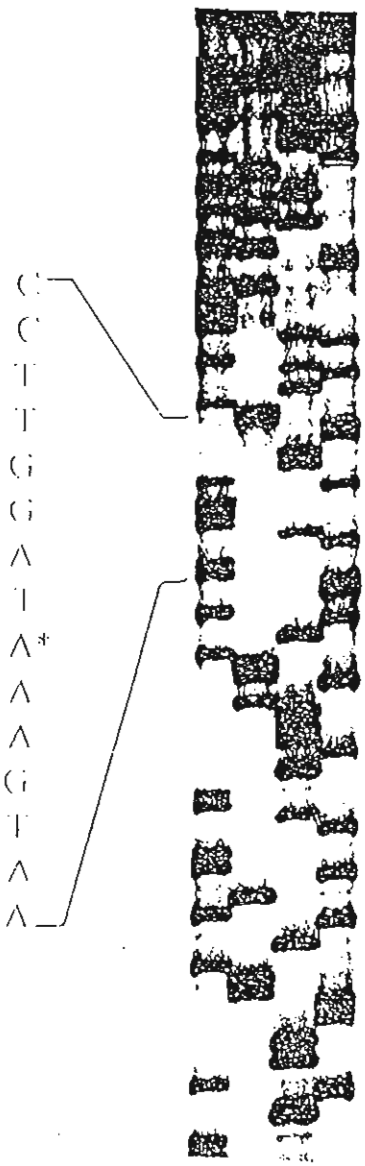


Figure 1C

P. Patmasiriwat

References

Brzovic PS, Meza J, King MC, Klevit RE. The cancer-predisposing mutation C61G disrupts homodimer formation in the NH2-terminal BRCA1 RING finger domain. J Biol Chem. 1998 Apr 3;273(14):7795-9.

Cancer Registry NCI, 1996 (Annual Report), pp12-25. National Cancer Institue, Department of Medical Services, Ministry of Public Health , Bangkok Thailand (1996)

Cancer Statistics 1985. National Cancer Institute report pp 5-9, Department of Medical Services , Ministry of Public Health , Bangkok, Thailand (1985)

Chindavijak, K., Martin, N. Breast. In: S. Deerasamee, N. Martin, S. Sortipong, S. triamporn, H. Sriplung, D. Srivatanakul, V. Vatanasapt, D.M. Parkin and J. Ferlay (eds.). Cancer in Thailand, Vol. II, 1992-1994.IARC Technical Report No.34, pp 53-55. International Agency for Research on Cancer , Lyon, France (1999)

Claus EB et.al, Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. Am J Hum Genet 1991; 48: 232-242

Couch FJ, DeShano ML, Blackwood MA, Calzone K, Stopfer J, Campeau L, Ganguly A, Rebbeck T, Weber BL. BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. N Engl J Med. 1997 May 15;336(20):1409-15

Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE, King MC. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 1994; 8: 399-404 .

Hakansson S, Johannsson O, Johansson U, Sellberg G, Loman N, Gerdes AM, Holmberg E, Dahl N, Pandis N, Kristoffersson U, Olsson H, Borg A. Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. Am J Hum Genet. 1997 May;60(5):1068-78.

Inoue, R., Fukutomi, T., Ushijima, T., Matsumoto, Y., Sugimura, T., Nagao, M.Germline mutation of BRCA1 in Japanese breast cancer. Cancer Res. 55, 3521-3524 (1995)

Inoue R, Ushijima T, Fukutomi T, Fukami A, Sugimura H, Inoue S, Okonogi H, Sugimura T, Matsumoto Y, Nagao M. BRCA2 germline mutations in Japanese breast cancer families. Int J Cancer. 1997 Apr 22;74(2):199-204.

Katagiri, T., Emi, M., Ito, I., Kobayoshi, K., Yoshimoto, M., Iwase, T., et al. Mutations in the BRCA1 gene in Japanese breast cancer patients. Hum. Mutat 7, 334-339 (1996)

P. Patmasiriwat

Li, S.S-L., Tseng, H-M., Yang, T-P., Liu, C-H., Teng, S-J., Huang H-W., Chen, L-M., Kao, H-W., Chen, J.H., Tseng J-N., Chen, A., Hou, M-F., Huang T-J., Chang H-T., Mok, K-T., Tsai, J-H. Molecular characterization of germline mutations in the BRCA1 and BRCA2 genes from breast cancer families in Taiwan. Hum. Genet 104,201-204 (1999)

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. A strong candidated for the 17q-linked breast and ovarian cancer susceptibility gene BRCA1. Science1994; 266: 66-71

Newman B, Austin MA, Lee M, King M-C. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high risk families. Proc. Natl Acad Sci USA1988; 85: 3044-3048

Public Health Statistics 1996, pp 153-160. The Ministry of Public Health, Bangkok, Thailand (1997).

