

行政院國家科學委員會專題研究計畫 成果報告

乳癌致癌感受性與荷爾蒙代謝相關基因 (androgen
receptor , SRD5A2 , vitamin D receptor , SULT1A1 , HSD17B1 ,

計畫類別：個別型計畫

計畫編號：NSC92-2314-B-002-271-

執行期間：92年08月01日至93年07月31日

執行單位：國立臺灣大學醫學院外科

計畫主持人：黃俊升

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 期中進度報告

計畫中文名稱：乳癌致癌感受性與雄性素受體之基因多型性關連性研究

計畫英文名稱：

Breast cancer risk associated with genetic polymorphism of androgen receptor

計畫類別： 個別型計畫 整合型計畫

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計畫參與人員：

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執行單位：國立台灣大學醫學院外科

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一、中、英文摘要：

(一) 中文摘要。

關鍵詞: 乳癌、基因多形性、雄性素受體、致癌感受性

因個人基因型不同而致的乳癌致癌感受性是近來的研究重點。不同基因型與乳癌發生相關性的研究，在不同種族常有不同結果，這或許與各族群基因型比例不同。台灣乳癌好發的年齡層較年輕，亦隱含基因在台灣乳癌的重要角色。雄性素受體(Androgen Receptor)可以促進乳房腫瘤的生長與擴散。雄性素基因有一個三核甘酸重複的部位，其重複的長度因人而異。此基因的多形性與乳癌致癌感受性可能有關。吾人檢測 312 個台灣乳癌的檢體的雄性素受體基因型，並與文獻上的健康對照組做比較，發現短等位基因的長度在乳癌與健康婦女無差異，但長等位基因的長度則有顯著差異。若兩等位基因都屬於短等位基因，則罹患乳癌危險性最低，兩等位基因都為長等位基因，則罹患乳癌危險性最高。吾人的發現顯示雄性素受體 CAG 三核甘酸重複長度多形性與乳癌感受性顯著相關。

(二) 英文摘要。

Key word: Breast Cancer、genetic polymorphism、susceptibility、androgen receptor

Recent attention has brought focus both on genetic predisposition and gene-environmental interaction as susceptibility factors to the development of breast cancer. The ethnic discrepancies in cancer risk associated with various genotypes may be explained in part by the racial difference in the distribution of frequencies of the genotypic polymorphism. Taiwanese breast cancer is characterized by younger age at tumor onset, which suggests that genetic predisposition may be more important in Taiwanese breast cancers than in others. Androgen receptor can mediate breast tumor growth and progression. The androgen-receptor gene AR contains a highly polymorphic

CAG trinucleotide repeat in its first exon. Polymorphisms of the AR gene may be susceptibility factors for breast cancer. We investigated the genotypes of CAG repeat in 312 cases of breast cancer in Taiwan and compared the results with those of healthy controls in the literature. The frequency distributions of CAG-repeat lengths in the shorter allele of the case and control were not different, while those in the long allele were significantly different between the case and the control. Women of S/S genotype carries the lowest breast cancer risk and women of L/L carries the highest risk (odds ratio 7.4, 95%CI 3.3-16.5), while women of S/L genotype have breast cancer risk in-between (odds ratio 16.17, 95%CI 7.0-37.3). Our findings suggest the genotype polymorphism of androgen receptor may be a susceptibility factor to breast cancer in Taiwanese women.

二、報告內容：

(一) 前言與目的

Breast cancer is similar to other human cancers in that it arises from a multi-factorial process. Recent attention has focused both on genetic predisposition as a risk factors for developing breast cancer (1,2), as well as association with factors arising from modern affluence including diet and alcohol consumption (3,4). More importantly, the influence of reproductive factors strongly supports a hormonal role in cancer etiology (5,6).

