The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine

eJIFCC

ROLE FORA BARD1 SNP IN BREAST CANCER SUSCEPTIBILITY

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J. M. F. Alonso visited for 3 months in 2004 the Ohio State University, James Cancer Hospital, Human Cancer Genetics Program, Columbis, US, and was supervised by Dr. Charis Eng.

Visit Report

1.1 Introduction

Breast cancer is the most frequent malignancy in women. Most breast cancer cases occur in a small percentage of the population that is at increased risk due to genetic factors (Pharoah et al, 2002). The known breast cancer predisposing genes, including BRCA1 and BRCA2, account for only 20-25% of this effect (Easton DG, 1999). Current models suggest that the remaining risk is more probably attributable to a polygenic effect reflecting the random combination of multiple alleles relatively common in the population, each with a small effect on breast cancer risk (Antoniou et al, 2002). The simplest approach to select candidate alleles is biological plausibility. Genes involved in the same cellular processes than BRCA1 are obvious candidates to be investigated. In this context, BARD1 appears to be especially relevant, as growing evidence suggests that in vivo, BRCA1 and BARD1 physically interact and cooperate in a myriad of different biological processes. Co-immunoprecipitation analysis indicates that most (>75%) of the cellular pool of endogenous BRCA1 exists in vivo in the form of BRCA1/BARD1 heterodimer. This interaction is essential for the ubiquitin E3 ligase activity of BRCA1 (Baer and Ludwig, 2002).

The role of BARD1 in cancer susceptibility is still unclear. Thai et al. (1998) have described one somatic mutation in BARD1 associated with sporadic breast cancer (V695L) and one germline mutation associated with ovarian cancer (Q564H). Ghimenti et al. (2002)have found four different mutations in breast or breast/ovarian families negative for BRCA1 and BRCA2 mutations (K312N, C557S, N295S and 1144del21bp. Several polymorphisms have been reported in BARD1 but only two have been suggested as possible responsible for breast cancer susceptibility: Ishitobi et al. (2003) proposed V507M to be associated with increased risk of breast cancer in postmenopausal women, and Karppinen et al. (2004) proposed C557S to be associated with familiar breast cancer, specially in families whose history did not include ovarian cancer.Those data supports a role for BARD1 in breast cancer susceptibility. However, the extent and nature of this risk is far from clear.

1.2 Patients and Methods

DNA from 682 controls (healthy blood donors) and 888 breast cancer cases have participated in our study. Breast cancer cases were either sporadic (679 breast cancer cases not selected by family history) or familial (the index case from 209 high risk breast ovarian families). The index cases of the 209 families included in this analysis were women diagnosed of breast cancer in which a complete screening of BRCA1 and BRCA2 germ-line mutations had been performed previously. This analysis was performed by a combination of techniques: DGGE, SSCP, CSGE and by HPLC.

Genotyping of the P24S SNP was performed by the 5' nuclease assay in an ABI-7700 (APPLIED BIOSYSTEMS) with TaqMan probes. Genotyping of the V507M SNP was performed by direct sequencing.

1.3 Results and Discussion

We performed a case-control study to investigate the possible association of the BARD1 P24S SNP (C143T) with breast cancer susceptibility but the frequencies found by our group resembles those of other Caucasian populations (Thai et al., 1998), so the data indicates a lack of association between the BARD1 P24S polymorphism and breast cancer risk in Spanish population.

Afterwards, we tried to know if there was any conection among the P24S SNP (C143T) from our study and V507M SNP (A1592G) from the Ishitobi et al study (2003). With this aim, we genotyped the V507M polymorphism in those samples homozygous for the P24S SNP so that we were able to unambiguously characterize four haplotypes.

The frequency of C-G and C-A haplotypes were similar in controls, sporadic breast cancer and familial breast cancer (irrespectively of the mutation status). The data suggests that these haplotypes do not influence breast cancer risk. Interestingly, we observed that the T-G haplotype was over represented in all breast cancer subgroups. Frequencies were as follow: 13% in controls (N=116), 22% in sporadic cases (N=134), 25% in BRCA unrelated familial cases (N=24), 25% in BRCA1 related cases (N=12) and 43% in BRCA2 related cases (n=14). The highest frequency was therefore observed in the subgroup of BRCA2 related cases. In the remaining subgroups (sporadic, BRCA1 related and BRCA unrelated familial cases) the frequency was similar and for the purpose of statistical analysis they were considered collectively as non-BRCA2 breast

cancer cases. The over-representation of the T-G haplotype in non-BRCA2 cases (22% vs. 13%, OR=1.94, p=0.04) and BRCA2 related c

ases (43% vs 13%, OR=5.05, p=0.01) were significative findings. The relevant data is summarize in the table.

Collectively, the data suggests a role for the T-G haplotypes in breast cancer risk, especially in those cases related to BRCA2 germ-line mutations

143-1592 Haplotypes analysis in samples homozygous for the BARD1 143SNP

Haplotypes	Controls *n* (%)	BC[1] *n* (%)	OR (95%CI) *p*	BRCA2 BC[2] *n* (%)	OR (95%CI) *p*	Non-BRCA2 BC[3] *n* (%)	OR (95%CI) *p*
CC carriers:	86	210		22		188	
-C-G-	80 (93)	188 (90)		19 (86)		169 (90)	
-C-A-	6 (7)	22 (10)	1.56 (0.6-4.5) *ns*	3 (14)	2.11 (0.4-10) *ns*	19 (10)	1.5 (0.5-4.3) *ns*
TT carriers:	116	184		14		170	
-T-A-	101 (87)	140 (76)		8 (57)		132 (78)	
-T-G-	15 (13)	44 (24)	2.1 (1.1-4.2) .02	6 (43)	5.1 (1.3-19) .01*	38 (22)	1.9 (1-3.9) .04

1 All BC cases included in our study. 2 BC related to a germ-line BRCA2 defect. 3 sporadic, BRCA1 and BRCA unrelated BC cases.
* Fisher exact test.

References

Antoniou AC, Pharoah P, McMullan G et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. Br. J Cancer. 2002; 7, 86(1): 76-83.

Baer R. and Ludwig T.. The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity. Current Opinion in genetics and development. 2002, 12:86-91.

Easton D.F. How many more breast cancer predisposition genes are there? Breast cancer res. 1 (1): 14-17 (1999)

Ghimenti C., Sensi E., Presciuttini S. et al. Germline mutations of the BRCA1-Associated Ring Domain (BARD1) gene in breast/ovarian

families negative for BRCA1 and BRCA2 alterations. Genes, chromosomes and cancer 33:235-242 (2002).

Ishitobi M., Miyoshi Y., Hasegawa S. et al. Mutational analysis of BARD1 in familial breast cancer patients in Japan. Cancer letters 200 (2003) 1-7.

Karppinen S-M, Heikkinen K, Rapakko K et al. Mutation screening of the BARD1 gene: evidence for involvement of the Cys557Ser allele i

n hereditary susceptibility to breast cancer. J Med Genet 2004; 41:e114.

Pharoah P, Antoniou A., Bowrow M et al. Polygenic susceptibility to breast cancer and implications for prevention. Nature genetics 2002, 31(1):33-36

Thai T. H., Du F., Tsan J. T. et al. Mutations in the BRCA1-associated RING domain (BARD1) gene in primary breast, ovarian and uterine cancers. Human Molecular Genetics 1998, Vol. 7, Nº 2; 195-202.