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# Could Allelic Discrimination in *ETS*2 Predispose Individuals to Breast Cancer?

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**Abstract:** The *V-ets erythroblastosis virus E26 oncogene homolog2* (ETS2) is known to have a stimulatory role in breast cancer progression. To find out its participation in the development of breast cancer, we have investigated a functional polymorphism in *ETS2,* rs461155, in a group of breast cancer patients. Allelic and genotypic frequencies obtained were compared with that obtained for ethnically matched healthy control individuals and data reported for four other ethnic groups in the HapMap project. Significant differences in genotypic frequencies were obtained between breast cancer patients and control individuals ($p<0.0001$). Ethnic groups reported to have higher frequency of breast cancer were observed to have higher frequency of the "G" allele ($p<0.01$). *In silico* analysis revealed that this allele interferes in proper splicing of the *ETS2* transcript and thus can alter function of the protein which in turn can influence breast cancer development.

The *V-ets erythroblastosis virus E26 oncogene homolog2* (ETS2) is a well known transcription factor (TF) that regulates expression of a number of cell cycle regulator genes like *Bcl-xL*, *c-myc*, *cyclin D1* and *P53* [1, 2]. Over expression of *ETS*2 causes upregulation of *P53*, *BAX* and down regulation of *Bcl2*, thus increasing sensitivity to apoptosis [3]; therefore, *ETS2* can act as a tumor suppressor. On the other hand, in breast cancer (BC), investigators have reported that ETS2 negatively regulates the *BRCA*1 promoter [4].

BC is of major concern in the world with a very high occurrence among residents of U.S having ancestry from northern and western Europe (CEU) and Yoruba populations from Nigeria (YRI**)** [5]. Considering the contribution of *ETS2* in the field of cell cycle regulation, apoptosis etc. the present study was aimed at investigating role of *ETS2* functional polymorphisms in association with BC.

Chromosomal localization of *ETS2* is at 21q22.3 [6]. ETS family members have a conserved DNA-binding domain (ETS domain) composed of a helix-turn-helix sequence and recognize a (C/A)(C/A)GGA(A/T)(A/G) motif and binds to GGAA/T core motif [7, 8]. Among different functional polymorphisms in *ETS2,* one intronic (rs35258008) and three exonic SNPs (rs457705, rs461155, rs34120017), located at the DNA binding domain, were investigated in the present study (details provided in Table **1**).

Post-operative normal tissue adjacent to malignant breast tumor was collected from BC patients (n=49) and genomic DNA was extracted from tissue [9]. Genomic DNA from ethnically matched healthy control individuals (n=144) was collected from peripheral blood [9]. Target sequence encompassing all the studied SNPs was amplified by polymerase chain reaction using primer sets; forward "5-GTTGTCTTTGCCAGGGACTC-3" and reverse "5-CGGTGAATGTGGTACTGTGG-3". The amplification condition was 5 minutes initial denaturation at 95°C followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 58°C for 40 seconds, extension at 72°C for 45 seconds and a final extension of 5 minutes at 72°C. Amplicons (365 bp) were subjected to sequencing in ABI prism 3130 Genetic Analyzer using Big Dye sequencing kit v3.1 followed by analysis using Sequencing Analysis software v 5.2.

Data obtained were analyzed statistically to compare allelic and genotypic distribution in the control and BC groups. rs457705, rs34120017, rs35258008 were found to be non-polymorphic in the studied eastern Indian population. rs461155 was polymorphic and allelic/genotypic frequencies obtained were compared with the data available from the HapMap project on four major populations in the world, namely CEU, YRI, Han Chinese from Beijing, China (HCB) and Japanese from Tokyo, Japan (JPT). Functional analysis for the TF was performed using web-based tools. Incidence of BC in different populations was obtained from published data (c.ref 5; GLOBOCAN 2002; Cancer research UK.org/statistics).

Genotypic data obtained in the present study indicated that while rs457705, rs34120017, rs35258008 were non-polymorphic in the eastern Indian population, the "T" allele of rs457705 had 100% prevalence. On the contrary, the "T" allele was found to be the minor allele in CEU and YRI population and in HCB and JPT populations it had only 61-63% prevalence [10]. Allelic and genotypic distribution of rs461155 in control and BC groups revealed statistically significant higher occurrence of the "G" allele in the later group (Table **2**).

