Chapter 4

Discussion
India with a population of 1.2 billion is the most populous democracy in the world. India is a pluralistic, multilingual, and multiethnic society. It includes over 15 native languages with more than 70 dialects (Government of India: Office of Registrar General, 2010). The Indian sub-continent is a vast country with billion people providing a large pool for diseases. Carcinoma of the breast is now the most common cancer among women in both developed and developing countries (Ferlay et al., 2010). Breast cancer in women is a major public health problem worldwide, being the principle cause of death from cancer among women globally. Despite the low prevalence of breast cancer in Asia, the cause-specific mortality in most Asian countries is reported higher than western countries (Yip, 2009). Despite such a huge number of patients from West Bengal, there has been no published report on molecular characterization of breast cancer.

Inspite of good advancements for diagnosis and treatment, cancer is still a big threat to our society (Kotnis, et al., 2005), being the second most common cause of deaths in the world after cardiovascular disorders (Jemal et al., 2007). Cancer is clearly a genetic disease (Workman, 2003). A neoplasm is caused by the mutation, amplification, deletion or abnormal expression of key genes that represent critical factors in the regulation of cell fate.

Genetic polymorphisms are variants in individual genomes and remain constant throughout a person’s lifetime. Every individual possesses a BRCA1 and BRCA2 gene (Hamilton, 2009). These genes are tumor suppressor genes that control cell growth, cellular processes, including chromatin remodeling, protein ubiquitination, cell cycle checkpoint control and apoptosis (Yang and Xia, 2010). Disruption of any or all of these processes may contribute to the increased risk for carcinogenesis, as seen in carriers of germ line BRCA1 mutations. Screening for mutations carried out by several groups worldwide suggests a
significant variation of the relative contribution of \textit{BRCA1} and \textit{BRCA2} genes to breast cancer risk (Levy-Lahad and Friedman, 2007). The contribution of \textit{BRCA1} and \textit{BRCA2} mutations pertaining to breast cancer in Indian Women remains largely unexplored. The few studies that have been reported, suggests a need to screen for mutations in Bengalee Caste Hindu Females which remain untouched till date (Kumar \textit{et al.}, 2002; Rajkumar \textit{et al.}, 2003).

Nevertheless, the effect of mutations in \textit{BRCA1}, \textit{BRCA2} and p53 gene to the incidence and prevalence of breast cancer (BC) has been well established worldwide (Chenevix-Trench \textit{et al.}, 2006). In other word, \textit{BRCA1}, \textit{BRCA2} and p53 genes are well established Breast Cancer susceptibility gene, which when mutated are inherited and strongly predisposes to breast cancer. Some of them directly influence breast cancer risk, whereas others are involved in the general process of cancer growth and metastasis. However, the role of these genes in predisposing Bengalee Hindus to breast cancer has not been explored and there is no reported study till date.

To the best of our knowledge, this is the first report of mutations in the \textit{BRCA1}, \textit{BRCA2} and p53 genes among Bengalee Caste Hindu females of West Bengal, India. Indian populations have not been extensively studied; consequently knowledge of the prevalence and spectrum of \textit{BRCA1}, \textit{BRCA2} and p53 gene mutations in these populations is sparse. In this present study, 11 sequence variants were identified in patients and controls. It may be possible that as yet unidentified genes play a greater role in the studied population. Alternatively, it may simply be that the spectrum of mutations differs in the population, such that other large genomic rearrangements remain unidentified which may be helpful to completely characterize the contribution of these genes to breast cancer.
Discussion

Tumor suppressor genes play important roles in regulation of cell growth and differentiation. Mutations in \textit{BRCA1}, \textit{BRCA2} and p53 genes may confer a greatly increased risk of breast cancer, some sequence variants in both genes might be candidates for moderate or low penetrance alleles.

In an attempt to screen the \textit{BRCA1}, \textit{BRCA2} and p53 genes in Bengalee population, 11 Single Nucleotide Polymorphisms has been identified; out of which 6 were novel variants, and has already been submitted to GenBank (NCBI). The present study also revealed that the mutation in \textit{BRCA1}, \textit{BRCA2} and p53 gene was apparently low among the studied population which is contrary to the earlier studies reported from Southern and Northern India. This might be due to ethnic differences vis-à-vis the genetic structure (Saxena \textit{et al.}, 2002; Rajkumar \textit{et al.}, 2003; Valarmathi \textit{et al.}, 2004; Saxena \textit{et al.}, 2006). The present study demonstrated a significant association (p<0.0001) of breast cancer among the \textit{BRCA1} mutation carriers, similarly \textit{BRCA2} and breast cancer also revealed significant association (p<0.0001) for the disease. P53 gene demonstrated no mutation in exons 5 – 7, only exon 8 presented with a change, which is significantly associated (p<0.0017) with breast cancer.

