

*MUTATION IN BRIEF*

# Recurrent BRCA1 and BRCA2 Germline Mutations in Ovarian Cancer: A Founder Mutation of BRCA1 Identified in the Chinese Population

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**Previous mutational analysis for BRCA gene mutations in sporadic ovarian cancer occurring in Chinese patients in Hong Kong identified six germline BRCA1 mutations and one germline BRCA2 mutation, six of which were novel (Khoo et al., 2000). Knowledge of BRCA gene mutations in the Chinese population is relatively scant. In this study, we focussed on whether any of these mutations could be recurrent in our Chinese population, making use of archival paraffin embedded tissue. A consecutive series of 214 ovarian cancer cases, half of Southern Chinese origin from Hong Kong whilst the other half of Northern Chinese origin from Beijing were used for the study. We identified one further novel mutation, 1081delG, in BRCA1. This was found to occur in two unrelated individuals with shared haplotype as revealed by allelotype analysis, thus demonstrating founder effect. Two other recurrent mutations were also identified, the 2371-2372delTG mutation in BRCA1 and the 3337C>T mutation in BRCA2 recurring in two and three unrelated individuals respectively, giving an overall prevalence 4.7% of recurrent BRCA mutations in ovarian cancer in the Southern Chinese population. Most importantly, all our recurrent mutation carriers were identified from Southern Chinese patients from Hong Kong whilst such mutations were absent in samples from the Northern Chinese. Our findings indicate possible heterogeneity in the BRCA genotype between Northern and Southern Chinese. The identification of a founder mutation and two recurrent mutations moreover, has important implications towards screening strategies for breast and ovarian cancer among Chinese of southern ancestral origin who are now dispersed throughout the world. © 2002 Wiley-Liss, Inc.**

KEY WORDS: BRCA1; BRCA2; recurrent and founder mutations; Chinese; ovarian cancer

## INTRODUCTION

Specific BRCA1 (MIM# 113705) founder mutations and recurrent mutations have been found which are apparently characteristic to particular ethnic origins, the most well known being those of the Askenazi Jews, Icelanders, Norwegians, Swedes, French Canadian and Dutch (Neuhausen, 1999). Amongst the Asian population, the Japanese are the only ones who have identified a founder mutation (Inoue et al., 1995). Knowledge of BRCA gene mutations in the Chinese population is relatively scant. The few reports of BRCA mutations in Chinese, are mostly in relation to breast cancer (Li et al., 1999; Tang et al., 1999; Khoo et al., 2000; Sng et al., 2000), including our report of somatic BRCA1 mutations in both breast and ovarian cancer (Khoo et al., 1999), and a single BRCA1 mutation reported from Mongolia (Elit et al., 2001). We recently identified a relatively high incidence of six

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(11.3%) germline BRCA1 mutations and one germline BRCA2 (MIM# 600185) mutation in an unselected series of ovarian cancer patients diagnosed in Chinese from Hong Kong (Khoo et al., 2000). Six of these mutations are novel. Our findings contrasts with the 2 - 6% incidence of germline BRCA1 mutations reported amongst the Caucasian population, in ovarian cancer unselected for age and family history (Matsushima et al., 1995; Takahashi et al., 1995; Stratton et al., 1997; Janezic et al., 1999). Although Moslehi R et al 2000 had found a high incidence rate of 41.3% BRCA1 mutations in unselected ovarian cancer cases in the Jewish population, all but one of these mutations were of the three well known founder mutations characteristic of Askenazie Jews. Our 11.3% relatively high incidence of BRCA1 mutations previously detected from screening just 53 cases from our Chinese population suggested it needed confirmation from further screening a larger series of cases and particularly whether possible recurrent or founder mutations may exist in our population.

