行政院國家科學委員會補助專題研究計畫成果報告

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※BRCA1 及 BRCA2 抑癌基因與乳癌危險因子之互動探討※

※The interaction of BRCA1, BRCA2 tumor suppressor genes and non-genetic risk factors of breast cancer※

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計畫類別：個別型計畫

計畫編號：NSC 88-2314-B-038-120

執行期間：87 年 8 月 1 日至 89 年 7 月 31 日

計畫主持人：陳品玲

共同主持人：楊沂淵、張金堅

執行單位：台北醫學院公共衛生學系

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BRCA1 及 BRCA2 抑癌基因與乳癌危險因子之互動探討

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1. Abstract

We have analyzed the germline mutations in the coding regions of BRCA1 candidate genes from 38 patients with breast cancer and equal numbers of normal individuals using single-strand conformation polymorphism (SSCP) analysis on polymerase chain reaction (PCR)-amplified genomic DNA. A total of 2 putative disease-related alterations (5.3%) were recognized which sequences differ in only one residue in a span of 112 amino acids, resulting from a change of I to T. The frequency of germline mutations in the BRCA 1 genes in the present study is consistent with the previous study which indicated the mutation rate of BRCA 1 gene is approximately 5-10 %.

Keywords: breast cancer, BRCA1, SSCP, PCR

2. Introduction and methods

Breast cancer is one of the most common cancers. Roughly one in eight females will develop breast cancer in their lifetime (American cancer society, 1994). In recent years, it is now the leading increased cancer incidence in Taiwan. Unfortunately, therapy for breast cancer remains only modestly effective. The nature of this disease can be fully understood only in light of its etiology or natural development. Understanding the etiology of breast cancer may provide a basis for rationale medical management and therapy. The key issue is how to determine the genetic predisposition of breast cancer. During the past six years, numerous studies have proved the relationship of familial breast cancer and BRCA1 tumor suppresser genes (Easton et al., 1995; Hakansson et al., 1997, Ursin et al., 1997). An estimated 5% to 10% of breast cancer cases are thought to be due to such mutations (Easton et al., 1995). Cumulative risk of breast cancer in women with BRCA1 mutations is estimated to be 3.2% by age 30 years, 19.1% by age 40 years, 50.8% by age 50 years, 54.2% by age 60 years, and 85% by age 70 years. Although the incidence of breast cancer in Taiwanese is lower than Caucasians, Taiwanese breast cancer is characterized by younger age at tumor onset (Yang et al., 1997). This evidence suggests that genetic predisposition may play an important role of causality of breast cancer in Taiwan. Therefore, we conducted a genetic epidemiological study of breast cancer to determine the influence of BRCA1 in the pathogenesis of breast cancer. If the role of tumor suppresser genes can be identified, it is advisable for

high-risk women to take suitable genetic counseling and life style.

Materials and methods:

Genomic DNA isolation:

DNA samples were isolated and purified from the peripheral blood lymphocytes according to the protocol described previously (Sambrook et al., 1989). In brief, 2 ml of blood was drawn in the test tube containing EDTA anti-coagulant. After centrifugation at 3000 rpm for 5 min, the buffy coat in middle layer was collected and incubated with RBC lysis buffer for 10 min. The peripheral blood lymphocytes were pelleted at 1800 rpm for 3 min and DNAs were released in cell lysis buffer by incubating at 60°C for 60 min. After incubation, 1/4 volume of 10 M NH_4OAc was added and centrifugation was performed to remove the pellet. The genomic DNAs were precipitated by the addition of equal volume of isopropanol, washed with 1 ml of 70% of ethanol and finally dissolved in 200 μl of ddH_2O.

Polymerase chain reaction (PCR) and agarose gel electrophoresis:

A total of 50 μl PCR mixture was prepared by adding 4 μl of 2.5 mM dNTPs, 0.75 μl of primers (0.1 μg/ μl), 37.5 μl of ddH_2O, 0.25 μl of Taq polymerase, 5 μl of 10x buffer and 2.5 μl of genomic DNA (1 μg). The reaction was performed using Perkin Elmer 9600 system and the condition was set as 95°C, 2 min for separation; 60°C, 2 min for annealing and 72°C, 3 min for extension. A total of 35 cycles was used and 10 min was in the extension step in the last cycle. 5 μl of each PCR product was subjected to 2% agarose gel electrophoresis for 30 min. The amplified DNA band was visualized by ethidium bromide.

