Summary
Breast carcinoma (BC) has now become one of the most frequently reported cancers amongst urban Indian women. As suggested by many that BC of younger women (< 40 years) show more aggressive clinico-pathological features than older women (> 40 years) with BC, the question still remains whether this difference is also seen at the genetic level in the two age groups. Cytogenetic and molecular analyses have shown deletions in chromosomes (chs.) 3 and 9 in a wide variety of cancers including BC. Deletions were seen at several regions of these chromosomes. Among them, chr.3p22.3, 9p21-22 and 9q22.32-22.33 regions were important for harboring multiple candidate tumor suppressor genes (TSGs).

To find out the candidate TSGs associated with development of BC, we have analyzed deletion, mutation, promoter methylation and expression of the candidate genes located at chr.3p22.3, 9p21-22 and 9q22.32-22.33 regions in 47 early- and 59 late-onset BC. Analyses of these genes in 14 other breast lesions were also done to see their association with the disease. Clinico-pathological correlations between alterations of these candidate TSGs and different clinical parameters and prognosis were also done.

The chr.3p22.3 harbors a number of candidate TSGs like hMLH1, APRG1, ITGA9 and HYA22 (RBSP3/CTDSPL). Deletion analysis of these genes showed 38%, 28%, 26% and 30% deletion in early-onset BC and, 32%, 22%, 20% and 24% deletion in late-onset BC respectively. High promoter methylation (32%-48%) was also observed in hMLH1 and HYA22/RBSP3. Significant association between deletion and methylation in hMLH1 was observed in early-onset BC. Quantitative real time PCR analysis (Q-PCR) showed reduced expression of in both hMLH1 and HYA22. Moreover, immunohistochemical analysis of hMLH1 also showed reduced protein expression that significantly correlated with its deletion (P= 0.02) and methylation (P= 0.007) status. High overall alterations of these genes (hMLH1 and HYA22) in both age groups suggested them to be candidate TSGs associated with BC. Presence of both deletion as well as methylation in hMLH1 and HYA22 genes in other breast lesions suggested them to be candidate TSGs for the development of breast tumors.

The candidate TSGs localized at chr.9p21-22 includes SH3GL2, p16, p14 and p15. Higher frequency of overall alterations (46–62%) in SH3GL2 and p16-p14 than p15 (22–26%) indicated their importance in BC. Deletion frequencies were in the following order: early-onset BC: p14 (43%) > p16 (42%) > SH3GL2 (38%) > p15 (33%) and late-onset: p14 (36%) > p16 (33%) > SH3GL2 (31%) > p15 (14%) while, methylation frequencies were: early-onset: SH3GL2 (34%) > p16 (28%) > p14 (26%) > p15 (15%) and late-onset: SH3GL2 (36%) > p16 (31%) > p14 (29%) > p15 (15%). Infrequent mutation was observed only in CDKN2A (p16-p14) common exon-2. Immunohistochemical analysis showed significant association between expression of SH3GL2 and p16 with their deletion (P = 0.01).
and 0.02, respectively) and methylation status (P = 0.007 and 0.01, respectively). In early-onset BC, overall alterations of SH3GL2 showed significant association with CDKN2A locus along with significant prognostic implications, whereas CDKN2A and CDKN2B (p15) loci were associated in both groups.

Four candidate TSGs PHF2, FANCC, PTCH1 and XPA have been identified at chr.9q22.32-22.33 region. Deletion frequencies of these genes in early-onset BC were: FANCC (47%) > PHF2 (43%) > PTCH1 (30%) > XPA (21%) and in late-onset BC: FANCC (49%) > PHF2 (42%) > PTCH1 (27%) > XPA (20%) while, methylation frequencies were: early-onset: PTCH1 (32%) > FANCC (30%) > XPA (24%) > PHF2 (21%) and late-onset: FANCC (39%) > PTCH1 (36%) > XPA (24%) > PHF2 (22%). High frequency of deletion and methylation in PHF2, FANCC and PTCH1 were observed in both age groups. Downregulation of these genes were detected by quantitative mRNA and immunohistochemical analyses. FANCC alterations in either age group, PTCH1 and XPA alterations in early-onset and PHF2 alterations in late-onset BC were associated with poorer patient survival indicating their importance as prognostic markers.

The alterations of these candidate TSGs in 4 cases with bilateral breast tumors and 1 case with multiple breast lesions showed concordant pattern of alterations in some genes indicating their clonal origin. On the other hand, discordant pattern of alterations seen in some genes indicated clonal divergence at certain point of tumor progression required for selective growth advantage of the tumor.

It is evident from this study that the candidate TSGs at chr.3p22.3, 9p21-22 and 9q22.32-22.33 are frequently altered (deleted/methylated) in primary BC, but differentially associated in early- and late-onset BC, indicating differences in their molecular pathogenesis. Thus, it is suggested that the dysregulation of the DNA repair pathways (hMLH1, FANCC, XPA), stem cell renewal pathway (PTCH1), G1-S cell cycle checkpoint (RBSP3/HYA22, p16, p14, p15) and some signal transduction genes (SH3GL2, PHF2) might give some selective growth advantage for progression and adverse prognosis of the disease.