Serova, O.M., Mazoyer , S., Puget, N., Dubois, V., Tonin, P., Shugart, Y., Goldgar D., Narod, S.A., Lynch, H.T., Lenoir, G.M. Mutations in BRCA1 and BRCA2 in breast cancer families: Are there more breast cancer susceptibility genes? Am. J. Hum. Genet. 60,486-496 (1997)

Schildkraut JM, Risch N, Thompson WD. Evaluating genetic association among ovarian, breast, and endometrial cancer: evidence for a breast/ovarian cancer relationship. Am J Hum Genet. 1989 Oct;45(4):521-9.

Scully R, Livingston DM. In search of the tumour-suppressor functions of BRCA1 and BRCA2. Nature. 2000 Nov 23;408(6811):429-32.

Sambrook, J., Fritsch, E.F., Miniatis, T.Molecular cloning: A Laboratory Manual, 2nd Ed., Cold Spring Habour Laboratory, Cold Spring Habour, NY

Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977; 74: 5463-5467

Stoppa-Lyonnet D, Laurent-Puig P, Essioux L, Pages S, Ithier G, Ligot L, Fourquet A, Salmon RJ, Clough KB, Pouillart P, Bonaiti-Pellie C, Thomas G. BRCA1 sequence variations in 160 individuals referred to a breast/ovarian family cancer clinic. Institut Curie Breast Cancer Group. Am J Hum Genet. 1997 May;60(5):1021-30.

Tang, N.L.S., Pang, C-P., Yeo,W., Choy, K-W., Lam, P.K., Suen M., Law L.K., King, W.W.K., Johnson, P., Hjelm, M. Prevalence of mutations in the BRCA1 gene among chinese patients with breast cancer. J. Natl. Cancer. Inst. 91,882-885 (1999)

Tavtigian SV, Simard J, Rommens J, Couch F, Shattuck-Eidens D, Neuhausen S, Merajver S, Thorlacius S, Offit K, Stoppa-Lyonnet D, Belanger C, Bell R, Berry S, Bogden R, Chen Q, Davis T, Dumont M, Frye C, Hattier T, Jammulapati S, Janecki

P. Patmasiriwat

References

Brzovic PS, Meza J, King MC, Klevit RE. The cancer-predisposing mutation C61G disrupts homodimer formation in the NH2-terminal BRCA1 RING finger domain. J Biol Chem. 1998 Apr 3;273(14):7795-9.

Cancer Registry NCI, 1996 (Annual Report), pp12-25. National Cancer Institue, Department of Medical Services, Ministry of Public Health , Bangkok Thailand (1996)

Cancer Statistics 1985. National Cancer Institute report pp 5-9, Department of Medical Services , Ministry of Public Health , Bangkok, Thailand (1985)

Chindavijak, K., Martin, N. Breast. In: S. Decrasamee, N. Martin, S. Sortipong, S. triamporn, H. Sriplung, D. Srivatanakul, V. Vatanasapt, D.M. Parkin and J. Ferlay (eds.). Cancer in Thailand, Vol. II, 1992-1994.IARC Technical Report No.34, pp 53-55. International Agency for Research on Cancer , Lyon, France (1999)

Claus EB et.al. Risch N, Thompson WD. Genetic analysis of breast cancer in the cancer and steroid hormone study. Am J Hum Genet 1991; 48: 232-242

Couch FJ, DeShano ML, Blackwood MA, Calzone K, Stopfer J, Campeau L, Ganguly A, Rebbeck T, Weber BL. BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. N Engl J Med. 1997 May 15;336(20):1409-15

Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE, King MC. Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 1994; 8: 399-404 .

Hakansson S, Johannsson O, Johansson U, Sellberg G, Loman N, Gerdes AM, Holmberg E, Dahl N, Pandis N, Kristoffersson U, Olsson H, Borg A. Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. Am J Hum Genet. 1997 May;60(5):1068-78.

Inoue, R., Fukutomi, T., Ushijima, T., Matsumoto, Y., Sugimura, T., Nagao, M.Germline mutation of BRCA1 in Japanese breast cancer. Cancer Res. 55, 3521-3524 (1995)

Inoue R, Ushijima T, Fukutomi T, Fukami A, Sugimura H, Inoue S, Okonogi H, Sugimura T, Matsumoto Y, Nagao M. BRCA2 germline mutations in Japanese breast cancer families. Int J Cancer. 1997 Apr 22;74(2):199-204.