Single-stranded conformational polymorphism (SSCP):

PCR was carried out as described above using different combinations of primer sets (Table 1; Castilla et al., 1994). 3μl of PCR products was mixed with 1 μl of formaldehyde and 1 μl of 6x DNA loading buffer. The mixture was denatured at 98°C for 5 min and loaded onto a precast gel (GeneGel Excel 12.5/24, Pharmacia). After electrophoresis, the gel was stained with silver to reveal the single-stranded DNA band pattern as described as by the manufacturer (Plus One DNA Silver Staining Kit).

DNA sequence determination:

The nucleotide sequences of PCR products with various SSCP pattern were determined by ABI auto-sequencer (PRISM 377-96 DNA sequencer; PE Applied Biosystems, USA). The sequences were analyzed and aligned to determine the mutation sites in BRCA1 exons using DNAPLOT program.

3. Results and discussions:

We have isolated a total of 200 genomic DNA samples from 51 patients with breast cancer and 149 apparently normal individuals. The DNAs were used as the templates for the PCR amplification of the breast cancer susceptible gene, BRCA 1, to assess the mutation rate in those patients in Taiwan. As seen in figure 1, parts of exon 11 genes was successfully amplified from all 200 DNA sample using primers 2142 and 2504 (please see table 1) and shown as a band with the size of approximately 363 base pairs. To amplify the whole regions spanning the exon 11 gene, we also designed 9 sets of primers as shown in table 1 and used them for the PCR amplification from 10 DNA genomic samples. A representative results was shown in figure 2. A variety of PCR products ranking from 327 bp to 647 bp in size were detected. However, considering the numerous amount of work in the subsequent SSCP analysis and sequence determination, we chose the product with 363 bp in size from patients with breast cancer and several normal control individuals for SSCP analysis. The results were represented by figure 3 in which the difference

in mobility was detected in lanes 2 and 3 when compared with the DNA pattern detected in other lanes, indicating that those PCR products contain one or more nucleotide sequence variations. To locate the precise mutation site in these 2 variants, the PCR products from lanes 2 and 3 were subject to direct nucleotide sequence determination using an auto-sequencing device. The deduced amino acid sequences of the 2 variants were aligned and compared as demonstrated in figure 4. They differ in only one residue at position 89 in a stretch of 112 amino acids, resulting in a change of residue I to T. The significance of that change is presently unknown; however, it may be involved in the genetic predisposition of this disease. In summary, we identified 2 BRCA 1 variants among the 38 patients with breast cancer. The prevalence observed in our study (5.3%) is consistent with those of some previous studies (Garcia-Patino et al, 1998; Hoskins et al., 1995). However, for a gene as large as BRCA 1, SSCP and sequence analysis is labor-intensive. Given these limitations, the establishment of cooperative studies will be necessary to determine the prognostic value of germline and somatic mutation of the BRCA 1 gene.

Table 1: primers used for amplification and SSCP analysis of BRCA1 genes

Intron 10-GTGAA TTTTC TGAGA CGGAT GTA	1121-GGGAG TCCGC CTAT : ATTAC AT
1046-ACAGC CTGGC TTAGC AAGGA G	1430-ATGAG GATCA CTGG : CAGTA AGTC
1352-TGTAT TGGAC GTTCT AAATG AGGT	1834-TCTAT TGGGT TAGG/ TTTTT CTCA
1731-CAAAC GGAGC AGAAT GGTCA	2216-CTCTG GGAAA GTATI : GCTGT CAT
2142-GCAAC TGGAG CCAAG AAGAG TAAC	2504-TTTTG CCTTC CCTAG AGTGC TAAC
2448-TATGG CACTC AGGAA AGTAT CTCG	2948-TTTGG CATTA TCAAC TGGCT TATC
2900-AGGCT TTCCT GTGGT TGGT	3548-AGATG CATGA CTAC : TCCCA TAGG
3413-TCCTG GAAGT AATTG TAAGC ATCC	3959-AGATG CCTTT GCCA/ TATTA CCTG
3860-TGCTA CCGAG TGTCT GTCTA AGAA	4213-AAGTT TGAAT CCATC CTTTG CTCT

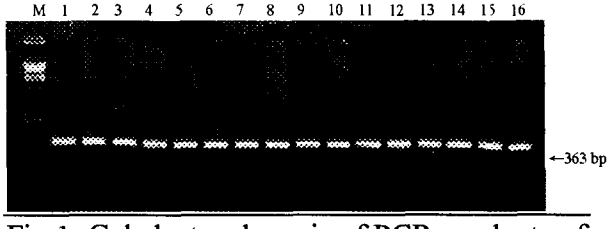

Fig 1. Gel electrophoresis of PCR products of exon 11 amplified by primers 2142 and 2504 from each BRCA individual. The size of PCR product is 363 bp. Lane M donates □ / Hind III DNA markers.