The androgen-receptor gene AR, which functions as a ligand-dependent transcriptional activator in response to androgens, contains an highly polymorphic CAG trinucleotide repeat (AR-CAG) encoding glutamines in its first exon. The length of the AR-CAG polymorphism is inversely associated with the degree of transcriptional activation by the AR (7,8). It is proposed that the shorter the length of this glutamine tract, the greater the affinity of androgens to the AR and the greater the androgenic effects. AR mediates breast tumor growth and progression(9,10). Increased AR-CAG repeat length has also been associated with decreased prostate cancer risk, presumably because of a decreased ability of androgens to stimulate transcription of genes involved in prostate growth (11,12,13). These findings suggest that AR-CAG repeat-length polymorphism may be involved in modifying the development of diseases caused by alterations in endocrine signaling.

(二) 研究方法

All breast cancer patients have pathologically confirmed primary breast carcinoma, and are diagnosed and treated at National Taiwan University Hospital. A 10-ml sample of peripheral blood collected in acetate-citrate dextrose will be obtained from each breast cancer patient before treatment. The buffy coats of these specimens are prepared immediately and stored at -80°C until extraction of the genomic DNA. Genomic DNA is obtained by conventional proteinase K extraction and stored at -80°C.

Analysis for CAG Repeat Length in the Androgen Receptor

The CAG repeat region resides in the first exon of the gene. A system will be established to rapidly analyze the CAG repeat sequence length in a large number of samples(14). A set of oligonucleotide primers that flank the CAG repeat

(TCCAGAATCTGTTCCAGAGCGTGC and GCTGTGAAGGTTGCTGTTCCCTCAT) will be constructed. The DNA is amplified using these primers by PCR to produce fragments of the N-terminal domain of the AR. Primers are fluorescently labeled. The length of these fragments varies only by the number of CAG repeats. For rapid and accurate assessment of fragment length, the DNA fragments are run on a 6% denaturing polyacrylamide gel by automated fluorescence detection (Genescan; Applied Biosystems 377). Using a series of sequenced PCR products of varying size, fluorescently labeled DNA markers are used to create a standard curve of peak arrival time, allowing us to calculate the length of an unknown PCR product. Resolution to one 1-bp length using this system will be confirmed with direct DNA sequencing.

CAG Repeat Length in the Androgen Receptor for the control

The data of CAG Repeat Length in the AR of Taiwanese women were published by Yu et al (15). They enrolled 166 healthy women generated from the general population.

(三) 結果與討論

The frequency distributions of CAG-repeat lengths in the shorter and the longer allele of AR-CAG genotype carried by each woman of breast cancer and control were shown at table 1. The data of healthy controls were published by Yu et al (15). The frequency distributions of CAG-repeat lengths in the shorter allele of the case and control were not different, while those in the long allele were significantly different between the case and the control. When the allele with repeat number smaller than 22 was designated as short allele and those equal to 22 or larger as long allele, the frequency of three genotypes (S/S, S/L, L/L) was different significantly between case and control. Women of S/S genotype carries the lowest breast cancer risk and women of L/L carries the highest risk (odds ratio 7.4, 95%CI 3.3-16.5), while women of S/L genotype have breast cancer risk in-between (odds ratio 16.17, 95%CI 7.0-37.3) (table 2).

Our findings suggest the genotype polymorphism of androgen receptor may be a susceptibility factor to breast cancer in Taiwanese women. The frequency distributions of CAG-repeat lengths in the long allele were significantly different between the case and control. Women of S/S genotype carries the lowest breast cancer risk and women of L/L carries the highest risk (odds ratio 7.4), while women of S/L genotype have breast cancer risk in-between (odds ratio 16.17). Our results seem to support previous studies by Giguere et al and Liede et al. Giguere et al observed an inverse association of CAG repeat length on breast cancer risk. Liede has similar findings in Philippines that shorter

CAG repeat length genotypes of AR are protective.