Screening the entire length for both BRCA1 and BRCA2 genes on a large number of cases to verify our high incidence is costly and also requires availability of frozen tissue or blood samples. We therefore focused our investigation specifically to find out whether any of our six novel BRCA mutations could be recurrent or characteristic to sporadic ovarian cancer in the Chinese population. Heteroduplex and SSCP analysis were used for this study. Moreover, we selected two geographically distinct population samples of Chinese in our study – that of Northern Chinese from Beijing and Southern Chinese from Hong Kong. Apart from the single BRCA1 mutation reported from Mongolia, there is to date no know publication of BRCA mutations from the Northern Chinese population.

## MATERIALS AND METHODS

### Samples

Cases of primary epithelial ovarian carcinomas diagnosed between the years 1986-1998 were retrieved from the files of the Departments of Pathology, Queen Mary Hospital, Hong Kong; Pamela Youde Nethersole Hospital, Hong Kong; Beijing Medical University, Beijing; and the PLA Hospital, Beijing. Histology of all cases was reviewed. There were a total of 106 consecutive cases from Hong Kong and 108 consecutive cases from Beijing. After deparaffinization, DNA was extracted from representative tissue blocks by standard procedures.

### PCR reaction

Specific BRCA1 and BRCA2 coding regions, ranging from 112-170 bp length, were amplified by polymerase chain reaction (PCR). The PCR reaction was carried out in a total volume of 30  $\mu$ l containing 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.0 mM MgCl<sub>2</sub>, 0.10% gelatin, 200  $\mu$ M of each dNTP, 200 nmol of each forward and reverse primers, 2.5 units of AmpliTaq DNA polymerase and 0.1-0.2 $\mu$ g of nucleic acids. Amplification consisted of 35 cycles each of 30 sec at 94°C, 30 sec at 55°C and 120 sec at 72°C. The primer pairs were designed to screen for the mutations 1080delT, 2371-2372delTG, 3976-3979delGTGA, 633C>T, IVS 22+7 A>G in BRCA1 and 3337C>T in BRCA2.

### Heteroduplex analysis

Heteroduplex analysis was used to screen for the deletion mutations and performed according to previously described protocols (Ozcelik et al., 1996; Ozcelik et al., 1997). A sample of the PCR product is mixed with an equal volume of PCR product from normal control DNA and electrophoresis loading buffer (15% sucrose + 0.05% xylene cyanol + 0.05% bromophenol blue). Heteroduplex formation is carried out by denaturing the mixture for 5 min at 94°C and cooling slowly to room temperature. The samples were then subjected to electrophoresis, on 12% polyacrylamide gels or MDE gels, at 60 – 80V for 18 h.

### SSCP analysis

Multiplex SSCP was used to screen for BRCA1 and BRCA2 mutations IVS 22+7 A>G and 3337C>T respectively, whilst a separate SSCP gel was used to screen for the BRCA1 633C>T mutation. For the multiplex SSCP, 3 $\mu$ l of each of the two PCR products and 6 $\mu$ l of loading dye were mixed together. The mixture was heated at 94°C for 8 minutes and then was snap cooled on ice. A total of 12 $\mu$ l of the mixture was then subjected to electrophoresis on a 12% (29:1) polyacrylamide gel with the 0.5xTBE as buffer, at 4°C for 3.5W for 21 hours. Samples screened for mutation C633T were subjected to electrophoresis at constant 350V for 6.25 hours. After electrophoresis, a fluorescent nuclei acid gel stain, SYBR Gold, (Molecular Probes, Eugene, OR.) was used to

visualize the DNA. Staining was performed for 2 times for 10 minutes each time using 20m and 10 ml of 1 x SYBR gold in 1xTBE respectively. DNA was visualized by exposure to UV light.