\textit{BRCA1} gene was sequenced for exons 2,8,11 and 13 (Table 5). The common mutation 185delG distinct with Ashkenazi Jews are absent in our study (Struewing \textit{et al.}, 1997). In India, 185delAG has been reported in all populations studied till date from Northern and Southern India (Kumar \textit{et al.}, 2002; Hedau \textit{et al.}, 2004; Valarmathi \textit{et al.}, 2004; Saxena \textit{et al.}, 2010; Lakhotia \textit{et al.}, 2010). Worldwide population studies have revealed that the 185delAG predates the severance of Sephardi and Ashkenazi Jewish population and is probably 2000 years old (Bar-Sade \textit{et al.}, 1998). The present study identifies 5 sequence variants in \textit{BRCA1} gene of which 3 are novel variants (reported to GenBank, NCBI). Mutation in \textit{BRCA1} intron 7 (IVS7-...
28 C>T) was identified in the present study among 22.22% of the patients only (Figure 10), which is a variant of unknown clinical significance. Majority (46%) of the mutation carriers reported of family history of the disease. Exon 11 is a large gene comprising of more than 50% of the coding region of the gene. Numerous variants have been reported worldwide with known and unknown clinical significance. However, c.775 G>A in exon 11 (rs80357338) has been reported with varied allele changes reflecting variations in amino acid changes, including gain of stop codon. The present study identifies a new allelic change with amino acid changing from Arginine to Histidine. 11.11% of the cases reported the change without any controls reporting the same. This mutation has been referred from BIC database, reference reports are not published till date. Mutation in codon 1045 (c.1045 G>T) is a novel variant reported in the present study with amino acid changing from Serine to Isoleucine. 16.66% of the patients and 3.15% of the controls reported the variant. Another mutation in codon 1105 of the BRCA1 gene changes the amino acid from Arginine to Threonine is evident among 12.96% of the patients. This variant lacks any published report; therefore association could not be established. During the course of the study, a novel variant in exon 13 was identified in codon 4300 that changes the amino acid from Proline to Serine, present among 13.88% of the patients and 3.15% of the controls. It is reported that BRCA1 and its transcriptional activation domain plays an important role in Breast Cancer predisposition (Roy et al., 2012). Very few studies have been reported from India on molecular heterogeneity of BRCA1 gene, and the variation in mutation spectrum makes every study unique.

BRCA2 has been sequenced for exons 2, 8, 9 and 13 (Table 7). Studies showed that exonic variants can alter pre-mRNA splicing either by changing splice sites or by modifying splicing regulatory elements (Di Giacomo et al., 2013). The result identifies 5 sequence variants of which 2 are novel. Exon 2 changes are present among 19.4% of the cases and 1.57%
of the controls. A study from Southern India reported a novel variant in exon 8 with T-C transition. The T and C transitions are also common in our present study with changes in different bases of the gene. Three intronic variants of unknown significance have been reported in the study from Intron 9 (Figure 18). The sequence variant in exon 9 changes the amino acid from Proline to Leucine (Figure 19). BRCA2 mutations are generally less common than BRCA1 mutations. Only in Iceland the situation is different where one single mutation in BRCA2 gene is responsible for almost all inherited breast cancers (Thorlacius et al., 1996). Majority of the studies associate BRCA2 mutations with male breast cancer risk (Serova et al., 1997). In a study conducted in Hungary, the country with highest male breast cancer mortality in Europe, the results showed 33% of the patients with BRCA2 germ line mutation (Csokay et al., 1999). A hospital based study on unselected breast cancer cases from Rome concluded in low spectrum of BRCA2 mutations, though the variants are common in Romanian breast cancer patients (Burcos et al., 2013). A large number of genetic alterations are still classified as “variants of unknown significance” (Chenevix-Trench et al., 2006).