Katagiri, T., Emi, M., Ito, I., Kobayoshi, K., Yoshimoto, M., Iwase, T., et al. Mutations in the BRCA1 gene in Japanese breast cancer patients. Hum. Mutat 7, 334-339 (1996)

P. Patmasiriwat

T, Jiang P, Kehrer R, Leblanc JF, Goldgar DE, et al. The complete BRCA2 gene and mutation in chromosome 13q-linked kindreds. Nature Genet 1996; 12: 333-337

Takano M, Aida H, Tsuneki I, Takakuwa K, Hasegawa I, Tanaka H, Saito M, Tsuji S, Sonoda T, Hatae M, Chen JT, Takahashi K, Hasegawa K, Toyoda N, Saito N, Yakushiji M, Araki T, Tanaka K. Mutational analysis of BRCA1 gene in ovarian and breast-ovarian cancer families in Japan. Jpn J Cancer Res. 1997 Apr;88(4):407-13.

Tumor Registry Statistical Report 1998. Siriraj Cancer Center, Faculty of Medicine Siriraj Hospital, Mahidol University. Bangkok, Thailand (1998)

Vatanasapt, V., Martin, N., Sriplung, H., Chindavijak, K., Sontipong, S., Sriamporn, S., Parkin, D.M., and Ferlay, J. Cancer Incidence in Thailand, 1988-1991. Cancer Epidemiology, Biomarker&Prevention 4,475-483 (1995)

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion Jcollins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2 . Nature1995; 378: 789-792

Zheng L, Li S, Boyer TG, Lee WH. Lessons learned from BRCA1 and BRCA2. Oncogene. 2000 Dec 11;19(53):6159-75..

T, Jiang P, Kehrer R, Leblanc JF, Goldgar DE, et al. The complete BRCA2 gene and mutation in chromosome 13q-linked kindreds. Nature Genet 1996; 12: 333-337

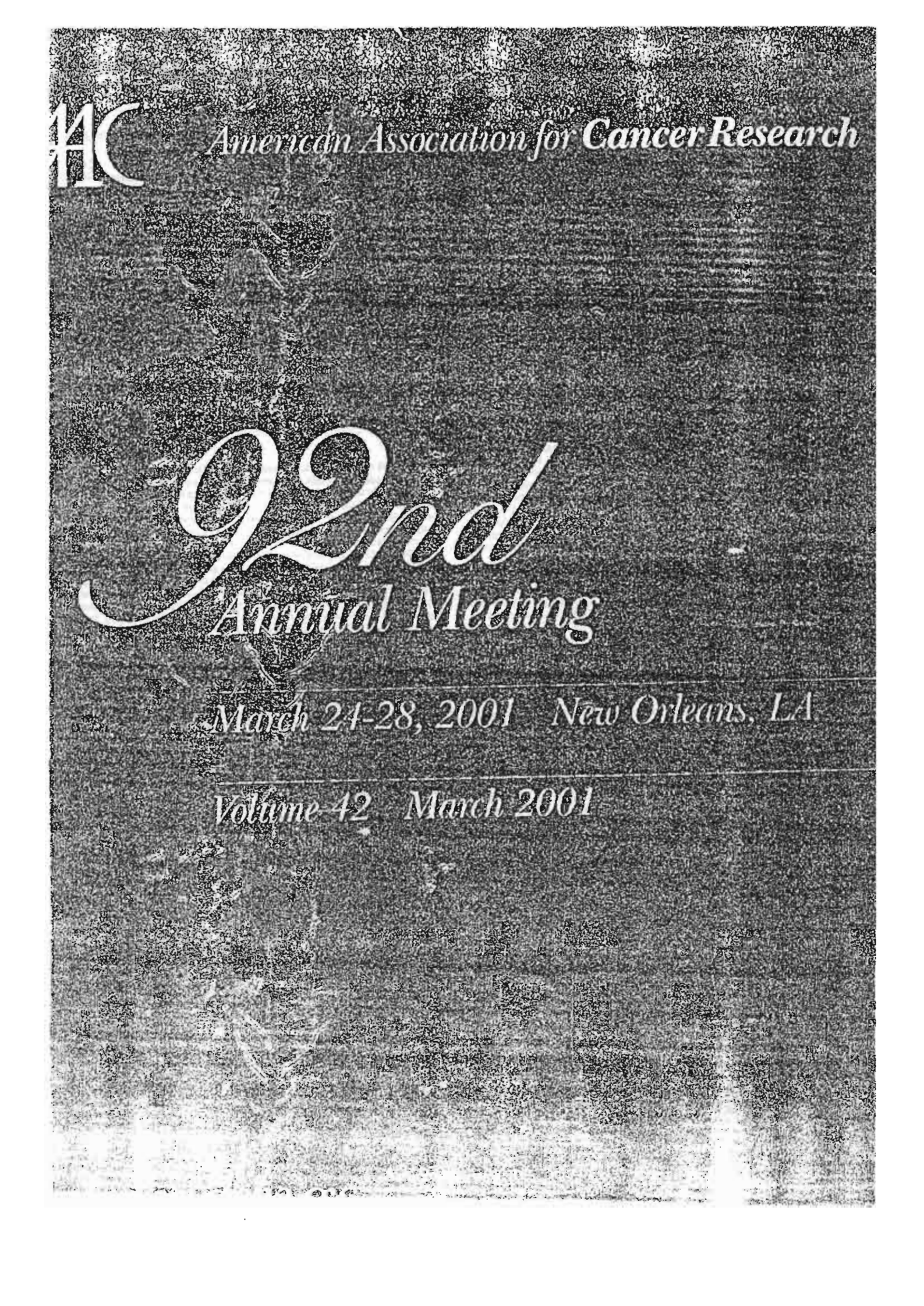
Takano M, Aida H, Tsuneki I, Takakuwa K, Hasegawa I, Tanaka H, Saito M, Tsuji S, Sonoda T, Hatae M, Chen JT, Takahashi K, Hasegawa K, Toyoda N, Saito N, Yakushiji M, Araki T, Tanaka K. Mutational analysis of BRCA1 gene in ovarian and breast-ovarian cancer families in Japan. Jpn J Cancer Res. 1997 Apr;88(4):407-13.