#### DNA sequence analysis and allelotyping

For those samples forming heteroduplex bands or SSCP band shifts, genomic DNA was further extracted from the patient's tumor and non-tumor tissue and further analyzed together by direct sequencing of both strands using the dRhodamine Terminator Cycle Sequencing kit on an ABI PRISM 377 Automated DNA Sequencer (Applied Biosystems, Foster City, CA). Tumor samples from each of the recurrent BRCA1 mutations identified were analyzed for allelic association using four different highly polymorphic microsatellite markers located on chromosome 17q, D17S1322, D17S855, D17S1185 and D17S1325, the former two intragenic to BRCA1 and the latter two flanking the BRCA1 gene (Figure 1). The cases harboring the recurrent BRCA2 mutation were analyzed using microsatellite markers, D13S260 and D13S267 flanking the centromeric and telomeric ends of the BRCA2 gene respectively. PCR products with fluorescent dye labeled primer were mixed with loading dye which contains fluorescent labeled internal size marker. The mixture was subjected to electrophoresis on an ABI PRISM 377 Automated DNA Sequencer (Applied Biosystems, Foster City, CA) and the data generated analyzed by the GeneScan and Genotyper (Version 3.1) software provided by the sequencer supplier.

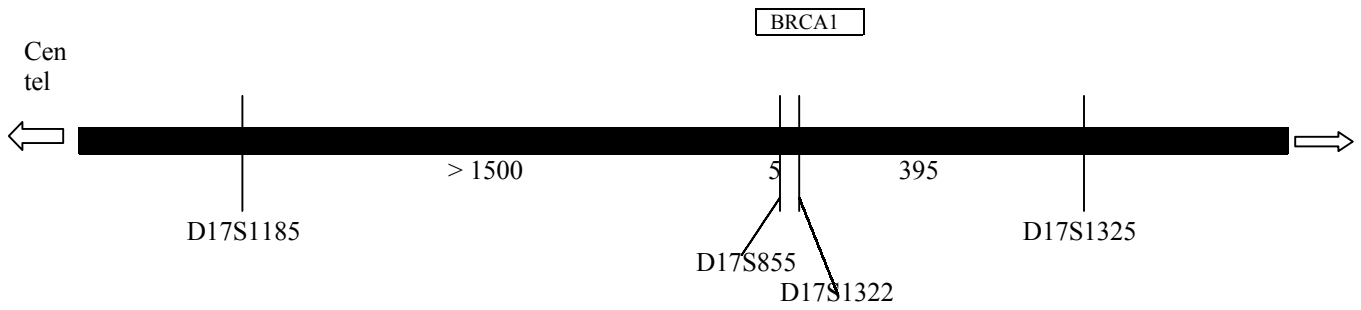
### RESULTS AND DISCUSSION

Screening for the three BRCA1 deletion mutations (1080delT, 2371-2372delTG and 3976-3979delGTGA) by heteroduplex analysis identified sequence alterations in three cases. One was a recurrent 2371-2372delTG frame-shift mutation. Whilst no additional 1080delT mutation or 3976-3979delGTGA mutation were detected, a novel frame-shift mutation, 1081delG, which causes a truncated product at codon 328, was identified in two further unrelated patients. SSCP analysis identified sequence alterations in two unrelated cases. Both were shown to be a BRCA2 recurrent non-sense mutation 3337C>T. No additional BRCA1 633C>T or IVS 22+7A>G mutations were detected.

All five cases were confirmed to harbor the mutations in the germline, on further PCR and DNA sequencing of non-tumor tissues. All recurrent mutations were of Southern Chinese origin from Hong Kong with none coming from Northern Chinese origin. The clinical characteristics of the recurrent BRCA1 mutation carriers (Table 2) show that each recurrent mutation appeared to be associated with a distinct phenotype, serous cystadenocarcinoma for the cases with the 2371delTG mutation, and endometrioid adenocarcinoma for the cases with the 1081delG mutation. This however was not the case for the BRCA2 recurrent mutation.