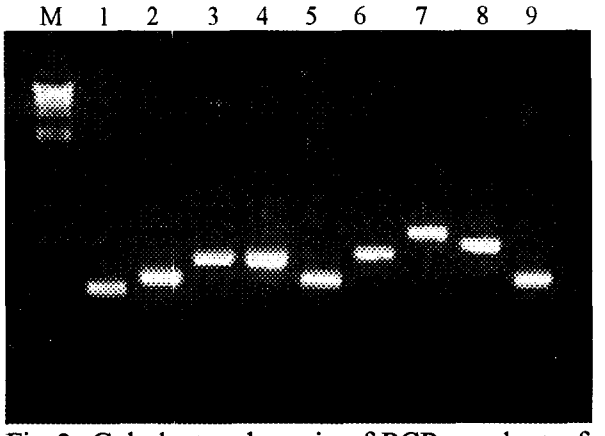

Fig 2. Gel electrophoresis of PCR product of BRCA1 exon 11 gene from one patient with breast cancer by using 9 sets of primers. In lane 1, the size of PCR product is 327 bp (intron10; 1121); lane 2, 385 bp (1046; 1430); lane 3, 483 bp (1352; 1834); lane 4; 486 bp (1731; 2216); lane 5, 363 bp (2142; 2504); lane 6, 501 bp (2448; 2948); lane 7, 647 bp (2900; 3548); lane 8, 547 bp (3413; 3959); lane 9, 354 bp (3860; 4213). Lane M is □ / Hind III DNA markers.

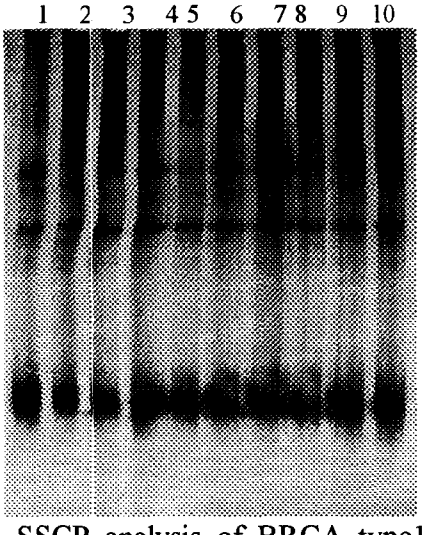

Fig 3. SSCP analysis of BRCA type1 gene exon 11 PCR-amplified by primers 2142 and 2504 from 10 different BRCA individuals. The product size is 363 bp. After DNA Electrophoresis System and silver stain analysis, difference in mobility can be detected in lanes 2 and 3.

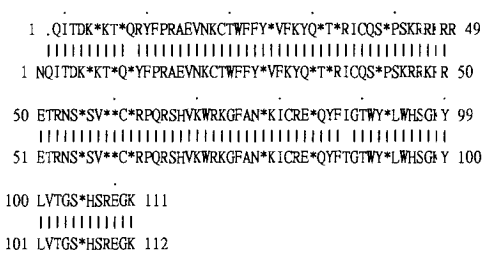

Fig 4. Deduced amino acid sequence alignment of PCR products of BRCA type 1 exon 11 with different mobility.

4. 計劃成果自評

本研究係邀請先前參與前一研究的乳癌個案提供血液樣本及環境因子暴露資料。所有研究對象之 DNA 均已完成分離萃取。唯實驗室分析基因型態，步驟繁複，考量經費限制下，以具有癌症家族史之 51 名乳癌患者之 DNA 執行 BRCA1 之基因分析。在 38 名完成 BRCA1 基因分析之患者中，僅 2 個個案出現變異（5.3%）。因此樣本數的不足成為本研究最主要的研究限制。雖然受限於樣本數的限制，但是本研究結果顯示台灣地區雖然屬於乳癌低發生率地區，BRCA1 變異現象與其他地區文獻資料吻合。未來可繼續探討 BRCA1 變異現象的影響。

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