Tumor Registry Statistical Report 1998. Siriraj Cancer Center, Faculty of Medicine Siriraj Hospital, Mahidol University. Bangkok, Thailand (1998)

Vatanasapt, V., Martin, N., Sriplung, H., Chindavijak, K., Sontipong, S., Sriamporn, S., Parkin, D.M., and Ferlay, J. Cancer Incidence in Thailand, 1988-1991. Cancer Epidemiology, Biomarker&Prevention 4,475-483 (1995)

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion Jcollins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2 . Nature1995; 378: 789-792

Zheng L, Li S, Boyer TG, Lee WH. Lessons learned from BRCA1 and BRCA2. Oncogene. 2000 Dec 11;19(53):6159-75..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MOLECULAR BIOLOGY 26

#3929 **BRCA1 is Post-Transcriptionally Upregulated by Estrogen in MCF-7 Human Breast Cancer Cells.** Zhiquan Zhao and David Spriggs. *Memorial Sloan-Kettering Cancer Center, New York, NY.*

BRCA1 has been known as a tumor suppressor gene that is implicated in hereditary forms of breast and ovarian cancer. For over a decade, it has drawn much attention and its regulatory mechanisms have been widely investigated. Most studies indicate that BRCA1 is transcriptionally regulated in a proliferation-dependent manner. Studies by several groups have demonstrated that BRCA1 is upregulated in response to exposure to estrogen. We sought to find out if post-transcriptional mechanism is involved in the regulation of this gene. Preliminary experiments indicated that the in vitro transcribed BRCA1 3"– UTR RNA binds to a 35-KDa protein on Northwestern blotting. Twelve-hour or longer exposure of human breast cancer cells to Estradiol significantly increased the binding signal. Since HuR has been well known as an RNA-binding protein which is 36KDa (confirmed by Western on the same blot), we did the in vitro RNA-protein binding assay and were able to show that HuR indeed binds to BRCA1 3"– UTR. Further studies had located the responsible element to be in the first 1/3 of the whole 3"– UTR. Estradiol treatment of MCF-7 cells stably transfected with Luciferase constructs containing the BRCA1 3" – UTR sequence could considerably increase the Luciferase activity, further indicating that the 3"– UTR sequence is involved in the post-transcriptional regulation of the BRCA1 gene.