Similar to our observations reporting our novel BRCA1 mutations in our previous paper (Khoo et al., 2000), our recurrent BRCA1 mutations are also noted to be located at the 5' region of the gene, which is concurrent with the reported connection between the risk of ovarian cancer and mutations in the 5' two thirds of the gene (Gayther et al., 1995)

Haplotype analysis of patients with recurrent mutation showed that in the two cases (H64 and H121) with the recurrent mutation 1081delG, shared haplotype was seen for all four markers. The haplotype 5-2-4-10, at loci D17S1185, D17S855, D17S1322 and D17S1325 was common for both cases (Table 1b). Patient H121 developed endometrioid adenocarcinoma of the ovary at the age of 39. She had a maternal cousin with ovarian carcinoma who was not available for genetic testing. Patient H64 did not have a family history of cancer and was aged 72 when she developed endometrioid adenocarcinoma of the ovary. As members from both families were unfortunately unavailable for testing, reconstruction of disease haplotype in the families was not possible. We however determined the frequencies of alleles of the four micro-satellite markers used in a further 75 Hong Kong Chinese known not to carry BRCA1 mutations. The frequency of the common alleles shared by our two cases compared with that of the Chinese population is shown (Table 1) and strongly supports the possibility that patients H121 and H64 share a common ancestral mutation. The cases with the recurrent BRCA1 mutation 2371-2372delTG showed no allelic association. Our three cases carrying the recurrent BRCA2 mutation 3337C>T, showed sharing of some alleles, suggesting some degree of shared ancestry but not sufficient to demonstrate founder effect.



Distances in kb

| Marker   | Allele size | Heterozygosity (GDB) | Heterozygosity (Chinese samples) |
|----------|-------------|----------------------|----------------------------------|
| D17S1185 | 203-249bp   | 87%                  | 82%                              |
| D17S855  | 143-155bp   | 82%                  | 81%                              |
| D17S1322 | 121-139bp   | 66%                  | 81%                              |
| D17S1325 | 155-185bp   | 89%                  | 90%                              |

**Figure 1.** Physical map of the four microsatellite markers used. The location of the BRCA1 locus with respect to these markers is shown. Values given between the markers denote the distances (in kb) between markers, taken from Neuhausen et al, 1994 and 1996. Allele size and heterozygosity data are taken from the Genome Database (GDB) <http://www.gdb.org/>). Heterozygosity values calculated from the normal Chinese population are also given. Cen = centromeric end; tel = telomeric end.

**Table 1. Allele frequency of microsatellite markers intragenic and flanking the BRCA1 gene in the Southern Chinese population**

| Marker          | Frequency     | Marker         | Frequency     | Marker          | Frequency     | Marker          | Frequency     |
|-----------------|---------------|----------------|---------------|-----------------|---------------|-----------------|---------------|
| <b>D17S1185</b> |               | <b>D17S855</b> |               | <b>D17S1322</b> |               | <b>D17S1325</b> |               |
| Allele          |               | Allele         |               | Allele          |               | Allele          |               |
| 1               | 0.01          | 1              | 0.03          | 1               | 0.01          | 1               | 0.01          |
| 2               | 0.05          | 2              | 0.11 H121 H64 | 2               | 0.06          | 2               | 0.01          |
| 3               | 0.13          | 3              | 0.31          | 3               | 0.45          | 3               | 0.02          |
| 4               | 0.26          | 4              | 0.16          | 4               | 0.27 H121 H64 | 4               | 0.02          |
| 5               | 0.26 H121 H64 | 5              | 0.29          | 5               | 0.16          | 5               | 0.04          |
| 6               | 0.17          | 6              | 0.09          | 6               | 0.04          | 6               | 0.06          |
| 7               | 0.05          | 7              | 0.01          |                 |               | 7               | 0.09          |
| 8               | 0.07          |                |               |                 |               | 8               | 0.07          |
|                 |               |                |               |                 |               | 9               | 0.07          |
|                 |               |                |               |                 |               | 10              | 0.13 H121 H64 |
|                 |               |                |               |                 |               | 11              | 0.26          |
|                 |               |                |               |                 |               | 12              | 0.02          |
|                 |               |                |               |                 |               | 13              | 0.06          |
|                 |               |                |               |                 |               | 14              | 0.08          |
|                 |               |                |               |                 |               | 15              | 0.01          |
|                 |               |                |               |                 |               | 16              | 0.03          |

