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Breast Cancer Susceptibility Gene (BRCA-1) mutations in selected Sri Lankan breast cancer patients; a preliminary report

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Breast cancer results from genetic and environmental factors leading to accumulation of mutations in genes involved in regulation of cell cycle. These mutations can be either germline or sporadic. In 1994 the first breast cancer susceptibility gene, named Breast Cancer 1 (BRCA-1) was identified. Subsequently second breast cancer susceptibility gene BRCA-2 was identified in 1995. At present, over 878 and 900 distinct mutations, polymorphisms and variants have been reported throughout the BRCA-1 & BRCA-2 respectively. Prevalence of BRCA-1 and BRCA-2 mutations is variable among different populations. In Sri Lanka, BRCA-1 and BRCA-2 germline mutations have not been characterised to date.

Venous blood samples were collected from 68 breast cancer patients (n=21, with a family history; n=47 without a family history of breast cancer) and two individuals without disease but who had a family history of breast cancer. Genomic DNA was extracted from the peripheral blood leucocytes. Specific primers were selected for PCR amplification of exons 2, 20 and 21 of BRCA-1. SSCP (Single Strand Conformation Polymorphism) was done by amplifying genomic DNA samples using PCR, denatured and separated by electrophoresis under nondenaturing conditions (15% polyacrylamide gel). HDA (Hetero Duplex Analysis) was done by using PCR products of genomic DNA samples. Sample PCR products were mixed, denatured and annealed with a known normal control PCR product. These fragments were separated under nondenaturing conditions (15% polyacrylamide gel) and visualized by silver staining.

Mutations were suspected in exon 21of BRCA-1 in three patients with family history of breast cancer. Conformational changes of the single strand mutant DNA was believed to be the reason for the different migrating patterns of SSCP analysis. Another mutation was suspected in exon 20 of BRCA-1 in one patient with a family history of breast cancer. This shows conformational mobility shift in SSCP and HDA (Conformational mobility shift of double-stranded heteroduplex molecules when mutant and wild-type strands annealed). These mutations need to be confirmed by direct sequencing.

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