The lack of the identified mutations in the Indian scenario describes paucity of association of BRCA2 mutations with breast cancer from India which is one of the major objectives of the present study. Since, all the mutations reported for BRCA1 and BRCA2 in the present study being the first attempt from Bengalee population, therefore comparative analysis seems to be unresolved. However, its significance and association has been referred from the BIC database.

The association between p53 gene mutations and breast carcinoma has been attempted by several studies; however, the issue is still unresolved. The high incidences of breast cancer have prompted many researchers to understand its risk factors at the genomic level. The present
study observed no mutations in exons 5, 6 and 7 of p53 gene. Although, p53 mutations are reported at various frequencies in different populations, but present study indicated that these mutations in p53 were not significantly contribute to breast cancer among the cases cutting across all the castes thereby, they are of little relevance for their pathogenic role in this disease inheritance and cannot be recommended for the diagnostic screening (Ghosh Roy et al., 2012). Apart from exons 5, 6 and 7 of p53 gene a novel mutation has been identified in exon 8 changing the amino acid from Arginine to Lysine and the change has been significantly associated with breast cancer pathogenesis (Chapter 3, Table 5,7 and 9).

To explore the possibility for understanding the role of Single Nucleotide Polymorphism(s) (SNPs) Linkage Disequilibrium (LD) plots were constructed (using Haploview 3.2) by gene sequence of 6 SNPs for chromosome 17 having BRCA1 and p53 genes (Figure 21). Examination of LD analysis revealed, except rs06, no two SNPs segregated. Consequently, rs06 might not be an effective molecular genetic marker for the studied population with regard to the breast cancer study. On the other hand, LD study depicted that rs03 and rs01 might be strong associated marker for the studied population. In general, strong association between pairs of markers suggests lack of recombination, whereas weak association may be evidence for a history of active recombination between them, i.e. possible hotspots (Jeffreys et al. 2005).

Furthermore, it would be evident from the gene expression study (Figure 24, 26) executed by Real Time PCR (RT-PCR) that there were down regulation of tumor protein of both the p53 and BRCA genes among the studied Bengalee population. However, many studies have reported about the allele frequency variation in SNPs between ethnic groups (Goddard et al. 2000, Excoffier and Slatkin 1995; Bonnen et al. 2000; Tishkoff et al. 2000; Niu 98
Therefore, the variation in SNPs and down regulation in gene expression of the genes would not only be the sole factor for breast cancer. Breast cancer could manifest from multiple reasons as it is complex disorder, which indicate other associated risk factors for the modification and expression of the situation. It would be apparent from the foregoing presentation that including genetic factors, development of breast cancer in females depends on several other factors. Since cancer is complex and non communicable disease, the present study, therefore, attempted to understand epigenetic condition of breast cancer. To achieve the purpose, different lifestyle variables have been taken into consideration. The present study has age matched cases (54.0370±10.383 years) and controls (54.609±8.005 years) with patients ranging in age from 30 to 78 years and controls from 38 to 72 years (Table 2).

Age and family history are commonly considered to have effect on prognosis and survival of breast cancer. The present study revealed substantial variation in breast cancer risk among the mutation carriers, particularly in terms of age variation and cancer type which basically envisaged that the concomitant effect of genetic variability and environmental factors which eventually modify the expression of the status.

The implication of natural hormones specially the sex hormones on developing cancers such as endometrial cancer (Key and Beral, 1992), breast and prostate cancer (Sharma and Ray, 2000) (among sex organ related neoplasm) or colon cancer (English et al., 2001), gall bladder cancer (Ray and Gupta, 2001), kidney cancer (Li et al., 1985) etc (non sex organ related neoplasm) have been reported globally. Furthermore, breast cancer risk is enhanced by increasing the duration of exposure to endogeneous hormones (Endogenous Hormones and Breast Cancer Collaborative Group, 2011). It has also been reported that age at menarche, parity and age at first full-term pregnancy are risk factors for breast cancer (Kelsey et al, 1993,
Russo et al., 2005). In addition to that breast cancer risk is associated with several reproductive factors. It is well established that breast cancer risk increases with early age at menarche (Dumitrescu and Cotarla, 2005). This association is consistent with the hypothesis that breast cancer risk is related to the extent of breast mitotic activity. This activity is driven by estrogen and progesterone exposure during the luteal phase of the menstrual cycle (Ferguson and Anderson, 1981), which determines the probability of tumorigenic somatic events (Pike et al., 1993). Therefore, an early age at menarche increases the period during which the breast is mitotically active and subsequently increases breast cancer. Therefore, early menarche or late menopause increases the risk of breast cancer. In this context, the present study also observed that an early age at menarche (Table 3) is significantly associated (p<0.0001) with an elevated risk of breast cancer in Bengalee population. Irregularity of menstrual periods was also found to be significantly (p<0.0002) associated with breast cancer risk and in corroboration many studies related to the increased risk associated with irregular menstrual cycles (Wu et al., 1996; Whelan et al., 1994; Tonkelaar et al., 1996; Yuan et al., 1988, Chen et al., 2013). However, a number of studies have reported little association with irregularity and increased breast cancer risk (Clavel, 2002; Grabrick et al., 2002; Titus et al., 1998; Gao et al., 2000).