#3930 **Oligomerization of Stress-Induced Human GADD45 Protein.** Oleg Kovalsky and Albert Fornace. *National Institutes of Health, Bethesda, MD.*

Gadd45 is an 18 kDa acidic protein which is induced in the cell by various kinds of genotoxic stress. The exact function of the protein is not known. However, there is evidence for its involvement in growth control, maintenance of genomic stability, DNA repair, cell-cycle regulation and apoptosis. Consistently, Gadd45 has been shown to interact in vitro and/or in vivo with a number of proteins playing central roles in these cellular processes: PCNA, p21, cdc2-cyclin8 complex, MTK1 and histones. Adding to this complexity, we have found that Gadd45 self-associates in solution, both in vitro and when expressed in the cell. Moreover, Gadd45 can complex with two other members of Gadd45 family of stress-induced proteins, human MyD118 and CR6. Gel-exclusion chromatography, native gel analysis, chemical cross-linking and ELISA showed that recombinant Gadd45 forms dimeric, trimeric and tetrameric species in vitro. Deletion mutant and peptide scanning analyses suggest that Gadd45 has two self-association sites: within 48 N-terminal aa and within 40 C-terminal aa. The Kd of self-association is estimated to be 4 – 10 uM. Despite low-abundancy of Gadd45 in the cell, oligomer-forming concentrations can be realized in the foci-like nuclear structures formed by the protein. The potential role(s) of homo- and hetero-associations of Gadd45 proteins will be discussed.

#3931 **Overexpression of Human BRCA1 Enhances Nucleotide Excision Repair.** Anne-Renee Hartman, Dan Haber, and James Ford. *Harvard Medical School, Boston, MA, and Stanford University, Stanford, CA.*

Inheritec mutations of the BRCA1 gene result in a predisposition to the development of breast and ovarian cancer, and over 70% of BRCA1 mutant breast cancers also exhibit mutations in the p53 tumor suppressor gene. The BRCA1 gene product may be involved in DNA repair processes through its interaction with other repair and recombination proteins or as a transcription factor. BRCA1 transcriptionally regulates GADD45, a growth arrest and DNA damage-inducible gene that plays a role in the nucleotide excision repair (NER) pathway. We have evaluated the effect of overexpression of BRCA1 protein on NER of UV-radiation induced photoproducts in genomic DNA in vivo. Assays for global genomic NER were performed using U2OS human osteosarcoma cells engineered to contain an inducible, tetracycline-regulated BRCA1 expression vector and are wildtype for p53 (UBR60 cells), or null for p53 due to expression of the HPV E6 gene (E621 cells). A 4-5 fold induction of BRCA1 protein level was achieved in both cell lines following removal of tetracycline from the growth media, as determined by immunoprecipitations. Treatment of cells with UV-irradiation (10 J/m2) resulted in increased p53 levels in UBR60 cells, but p53 levels remained undetectable in E621 cells. Global genomic repair of UV-induced cyclobutane pyrimidine dimers (CPD's) was assessed using an immunoslotblot method and monoclonal antibodies specific for this photoproduct. E621 cells null for p53 exhibited poor NER, as we have previously shown for other p53 dysfunctional cells, removing only 3% of CPD's 24 hours following 10 J/m2 of UV-irradiation. However, induction of BRCA1 expression enhanced repair in E621 cells with 34% of CPD's removed 24 hours following UV-irradiation. Similarly, overexpression of BRCA1 in p53 wild-type UBR60 cells further enhanced repair with 42% of CPD's removed by 24 hours following UV, whereas, UBR60 cells which did not overexpress BRCA1, repaired only 23% of CPD's by 24 hours. Therefore, overexpression of BRCA1 protein resulted in enhanced global genomic repair, particularly in cells lacking p53, suggesting that induced BRCA1 may partially compensate for loss of p53 in the repair of UV-induced DNA damage. These results are the first to demonstrate an effect of the BRCA1 gene on NER of UV photoproducts. We hypothesize that BRCA1 may be involved in NER through transcriptional regulation of the GADD45 gene and are currently investigating this potential mechanism.

#3932 **Comparative Analysis of Mutation Detection Strategies for Localizing BRCA1 Mutations and Variants.** Elaine A. Ostrander, for the Breast Cancer Information Core Steering Committee. *Fred Hutchinson Cancer Research Center, Seattle, WA.*