**Table 2. Recurrent BRCA1 and BRCA2 mutations detected and clinical characteristics of carriers**

| CASE   | AGE | MUTATION                | Codon change                  | Family history of cancer      | Cancer type                                     | Personal history of cancer |
|--------|-----|-------------------------|-------------------------------|-------------------------------|---|----------------------------|
| G116*  | 44  | 2371-2372delTG<br>BRCA1 | Frame-shift X<br>at codon 760 | Liver (F),<br>Esophagus (MGM) | Serous cystadenocarcinoma, grade 3              | Endometrial carcinoma      |
| H66    | 71  | 2371-2372delTG<br>BRCA1 | Frame-shift X<br>at codon 760 | Nil                           | Serous cystadenocarcinoma, grade 2              | None                       |
| H64 #  | 72  | 1081delG<br>BRCA1       | Frame-shift X<br>at codon 340 | Nil                           | Endometrioid adenocarcinoma,<br>grade 3         | None                       |
| H121 # | 39  | 1081delG<br>BRCA1       | Frame-shift X<br>at codon 340 | Ovary (C)                     | Endometrioid adenocarcinoma,<br>grade 1         | None                       |
| 50*    | 64  | 3337C>T<br>BRCA2        | Q1037X                        | Nil                           | Mucinous cystadenocarcinoma,<br>grade 3         | None                       |
| PY23   | 69  | 3337C>T<br>BRCA2        | Q1037X                        | Nil                           | Bilateral serous cystadenocarcinoma,<br>grade 3 | None                       |
| H71    | 44  | 3337C>T<br>BRCA2        | Q1037X                        | Nil                           | Bilateral serous cystadenocarcinoma,<br>grade 3 | None                       |

\*These two mutation carriers were identified in our previous study (Khoo et al, 2000). # novel mutation in this article. F, father; MGM, maternal grandmother; C, maternal cousin.

The clinical characteristics of the recurrent BRCA1 and BRCA2 mutations show that the new founder mutation seems to be related to endometrioid adenocarcinoma whilst the other recurrent mutations appear to exhibit another distinct phenotype. However more recurrent cases need to be identified to confirm this association. It is of interest to note that for all the recurrent mutations and new founder mutation identified, breast cancer was not involved in any of the carriers nor in their families. This may be related to the relatively lower incidence of breast cancer in the Chinese population in comparison with that of Caucasians. The age standardized incidence rates of breast cancer in the United States of America and the United Kingdom, range from 90.7- 67.9 per 100,000 respectively. In contrast, the incidence of breast cancer reported amongst the Chinese population is 34.0 and 24.6 per 100,000 from Hong Kong (Southern Chinese) and Tianjin (Northern Chinese) respectively (Parkin et al., 1997). The lack of strong family history in this current study as well as that in our previous report (Khoo US et al., 2000) suggests that our mutations identified, which are apparently unique to Southern Chinese are probably of low penetrance.

Whilst BRCA1 and BRCA2 mutations have largely been reported to be scattered throughout the whole gene, there are an increasing number of reports of the presence of recurrent mutations peculiar of populations of difference ethnic or geographic origin. These differences may be partly due to founder mutations, which presumably arose in a single ancestor of a specific ethnic group initially established by a small number of people which later expanded (Neuhausen, 1999). Founder effects are most prominent in isolated populations that undergo rapid expansion from a limited number of ancestors. Historically, China had been largely in isolation from the rest of the world for a few centuries. In fact, a founder mutation in the hMLH1 gene has just recently been reported in the Hong Kong Chinese population (Chan et al., 2001). Our characterization of a BRCA1 founder mutation with a possible distinct phenotype of Southern Chinese origin and our overall prevalence 4.7% of recurrent BRCA mutations in ovarian cancer in is of importance towards assisting BRCA screening strategies in our region.