Study of risk factors of breast cancer with marital status might have an impact on the incidence of breast cancer and it was observed that women who never married were at higher risk of breast cancer (Ebrahimi et al., 2002) which has also been reported in Indian context (ICMR Bulletin, 2003). Since there is a strong interaction between marital status and parity, the increased breast cancer risk associated with single women possibly might be due to nulliparity (Hadjisavvas et al., 2010). The present study also revealed significant association (p<0.0015) with breast cancer and unmarried females in comparison to the controls with an increased risk of 0.19 times (OR-0.192; 95% CI= 0.0527 – 0.6995; p<0.01).
The protective effect of pregnancies against breast cancer was first described by Lane-Claypon (1926), and was confirmed through a large number of subsequent studies (Kelsey et al, 199; Press and Pharoah, 2010). The underlying mechanisms may include differentiation of the mammary epithelial cells, reduced number of mammary stem cells, altered mammary response to oestrogen, and reduced levels of circulating hormones (Britt et al., 2007, Rosner et al., 1994). Significant associations (p<0.0001) have been found in parity status of the cases and controls (Table 19), and logistic regression analysis suggesting that nulliparous women are 0.2138 times more likely to attain the disease (OR-0.2138; 95% CI=0.88-0.52; p<0.001). It has been known for decades that nulliparity is associated with an increased risk for certain reproductive malignancies, including breast, ovarian and uterine cancers. A recent commentary in The Lancet summarized the available evidence based on data in nulliparous women and concluded that the risk of nulliparity was related to the increased number of ovulatory cycles, and so might be preventable by utilization of oral contraceptives (Gleicher, 2013).

Breast feeding as a protective factor for breast cancer does have a sound biological plausibility. Various pathophysiological mechanisms which are been proposed such as decreased frequency and intensity of ovulation thus maintaining the consistent lower level of estrogen; mobilization of endogenous carcinogens from the ductal and lobular epithelial cell environment, and facilitating the excretion of organochlorides (xenoestrogens) having the same potentials as estrogen (Helewa et al., 2002). From the population of Delhi it was reported that the mean duration of the sum total breastfeeding for all children as 6.58 years in patients and 7.4 years in controls (OR=1.91; 95% CI, 1.17–3.13).(Pakseresht et al., 2009). Similarly, another study from South India has come out that lack of breastfeeding is positively associated with breast cancer (Meshram et al., 2009). Multicentric trial observed that prolong breastfeeding is associated with reduction in breast cancer risk among reproductive age females.
A review from France accomplished that protective effect of breastfeeding is more for the women having extended periods of breastfeeding, particularly in case of BRCA1 mutation (thus avoiding a negative epigenetic change) (Freund et al., 2005).