To date, over 1600 germline mutations and variants of unknown significance have been identified in the BRCA1 and BRCA2 genes, which together account for the majority of hereditary breast and/or ovarian cancer syndromes (http://www.nhgri.nih.gov/intramural_research/Lab_transfer/Bic). The data generated derive from population-based case control studies, hospital-based series of breast cancer patients, women from high-risk breast cancer clinics, and breast and/or ovarian cancer families undergoing genetic testing. Decision making strategies, health policies, and research planning utilize these data, which were generated using a variety of mutation scanning technologies. We sought to determine the comparative sensitivity, specificity, and cost efficiency of the common mutation scanning technologies by blind testing of a panel of 65 samples containing 58 BRCA1 mutations/variants. Individual labs with established expertise in single strand conformational polymorphism analysis (SSCP), conformation-sensitive gradient gel electrophoresis (CSGE), two-dimensional gene scanning (TDGS) and denaturing high performance liquid chromatography (DHPLC) undertook the blind analysis and follow-sequencing. Insertions, deletions, missense changes and intronic changes were all present in the panel. Results were as follows: CSGE detected 76% of possible changes, SSCP 72%, and TDGS 88.2%. Using the reference standard of DNA sequencing, DHPLC provided the strongest results; detecting all 58 mutations/variants. We noted strong trends in the type of mutations that were undetected by CSGE, SSCP and TDGS, with all technologies having the highest level of detection for DNA deletions and the weakest for missense changes. We note also a certain amount of human and administrative errors which contributed to the lack of detection of some variants. Such errors, while reflected in the final detection rates, but are not inherent in the methods themselves. Principles garnered from these experiments will prove useful for interpreting applying similar data generated from the study of other cancer susceptibility genes.

#3933 **Mutational Profile of BRCA1/2 Mutations in Worldwide Populations: The Magic Project.** Csilla I. Szabo, Olga Smirnikova, Michael Badzioch, Sunita Saxena, Ashok Mukherjee, Xiangcheng Zhi, Ching-Sheng Wang, Pimpicha Patmasiriwat, Krich Bhothisuwan, Pablo Ruiz-Flores, Hugo Barrera-Saldaña, S. Kolman, Juan Llerena, David E. Goldgar, and Gilbert Lenoir. *Autonomous University of Nuevo Leon, Monterrey, Mexico, FIOCRUZ, Rio de Janeiro, Brazil, Institute of Pathology New Delhi, India, International Agency for Research on Cancer, Lyon, France, Mahidol University, Bangkok, Thailand and Tianjin Cancer Center, Tianjin, China.*

Our understanding of breast cancer genetics has been greatly enhanced by identification of BRCA1 and BRCA2. While approximately 60-80% of high-risk breast and/or ovarian cancer families are attributable to mutations in these genes, BRCA1/2 account for only 2-3% of all breast cancer cases and 10% of early onset breast cancer in the general population. However, due to population-specific founder effects, the contribution of these genes to early onset breast cancer may attain 30%. To date, the vast majority of the information that has been gamered about these genes derives from populations of Western European descent, whereas the role of these genes in ethnically diverse populations from other regions of the world remains largely unexplored. As the representation, and hence the relative proportion of global cancer burden due to disease in these populations is increasing, it is becoming increasingly important to understand the genetic basis of cancer in populations that have, to date, been underrepresented in such studies. At the IARC, we have initiated an international collaborative research program, 'Mutational Atlas of Genes in Cancer' (MAGIC), to characterize the mutational patterns and associated risks of known cancer susceptibility genes in geographically diverse populations. The initial focus of these studies has been on breast cancer genetics. We present data from analysis of the BRCA1 and BRCA2 genes in small series of high risk families/early onset breast cancer cases ascertained in Tianjin, China, Delhi, India; Bangkok, Thailand; Monterrey, Mexico; and Rio de Janeiro, Brazil. The highest proportion of truncating mutations of these genes was found in Thai patients (44%), which may be due in part to identification of two possible population-specific founder mutations in BRCA1. In contrast, only 3.5% truncating mutations were identified in a Mexican patient series biased towards early onset patients without reported family history. Frequency of deleterious mutations in BRCA1/2 in the remaining populations were intermediate (10% India, 19% China and 23% Brazil). However, this variation is likely influenced more substantially by differences in the composition of the patient series analysed rather than intrinsic differences in mutation prevalence between the respective populations. Analysis of more extensive patient series have been initiated to better characterize the population genetics of BRCA1 and BRCA2 world-wide.

#3934 **BRCA1 Activation of the GADD45 Promoter.** Wenhong Fan, Shuangan Jin, Hongchang Zhao, Felyce Fan, Tong Tong, Patricia Blanck, Albert J. Fornace Jr., and Qimin Zhan. *University of Pittsburgh School of Medicine, Pittsburgh, PA.*

Breast cancer susceptibility gene BRCA1 has been implicated in the control of gene regulation and such regulated genes are thought to mediate the biological role of BRCA1. Overexpression of BRCA1 induces GADD45, a p53-regulated and