Important also is our finding of recurrent BRCA mutations identified only in the patients from Hong Kong and not from Beijing. The Chinese population in Hong Kong, which is Cantonese speaking, consists predominantly of immigrants from the southern provinces of China, namely Guangzhou, in contrast with the population from Beijing in Northern China. This observation supports previous publications suggesting Southern Chinese have distinctive genetic characteristics from that of the Northern Chinese. Studies on glucose-6-phosphate dehydrogenase deficiency, Thalassemia and debrisoquin hydroxylation phenotype have reported the finding of genetic variation amongst Chinese subjects from different regions (Wang et al., 1993; Tang et al., 1992; Tang et al., 1993; Liu et al., 1997). Tang et al. 1999 have also identified a recurrent BRCA1 mutation 589-590delCT related to early onset breast cancer in 3 unrelated patients of Southern Chinese origin. No other recurrent BRCA mutations have hitherto been reported amongst the Chinese.

Our discovery of a founder mutation and three other recurrent BRCA mutations unique to Southern Chinese have important implications towards genetic testing for BRCA and clinical management in Chinese of southern origin who are now dispersed all over the world.

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#### REFERENCES

- Elit L, Jack E, Kwan E, Baigal G, Narod S. 2001. A unique BRCA1 mutation identified in Mongolia. *Int J Gynecol Cancer* 11(3):241-3.
- Inoue R, Fukutomi T, Ushijima T, Matsumoto Y, Sugimura T, Nagao M. 1995. Germline mutations of BRCA1 in Japanese breast cancer families. *Cancer Res* 55:3521-24.
- Janezic SA, Ziogas A, Krumroy LM, Plummer SJ, Cohen P, Gildea M, Barker D, Haile R, Casey G, Anton-Culver H. 1999. Germline BRCA1 alterations in a population based series of ovarian cancer cases. *Hum Mol Genet* 8(5):889-897.
- Gayther SA, Warren W, Mazoyer S, Russel PA, Harrington PA, Chiano M, Seal S, Hamoudi R, van-Rensburg EJ, Dunning AM, Love R, Evans G, Easton D, Clayton D, Stratton MR, Ponder BAJ. 1995. Germline mutations of the BRCA1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. *Nat Genet* 11: 428-423.
- Khoo AS, Balraj P, Volpi L, Nair S. 2000. A new BRCA1 germline mutation (E879X) in a Malaysian breast cancer patient of Chinese descent. *Hum Mutat* 15(5):485.
- Khoo US, Ngan HYS, Cheung ANY, KYK Chan, Jing Lu, Chan VWY, Lau S, Andrulis IL, Ozcelik H. 2000. Mutational analysis of BRCA-1 and BRCA-2 genes in Chinese ovarian cancer identifies 6 novel germline mutations. *Human Mutat* 16:88-89.
- Khoo US, Ozcelik H, Cheung ANY, Chow LWC, Ngan HYS, Done SJ, Liang ACT, Chan VWY, Au GKH, Ng WF, Poon CSP, Leung YF, Loong F, Ip P, Chan GSW, Andrulis IL, Lu J, Ho FCS. 1999. Somatic mutations in the BRCA1 gene in Chinese sporadic breast and ovarian cancer. *Oncogene* 18:4643-6.
- Li SSL, Tseng HM, Yang TP, Liu CH, Teng SJ, Huang HW, Chen LM, Kao HW, Chen JH, Tseng JN, Chen A, Hou MF, Huang TJ, Chang HT, Mok KT, Tsai JH. 1999. Molecular characterization of germline mutations in the BRCA1 and BRCA2 genes from breast cancer families in Taiwan. *Hum Genet* 104:201-4.
- Liu TC, Lin SF, Yang TY, Lee JP, Chen TP, Chang JG. 1997. Prenatal diagnosis of thalassemia in the Chinese. *Am J Hematol* 55(2):65-8.
- Matsushima M, Kobayashi K, Emi M, Saito H, Saito J, Suzumori K, Nakamura Y. 1995. Mutation analysis of the BRCA1 gene in 76 Japanese ovarian cancer patients: four germline mutations, but no evidence of somatic mutation. *Hum Mol Genet* 4:1953-1956.
- Moslehi R, Chu W, Karlan B, Fishman D, Risch H, Fields A, Smotkin D, Ben-David Y, Rosenblatt J, Russo D, Schwartz P, Tung N, Warner E, Rosen B, Friedman J, Brunet JS, Narod SA. 2000. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. *Am J Hum Genet* 66(4):1259-1272.
- Neuhausen SL. 1999. Ethnic differences in cancer risk resulting from genetic variation. *Cancer* 86(8):1755-1762.
- Ozcelik H, Antebi YJ, Cole DE, Andrulis IL. 1996. Heteroduplex and protein truncation analysis of the BRCA1 185delAG mutation. *Hum Genet* 98:310-2.
- Ozcelik H, Schmocker B, Di Nicola N, Shi XH, Langer B, Moore M, Taylor BR, Narod SA, Darlington G, Andrulis IL, Gallinger S, Redston M. 1997. Germline BRCA2 6174delT mutations in Ashkenazi Jewish pancreatic cancer patients. *Nat Genet* 16:17-18.
- Parkin DM, Whelan SL, Ferlay J, Raymond L, Young J. (eds) 1997. *Cancer Incidence in Five Continents. Vol VII IARC Scientific Publications no.143, Lyon.*

