SUMMARY & CONCLUSIONS
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While it has long been recognized that a proportion of breast cancer cases are the result of an inherited familial predisposition, precise knowledge of the underlying genetic processes has been lacking. Recent advances in molecular biology, however, have shown that hereditary breast cancer may eventuate as a result of mutations on several specific gene loci including BRCA1, BRCA2, ATM gene, PTEN and p53. Several other less frequently occurring predisposition genes such as the androgen receptor gene (AR), the HNPCC genes and the oestrogen receptor gene may also be involved, but to a lesser extent. Overall, approximately 5–10% of all breast cancers are thought to involve one of these inherited predisposition genes, with BRCA1 and BRCA2 being responsible for as much as 90% of this group.

The identification of BRCA1 and BRCA2 marked the first time that highly penetrant cancer predisposition genes were linked to the development of a common cancer, raising the possibility that many women might be carriers of a deleterious germline mutation and hence at increased risk for developing breast and ovarian cancer. This explosion of information relating to the genetic basis of breast cancer has profound implications for the practicing clinician and will significantly impact on the future approach to breast cancer patients and their relatives. As a result, studies of the two BRCA genes have been at the forefront of efforts aimed at integrating molecular genetics and clinical oncology. Epidemiologic studies have focused on the frequency of genetic variations with in the population and their associated risk for developing breast and ovarian cancer. Previous study from Kerala population showed that BRCA1 germline mutations contributed to a significant proportion of HBOC families in Kerala (Kumar et al., 2002; Vinodkumar et al., 2007). However, this gene alone is not sufficient to explain breast cancer susceptibility in every cancer-prone family (Easton et al., 1997). More over, study of BRCA genes raise the possibility that diagnostic screening recommendations and even therapeutic options may eventually be based on germline genotype. As an initial step in this direction, the present study
was undertaken with the primary objective to determine the genetic heterogeneity and prevalence of germline mutations of BRCA2 gene and their prognostic significance in hereditary breast/ovarian cancer patients from Kerala population. In addition, an attempt to identify the prevalence of BRCA2 germline mutations among early age onset sporadic breast cancer patients was also carried out.

The study was conducted in the Research Division of Regional Cancer Centre, Thiruvananthapuram, in a series of histopathologically confirmed incident breast/ovarian cancer cases, who attended, the outpatient clinics during the period from 2001-2006. Through pedigree analysis patients with a family history of breast/ovarian cancers were identified. From these patients, 102 breast/ovarian cancer patients from 96 breast/ovarian cancer families which met the criteria as per selection guidelines of IARC and IBCCS, were selected as hereditary breast ovarian cancer (HBOC) cases. Apart from this, 72 early age of onset (<45 years) sporadic breast cancer patients and 90 age matched control individuals were also selected. Thus altogether, 264 study subjects were screened for germline mutations in the BRCA2 gene. Patients with a prior history of treatment elsewhere and those with secondary or metastasized breast cancers were excluded from the analysis. After getting a written informed consent, peripheral blood was collected from the study subjects and DNA was isolated using standard phenol-chloroform extraction method. Entire 26 coding exons of the BRCA2 gene were then PCR amplified using appropriate primers. Mutation analysis of the BRCA2 gene was carried out using Conformation Sensitive Gel Electrophoresis. Potential sequence variants identified by altered electrophoretic mobility in CSGE analyses were re-amplified and subjected to automated DNA sequencing to characterize the mutations or polymorphisms. The associations between the BRCA2 mutation status and clinicopathological characteristics of breast/ovarian cancer patients were examined by unconditional logistic regression analyses to calculate the Odds Ratios (ORs) and 95% Confidence Intervals (CIs). Survival curves were generated according to the method of Kaplan-Meier using Log Rank test and Cox proportional hazards regression method.
Salient findings of the present study were as follows:

**BRCA2 gene mutation analysis**

- A total of 19 distinct germline BRCA2 sequence variants (including Frame shifts, Missense, Nonsense, Silent and intronic alterations) were identified in 31.3% of patients (32/102).

- Germline mutations in BRCA2 gene (Frame shifts, Missense and Nonsense mutations alone) were observed in 18.62% (19/102) of hereditary breast/ovarian cancer patients from 18 different HBOC families.

- 12 distinct pathogenic mutations including 2 frame shifts, 9 missense and one nonsense were identified.

- 52.63% (10/19) sequence variants identified were novel and hence could be considered unique to the Kerala population.

- 68.4% (13/19) of the sequence variants identified were detected in exon 11 of the BRCA2 gene.

- In sporadic breast cancer patients with an early age of disease onset, BRCA2 sequence variants were detected only in 6.94% (5/72) of patients.

- Two common polymorphisms in exon 10 of the BRCA2 gene (N289H and S455S) were identified in 11.8%, 27.8% and 13.33% of HBOC, sporadic and control subjects respectively.

- These two BRCA2 gene polymorphisms were found to be significantly associated with sporadic breast cancer susceptibility risk (OR=2.5; 95% CI=1.125-5.54, P = 0.02).

**Clinicopathologic Correlation**

- None of the clinicopathologic parameters analyzed revealed a statistically significant correlation with the BRCA2 gene mutation status in both HBOC and sporadic cases.
Survival Analysis

- Overall survival analysis in a cohort of 102 hereditary breast/ovarian cancer patients of whom 19 patients were carriers of a BRCA2 mutation and 83 patients were non carriers, showed a significantly worse survival for the BRCA2 mutation positive patients (P=0.02).

- Apart from the BRCA2 gene mutation status, various clinicopathological factors such as tumor size (P = 0.017), stage (P = 0.03), metastasis (P = 0.011) and laterality of the disease (P = 0.026) emerged as the main predictors of survival in HBOC cases.

- In survival analysis of sporadic breast cancer patients with an early age of disease onset, tumour size (P = 0.03), metastasis (P=0.02) and disease stage (P=0.01) were significantly associated with poor prognosis.

- No statistically significant differences on overall survival were observed between the sporadic and HBOC groups of patients.

- No significant influence of any non genetic modifying factors on HBOC cases compared to control subjects as well as on HBOC cases with and with out BRCA2 gene mutation was observed.

In conclusion, this is the first study to report the frequencies and types of BRCA2 germline mutations in Kerala population. The high incidence of unique pool of novel mutations with a slight preponderance of missense mutations identified, along with the significantly worse survival in BRCA2 mutation carriers obtained in the present study suggest the unique character of the BRCA2 mutations in the HBOC families in Kerala. However, a very small proportion of early-onset sporadic breast cancer patients only showed mutations in BRCA2 gene. Thus, germline mutations in BRCA2 gene have an important influence on the predisposition and development of hereditary breast and ovarian cancer in Kerala. Given current constrains on health-care resources, these results support the notion that BRCA2 mutation screening may have the strongest impact on health care when targeted to high risk populations.