MUTATIONAL PROFILE OF BRCA1/2 MUTATIONS IN WORLDWIDE POPULATIONS: THE MAGIC PROJECT

Szabo, Csilla I; Sinilnikova, Olga; Badzioch, Michael; Saxena, Sunita; Mukharjee, Ashok; Zhi, X.; Wang, Qing-Sheng; Patmasiriwat, Pimpicha; Bhothisuwan, Krich; Ruiz-Flores, Pablo; Barrera, Hugo; Koifman , S.; Llerena, Juan; Goldgar, David E; Lenoir, Gilbert

International Agency for Research on Cancer, Lyon, France; Institute for Molecular Pathology, New Delhi, India; Tianjin Cancer Center, Tianjin, China; Mahidol University, Bangkok, Thailand; University of Monterrey, Monterrey, Mexico and Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

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WHO collaboration group "MAGIC" with integration of our data of Breast Cancer genes

Will report to American Society of Cancer Research Mar, 2001

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P-139

NOVEL *BRCA1* AND *BRCA2* MUTATIONS IN FAMILIAL THAI BREAST AND/OR OVARIAN CANCERS.

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This study includes 23 Thai patients with breast and/or ovarian cancers. They were from 12 breast and/or ovarian cancers families (17 cases), 2 isolated cases of breast-ovarian cancers, and 4 isolated early-onset breast cancer cases. Variants of all exons of *BRCA1* and *BRCA2* were screened by heteroduplex analysis (HA) followed by DNA sequening. Five separated types of HA variants were observed within *BRCA1* and 5 within *BRCA2*. Sequencing information of these variants is shown in TABLE 2 and TABLE 3. Of these variants, 6 apparently exhibited the disease related mutations and they are localized only within exon 10 and 11 of *BRCA1* and *BRCA2*. Two types of conservative single base change missense mutations (MS) were detected in 4 families. Two types of polymorphisms and one intronic variant with unknown significance were found in other 3 families. Among families or isolated cases with disease-related mutations, two unrelated families share a same type of nucleotide change, the *BRCA1 3300delA*, and patients who exhibited this particular mutation had been experiencing ovarian cancer. An additional conservative single base MS was seen in one of the two families. Accordingly, based on sequencing information we found 3 separated types of disease-related mutations and four of them are novel mutations which have never been reported previously (*BRCA1-Tyr1ins20, 3300delA, BRCA2-6382delT, 5527del9*). The other type, *BRCA2 20-bp ins*, has been recorded once in BIC (S. Narod, Toronto). Our findings imply a strong evidence of *BRCA1* and *BRCA2* linkage in Thai breast and ovarian cancers and that the mutations reported herein could be specific mutation of Thais. Furthermore, the *3300delA* might be an ovarian cancer associated mutation within this region.

Of less importance are the *BRCA1* conservative MS (Thr1051Ser and Asp67Glu) and intronic polymorphism (IVS20−78(G>A)) as well as the *BRCA2* intronic variant (IVS20-26 (A>G)) and polymorphism (2565 G>T). Investigation of cases from different parts of the country or from neighboring countries within SEA is necessary for the future familial breast/ovarian cancers prevention and control in this region.

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P-140

CERVICAL ADENOCARCINOMA IN FILIPINOS

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NOVEL BRCA1 AND BRCA2 MUTATIONS IN FAMILIAL AND EARLY-ONSET BREAST AND/OR OVARIAN CANCER IN THAILAND

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Introduction

Breast cancer is one of the important world health problem, not only in industrialized countries, but also in developing countries where incidence increased gradually. In Thailand, the disease is the second leading cancer of women, followed only cancer of cervix uteri National estimation of age standardized incidence rate (ASR, world standard) of female breast cancer in this country shows an obvious increment; the estimated ASRs (per 100,000 women populations) were 7.0, 13.5 and 16.3 by the year 1985, 1990, and 1993 respectively. Incidence rate of breast cancer is highest in Bangkok (population 5.88 millions) with the ASR of up to 20.6, and it is the most frequent female cancer in this city followed by cervix uteri (ASR = 18.6). In Chiang Mai (popula-tion 1.37 millions) , breast cancer has been reported as a third frequent cancer after carcinoma of lung and cervix, but the incidence of breast cancer within this province appears to be the second aggregation in the country (ASR=15.2) following Bangkok metopolis. North-Easthern Thailand such as Khon Kaen province (population 1.62 millions) has shown a lowest incidence of breast cancer recorded since 1985 and the ASR in this province is only 8.6 . Male breast cancer, however, is very rare in Thailand. The ASR (world) of Thai male breast cancer is 0.1 .