Furthermore, long-term users of Oral Contraceptives (OCs) were at a higher risk of breast cancer than never users. Association studies regarding current/recent use of OCs with breast cancer risk demonstrated heterogeneous results to the extent of increased risk (Merethe et al., 2002) to no or weak association of OCs use among BRCA1 mutation carrier in Breast Cancer (Haile et al., 2006; Figuiredo et al., 2009; Grenader et al., 2005). In this context, the present study revealed significant association (p<0.0019) of prolong use (more than six months) of OCs and breast cancer in comparison (Table 25) to the controls and the use of OCs increased the risk of breast cancer by 2.77 times than those of non-users (OR- 2.77; 95% CI= 1.63 – 4.71; p<0.0001). The controversial effects of OCs on breast cancer have been studied extensively. But currently there is conflicting epidemiological evidence regarding the role of oral pills in causation of breast cancer, so it is difficult to make a blanket statement (Collaborative Group on Hormonal Factors of Breast Cancer, 1996). For instance, several studies have found no significant association between history of oral contraceptive uses and breast cancer (Reid, 2007; Marchbanks et al., 2002; Brinton et al., 1982) but other studies have shown diametrically opposite results (Ahmad, 2003; Jick et al., 1980; Hankinson et al., 1997; Yavari et al., 2005). The present study also could not measure the relationship of breast cancer with duration, type, dosage, and pattern of OCs usage because most of the subjects were not able to recall the details. For the females those have mutated tumor suppressor genes like BRCA1, the gene already fails to perform its tumor suppressing activity; elevated estrogen and progesterone stimulate breast cell proliferation, finally uncontrolled growth leads to carcinoma. Studies have found association between the OCs usage and BRCA1 mutation carriers.
(American Cancer Society Report, 2010). However, paucity of literature has been found from Indian context (Narod et al. 2002; McLaughlin et al., 2007; Modan et al., 2001). The present study reports significant association ($p<0.0019$) between OCs and breast cancer from eastern India for the first time. Since, hormones are considered to play a role in the etiology of breast cancer therefore, it is likely that $BRCA1$ might have important regulation of growth and differentiation in hormonally responsive epithelial cells. In addition to that, breast and ovary being the main estrogen receptor sites, the increased levels of the estrogen due to prolonged consumption of oral contraceptives gets accumulated in these sites (Marquis et al., 1995; Rajan et al., 1997). Such finding is similar to the present study mentioned earlier, which has significant association with use of OCs and increased breast cancer risk, while many studies have represented that exogenous hormonal factors such as estrogen replacement therapy and combined oral contraceptive use might cause to some extent increase in the risk for breast cancer (Key and Beral, 1992).

Induced and spontaneous abortion increases the risk of developing breast cancer. In early pregnancy, levels of estrogen increase leading to breast growth in preparation for lactation. The hypothesis proposed that if this process interrupted by an abortion before full maturity in the third trimester then more relatively vulnerable immature cells could be left than there were prior to the pregnancy, resulting in a greater potential risk of breast cancer over time. Though many studies (Jernstrom et al., 1999; Beral et al., 2004) have reported association between abortion and breast cancer risk, the exact influence is still speculative. Present study also revealed significant ($p<0.0001$) association and increased risk as well between abortion and breast cancer. There are a very few studies on abortions and breast cancer risk from India, but the few available reports also showed similar finding (Brind, 1996).
Post Menopausal status and characteristics are known to be induced by hormonal factors, which are the key factors for breast cancer and may synergistically interact with genetic factors in triggering the development and progression of breast cancer through estrogen synthesis, metabolism and signal transduction (Butt et al., 2012). The present finding revealed no significant association (Table 21) of post menopausal hormone therapy with breast cancer risk, which is contrary to the result from Pakistan (Butt et al., 2012). But the present study envisaged that post menopausal hormone therapy triggers the risk of breast cancer by 2 times (OR-2.0; 95% CI=1.08 – 3.73; p<0.0001) might be due to ethnic variability.

The study executed logistic regression analysis which revealed occupation (housewife), positive family history, irregularity of menstrual periods, use of oral contraceptives, abortions, breast feeding for less than 3 months, post menopausal hormone therapy, hysterectomy and smoking/ alcohol consumption has been analyzed to be significant predictor variables for breast cancer risk (Table 31).