The mechanisms underlying the association are not clear though. The length of the CAG repeat has been proposed to be inversely associated with the androgenic activity, the shorter the greater. Studies on breast cancer cell lines found that androgen can decrease breast epithelial cell proliferation. However, high androgen level may be associated the increased breast cancer risk among postmenopausal women since androgen is the only source of estrogen, through the conversion by aromatase, in postmenopausal women. Whether the results in our study can be modified by recognized breast cancer risk factors, especially hormonal factors, is unknown so far, since the controls in this study were derived from literature which has no information of recognized breast cancer risk factors. We will use our own controls, which has information of recognized breast cancer risk factors, enrolled in our hospital to do the molecular analysis and statistical analysis again. Hopefully, we can find some clue. Our healthy controls were randomly selected from the physical check-up ward at the same hospital during the same study period. Control subjects received a one-and-one-half day comprehensive health examination, and showed no evidence of breast cancer, any suspicious precancerous lesions of the breast, or other cancers. An experienced research nurse was assigned to administer a structured questionnaire to both case and control subjects. The information collected included age at diagnosis, family history of breast cancer (first-degree relatives), history of breast biopsy, history of breast screening, age at menarche and/or menopause, parity, age at FFTP, number of pregnancies, history of breast feeding, use of oral contraceptives, HRT, history of alcohol consumption and cigarette smoking, ethnic background, residence area, family income, and education level. The BMI and menopausal status were also recorded. Women younger than 55 years who had undergone hysterectomy, but not bilateral oophorectomy, were classified as unknown in terms of menopausal status.

TableI. Frequency distributions of CAG-repeat lengths in the shorter and the longer allele of AR-CAG genotype carried by each women

CAG repeat*	breast cancer case N=312		control subjects N=166	
shorter allele	no	%	no	%
≤16	31	9.9	16	9.6
17-18	22	7.1	9	5.4
19	25	8	12	7.2
20	31	9.9	31	18.7
21	60	19.2	36	21.7
22	72	23.1	29	17.5
≥23	71	22.8	33	19.9
	312	100	166	100

No significant for Chi-Square test ($\chi^2_6 = 9.268, \rho = 0.159$)

CAG repeat*	breast cancer case N=312		control subjects N=166	
Longer allele	no	%	no	%
20	11	3.5	12	7.2
21	23	7.4	21	12.7
22	47	15	22	13.2
23	27	8.7	24	14.5
24	65	20.8	18	10.8
25	39	12.5	20	12
26	42	13.5	23	13.9
≥27	58	18.6	26	15.7
	312	100	166	100

No significant for Chi-Square test ($\chi^2_7 = 16.8214$, $\rho = 0.0186$)

由 chi-square test 得知 在 longer allele case 與 control 組, 頻率分布有統計上顯著不同

Table II.

Genotype	case	control	OR	95%CI	P value
<22(S/S)	8	38			
S/L	161	103	7.4*	(3.3-16.5)	<0.0001
L/L	143	42	16.17*	(7.0-37.3)	<0.001

No significant for Chi-Square test ($\chi^2_2 = 57.8$, $\rho < 0.0001$)

以 repeat no=22 為 cut point , 發現基因型分布在 case 及 control 組間有顯著差異

計畫成果自評

While numerous studies have been conducted in Western countries to assess the epidemiology of breast cancer, there is continuing interest in studying Asian populations because their different risk profiles may help to explain the lower occurrence of the disease. In contrast to incidence rates prevailing in Western countries, Taiwan is considered to have the lowest incidence of breast cancer in the world. However, the incidence of Taiwanese breast cancer has increased about three folds in the past two decades. More importantly, Taiwanese breast cancer is characterized by younger age at tumor onset. More than fifty percent of total breast cancer diagnosed annually in Taiwan is composed of patients younger than 50 years of age, and this proportion is higher than those observed in Western populations. The evidences of earlier tumor onset suggest that genetic predisposition may be more important in Taiwanese breast cancers than in others.

Different ethnic groups with different genotype frequencies and different exposure doses will probably reveal different findings in the study of gene-environmental interactions. In addition, the advantage of conducting such studies of gene-environmental interactions in an area with a low, but increasing incidence of breast cancer is the less contribution from major risk factors and the relative importance of minor risk factors. Therefore, different risk profiles in Taiwanese woman may not only help to explain the lower occurrence of the disease in Taiwan, but also shed light on the etiology of breast carcinogenesis, which probably won't be discovered in Western populations.

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