Ovarian cancer is the second most frequent gynaecological malignancies of Thai women, exceeded only by cancer of cervix uteri. However, incidence rate of ovarian cancer is low with ASR (world) of 4.7 compared

to 19.9 per 100,000 population in Canada . Chiang Mai province has highest incidence rate with ASR of 6.0

Family history is an important risk factor of breast and ovarian cancers. Approximately, seven percent and ten percent of all breast and ovarian cancers respectively are inherited as autosomal dominant trait. The breast cancer susceptibility genes, *BRCA1* and *BRCA2* , are the potent genetic agents of hereditary breast and ovarian cancers with highly penetrance of 80 to 85 percent. These genes are tumor suppressor genes which functions of their protein products have not been exactly known. They are thought to involve in one or more of DNA repairing mechanisms in cooperating with RAD51 or some others such as ubiquitin DNA repairing protein . Also, their function as transcriptional regulators have been proposed and the development of breast cancer might be a reflection of loss function of BRCA1 or BRCA2 protein products in the family members with mutated of the gene.

Majority of mutation analyses with the coding sequence of *BRCA1* and *BRCA2* in breast and ovarian cancer come from north America and Europe. In Asia , however, there are only several reports from Japan , China, and Taiwan . We report herein *BRCA1* and *BRCA2* mutations as well as polymorphic amino acid changes in both members of breast and / or ovarian cancer risk families and isolated (single case) of early-onset breast or breast plus ovarian cancers in Thais. Mainly of the patients in this report were from central region of the country.

Patients

This work includes a total of 23 patients with breast and / or ovarian cancer. Seventeen patients were from 12 risk families. These families comprise of at least two affected cases of breast or ovarian cancer who were first degree relatives . The other six out of 23 patients were isolated cases without family history. Of these isolated cases , two had both breast and ovarian cancers within age under 45 years and four were breast cancer–only patients with the onset age under 35 years.All the patients had given informed consent before providing of their blood samples.

Mutation Analysis

Peripheral blood leukocytes were separated from whole blood specimens by standard dextran sedimentation technique. Genomic DNA was isolated by standard phenol-chloroform procedure described by Sambrook et al (1989). The purified genomic DNA samples were subsequently screened for *BRCA1* and *BRCA2* variants by PCR- based heteroduplex analysis according to Serova et al (1997). All 22 coding exons of *BRCA1* and 26 coding exons of *BRCA2* as well as the exon-intron junction regions were amplified using primer sequences described by Freidman et al(1994)and Tavtigian et al (1996), respectively. The PCR products were denatured at 90 °C for 5 minutes and cooled down to room temperature over at least 30 minutes to allow renaturation of the DNA strands. Homoduplex and heteroduplex were separated in non-denaturing 1xMDE polyacrylamide gel matrix(FMC Bioproduct , Rockland, ME) containing 7.5% glycerol at 300 to 600 V for 14 hours as per manufacturer's protocol. After vacuum drying at 80 ° C for 45 minutes, the gels were autoradiographed using Kodak BioMax MR film for at least 4 hours in room temperature. PCR products exhibiting variant bands in *BRCA1* or *BRCA2* genes were subsequently re-amplified and DNA sequenced by modified manual Sanger's dideoxy chain termination method(Sanger et al 1977) usingUSB PCR-product sequencing kit (Amersham).

Results

We analysed *BRCA1* and *BRCA2* gene mutations in 23 Thai patients with breast and/or ovarian cancers from 12 risk families (include 17 patients) and 6 isolated early-onset cases. Table 1 details age of onset of each patients, family history and *BRCA1* and *BRCA2* variant changes as screened by heteroduplex analysis. Among familial cases, age of onset of the patients were between 27 to 72 years. The cancer onset age among isolated cases were between 25 to 44 years.

Five separated variants were identified within *BRCA1* exons 5,10,11 and 20 and other five variants were found in *BRCA2* exons 10,11 and 21. Sequencing information of *BRCA1* and *BRCA2* variants are shown in Table 2 and Table 3, respectively. Of these ten variants , six were protein truncating mutations likely to be cancer-related.

All six cancer-related mutations were found within exons 10 and 11 of the *BRCA1* and *BRCA2* genes. Only one out of six mutations , the *BRCA2* 2041delA (exon 10) has been reported previously once (NIH- Breast Cancer Information Core, BIC). All others are novel mutations that have never been reported previously . Interestingly, one *BRCA1* mutation, 3300delA (exon 11) was a recurrent mutation which found in two unrelated families (family F7 (patient 17) and family F15 (patient 27and 28)). Patient 17 exhibited soly 3300delA while patient 27 and 28 (daughter, mother) apparently have 3300delA together with a conservative missense mutation 3271C>G(Thr1051Ser) . Notably, patient 17 is a member of a breast-ovarian cancer