Breast cancer is considered a multifactorial disorder which is thought to be a strong interplay between both genetic and non-genetic factors. The two genes, which when mutated confer a high risk of breast cancer has been isolated namely BRCA1 and BRCA2. These genes are thought to attribute a similar susceptibility to breast cancer risk (Robson et al., 1998; Krainer et al., 1997). Therefore, identification of BRCA1 and BRCA2 gene mutations and their association with reproductive and lifestyle variables is an important focus in prevention and early detection of breast cancer risk. For this purpose, the association of BRCA1 and BRCA2 mutation carriers with reproductive, lifestyle variables has been undertaken in the present study among the Bengalee Caste Hindu Breast Cancer patients of West Bengal using multivariate analysis. Analysis revealed that parity, abortions, oral contraceptive use, menstrual irregularity and family history were most significant predictor variables associated with BRCA1 and
BRCA2 mutation carriers (Table 28). Estrogen stimulates breast cell proliferation (Yager and Davidson, 2006) and BRCA1 and BRCA2 is involved in DNA repair and that unrepaired DNA damage in proliferating cells can be tumorigenic. The effect of estrogen on breast cancer risk might be even stronger among BRCA1 and BRCA2 mutation carriers than non-carriers. The present study vindicated that mostly factors related to hormonal regulation were associated with mutation carriers to present the phenotypic scenario of breast cancer. The present study, furthermore, revealed that increasing number of full term pregnancy was protective in the mutation carriers, which is corroborative with the results from the studies concerning Estrogen stimulates breast cell proliferation (Yager and Davidson, 2006). mutation carriers (King et al., 2003; Narod et al., 1995; Andrieu et al., 2006; Rebbeck et al., 2001; Antoniou et al., 2006), with usual exception to few studies with regard to BRCA2 mutation carriers (Cullinane et al., 2005; Tryggvadottir et al., 2003). Several mechanisms have been reported like decreased estrogen level, increased sex-hormone binding globulin levels (Bernstein et al., 1985), and pregnancy induced differentiation of breast tissue (Russo et al., 2005). The present study, however, also noticed that these protective mechanisms of pregnancy may also work in BRCA1 and BRCA2 mutation carriers. The present attempt also observed that increased duration of breast feeding was associated with decreased breast cancer risk among the carriers of BRCA1 and BRCA2 mutation, which is similar to the Collaborative study from 30 countries (Collaborative Group on Hormonal Factors in Breast Cancer, 2002) and other studies from global context (Jernstrom et al., 2004; Andrieu et al., 2006) as well. However, an Icelandic study found a suggestive but non-significant effect (Tryggvadottir et al., 2003). Mechanisms underlying the cause has been explained to be the postponed resumption of menstrual cycles, breast tissue differentiation (Russo and Russo, 1999), and decreased estrogen levels (Petrakis et al., 1987), others being excretion of carcinogens from breast ductal tissue (Murrell et al., 1991).
Although a modest increase of breast cancer risk among the oral contraceptive users has been reported in a large pooled analysis and a meta-analysis (Collaborative Group on Hormonal Factors in Breast Cancer, 2010; Kahlenborn et al., 2006). Nevertheless, the present study revealed significant associations among some specific mutations (rs03 and rs05) (Table 32).

The present study also found that menstrual irregularities were associated with breast cancer risk among the *BRCA1* and *BRCA2* mutation carriers, in corroboration with other study (Kelsey and Ross, 1993). These associations might be due the hypothesis of association of breast cancer with the total extent of breast mitotic activity, driven by estrogen exposure during menstrual cycle (Ferguson and Anderson, 1981) and the probability of tumorigenic somatic events (Pike et al., 1993) as well. Combined analysis of case-control studies have shown that the relative effects of age at menarche and menopause being similar in women with a family history of breast cancer and women without any such history, suggesting that these associations might be largely independent of genetic susceptibility (Andrieu et al., 2000; Collaborative Group on Hormonal Factors in Breast Cancer, 2010).

The present study being the first report from the Bengalee Hindu Caste Females of West Bengal revealed that the spectrum and prevalence of the *BRCA1*, *BRCA2* and p53 genes in the Bengalee Hindu Caste Females were found to have unique features with reasonable corroboration compared to other population. It is evident from the above mentioned findings that having a mutation in tumor suppressor genes cannot solely trigger a person’s risk of developing the disease during the lifetime. Certain other environmental factors modify the risk for the same.

Both personal and family histories influence a woman’s risk of developing breast cancer. In other word, woman with first degree relative with breast cancer will have twice the risk than those who do not possesses family history (Ambrosone, 2007). On the other hand,
twin studies indicated that up to 30% of breast cancer cases may be due to genetic factors (Lichtenstein et al., 2000). This study also emphasized the importance of a positive family history and other lifestyle factors for the breast cancer predisposition. Therefore, the present study envisaged that appropriate genetic counseling and modification of lifestyle factors, symptomatic mutation carriers would be able to minimize the risk for disease susceptibility among the Bengalee Hindu Caste Females of West Bengal, India.