GENOME-WIDE ANALYSIS OF DNA COPY NUMBER VARIATIONS IN INDIAN BREAST CANCER PATIENTS USING HIGH-DENSITY SNP ARRAYS

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INTRODUCTION In India, cancer of the breast is the most common cancer among women in many regions and has overtaken cervix cancer, which was the most frequent cancer a decade ago. Presently, 75,000 new cases of breast cancer occur in Indian women every year in India. The AAR (Age Adjusted Rate) of breast cancer during 1998-2002 ranged from 24.4 (Pune) to 29.2 per 100,000 women (New Delhi). Interestingly, although overall incidence of breast cancer in Indian population is low compared to Western populations (ASR of 23.5 vs. 90.7), the incidence of early onset disease (< 40 yrs) does not show significant geographic variation (ASR range worldwide of 12 to 33) suggesting that in the Indian population a greater proportion of breast cancer is due to early onset disease compared to Western populations. Trend analysis by period reveals significant increase in incidence in younger women (<40 years) 4.2%, compared to older women (0.8- 1.8%).This suggests a different pathogenesis of breast cancer in Indian women. Analysis of the whole tumour genome for copy number alterations might reveal information about specific regions which would be associated with breast cancer carcinogenesis in India.

OBJECTIVE Our aim was to study the genome-wide analysis of DNA copy number variations in Indian breast cancer patients using high-density snp arrays.

METHEDOLOGY Twenty breast tumors were analyzed for copy number analysis using SNP 10K array from Affymetrix. Briefly, 250 ng of genomic DNA was digested with XbaI and then ligated to a XbaI adaptor before subsequent PCR amplification. PCR product was fragmented with DNase I and visualized on a 4% Tris-borate EDTA agarose gel to confirm that DNA fragment sizes ranged from 50 to 100 bp. Fragmented PCR products were then end labeled with biotin. Hybridization and detection were done with an Affymetrix Fluidics Station 450 and GeneChip Scanner 3000.

DATA ANALYSIS Signal intensity data from the GeneChip Operating software were analyzed by GeneChip DNA analysis software (GDAS; version 3.0). GDAS Mapping Algorithm uses a model-based approach to do allele calling for all SNPs on GeneChip 10K mapping arrays. Copy number analysis was done using Copy number analysis tool (CNAT) 4.0 software which implements a Hidden Markov Model (HMM) based algorithm. Further chromosome regions of gain and loss were identified using DNA Analytics software.

RESULTS Complex chromosomal alterations involving multiple levels of change were observed. Copy number gains were observed on chromosome arms 1q31, 1q41-42,20q13, 20p11-12, 3q26-27, 6p22, 8q22-24, 8q13. High level amplifications were observed in chromosome arms 1q and 3q. These regions include genes like MIA3, LAMP3, ABCC5, TP63, KCNK9, IL7, and ADCY8. Losses were observed less frequently than gains and the minimal common regions of the most frequent losses were 11q23-q24, 17p12-p13, 18q21, 13q12-q13, 13q21, 8p21-p22, and 9p21-p23. These regions include genes like NCAM1, OPCML, TP53, MAPK4, CCBE1, MAST4, FBXL17, and FER.

CONCLUSION These data show that it is feasible to utilize high-density SNP arrays to generate concordant CNA profiles at high resolution.