- Sng JH, Chang J, Feroze F, Rahman N, Tan W, Lim S, Lehnert M, van der Pool S, Wong J. 2000. The prevalence of BRCA1 mutations in Chinese patients with early onset breast cancer and affected relatives. *Br J Cancer* 82(3):538-42.
- Stratton JF, Gayther SA, Russell P, Dearden J, Gore M, Blake P, Easton D, Ponder BA. 1997. Contribution of BRCA1 mutations to ovarian cancer. *N Engl J Med* 336:1125-1230.
- Takahashi H, Behbakht K, McGovern PE, Chiu HC, Couch FJ, Weber BL, Friedman LS, King MC, Furusato M, LiVolsi VA, Menzin AW, Liu PC, Benjamin I, Morgan MA, King SA, Rebane, BA, Cardonick A, Mikuta JJ, Rubin SC, Boyd J. 1995. Mutation analysis of the BRCA1 gene in ovarian cancers. *Cancer Res* 55:2998-3002.
- Chan TL, Yuen ST, Ho JWC, Chan ASY, Kwan K, Chung LP, Lam PWY, Tse CW, Leung SY. 2001. A novel germline 1.8-kb deletion of hMLH1 mimicking alternative splicing: a founder mutation in the Chinese population. *Oncogene* 20: 2976-2981.
- Tang NLS, Pang CP, Yeo W, Choy KW, Lam PK, Suen M, Law LK, King WWK, Johnson P, Hjelm M. 1999. Prevalence of mutations in the BRCA1 gene among Chinese patients with breast cancer. *J Nat Cancer Inst* 91:882-5.
- Tang TK, Huang CS, Huang MJ, Tam KB, Yeh CH, Tang CJ. 1992. Diverse point mutations result in glucose-6-phosphate dehydrogenase (G6PD) polymorphism in Taiwan. *Blood* 79(8):2135-40.
- Tang W, Luo HY, Eng B, Wayne JS, Chui DH. 1993. Immunocytological test to detect adult carriers of (--SEA/) deletional alpha-thalassaemia. *Lancet* 342(8880):1145-7.
- Wang SL, Huang JD, Lai MD, Liu BH, Lai ML. 1993. Molecular basis of genetic variation in debrisoquin hydroxylation in Chinese subjects: polymorphism in RFLP and DNA sequence of CYP2D6. *Clin-Pharmacol-Ther* 53(4):410-8.