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**Table 1. Different Sites in *ETS2* Investigated in the Present Study**

| SNP ID | Type | Alleles | Amino Acid Position | Effect |
|---|---|---|---|---|
| rs457705 | Synonymous SNP | G/T | 272 | Splicing regulation by SC35 (Pupasuite analysis) |
| rs461155 | Synonymous SNP | A/G | 341 | Splicing regulation by SRp40 (Pupasuite analysis) |
| rs34120017 | Deletion/Insertion polymorphism | -/C | 345 | Frame shift |
| rs35258008 | Deletion/Insertion polymorphism | -/A | - (Intronic) | - |

**Table 2. Allelic and Genotypic Frequencies of rs461155 in Control and Breast Cancer Subjects**

| Groups | N | Allele | | $\chi^2$, p Value | Genotype | | | $\chi^2$, p Value |
|---|---|---|---|---|---|---|---|---|
| | | A | G | | AA | AG | GG | |
| Control | 144 | 0.618 | 0. 382 | 4.21, 0.040 | 0.361 | 0.514 | 0.125 | 31.1, <0.0001 |
| BC | 49 | 0.50 | 0.50 | | 0.020 | 0.960 | 0.020 | |

Comparative analysis of allelic frequencies for rs461155 among the CEU, HCB, JPT, YRI and eastern Indian populations revealed statistically significant higher frequency of the "G" allele in CEU (p<0.0001) and YRI (p<0.05) populations (Table **3**). Further, comparison between these populations for the occurrence of BC (Fig. **1**) showed that CEU and YRI have significantly higher incidence (p<0.0001).

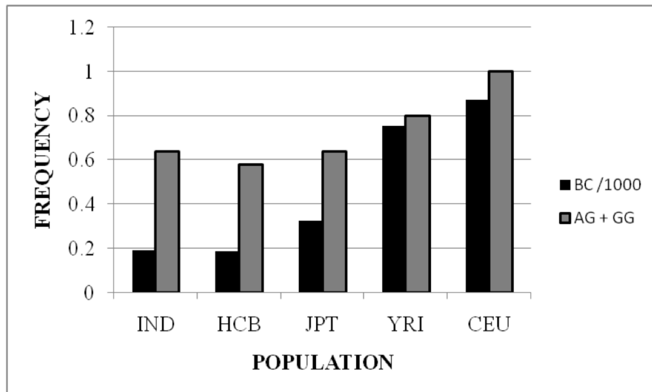


**Fig. (1).** Comparative analysis of genotypes (AG+GG) and age-standardized incidence of breast cancer (2002 estimates) in different populations.

Pupasuite analysis showed that the score for SRp40 activity, as exonic splicing enhancer, is 4.00 for the "A" allele and 1.39 for the "G" allele [11]. Therefore, higher occurrence of the rs461155 "G" allele may lead to loss of SRp40 mediated splicing of pre-mRNA and alter/reduce ETS2 activity. FastSNP analysis [12] also revealed that rs461155 could be considered as a "medium risk" SNP (risk level: 2-3).

Since rs461155 "G" allele may alter splicing of this important TF, it can be speculated that target genes further downstream of *ETS2* could be regulated incorrectly; along with the *P53* mediated apoptotic pathway and cell cycle regulatory pathways, *ErbB2*-mediated regulation of BC may also be altered since down regulation of *ETS2* leads to over-expression of *ErbB2* [13, 14]. Therefore, in addition to its action on *BRCA*1 [4], *ETS2* may influence development of BC through *P53* and *ErbB2* mediated pathways.

The "G" allele was found to be present in higher frequencies in both heterozygous as well as homozygous conditions in the CEU and YRI populations in comparison to the Asian population groups. Further investigations are necessary to find out whether this could be related to the reported higher occurrence of BC in these two populations.

The present study is the first to report frequency of rs461155 in the Indian population. From the result obtained it is evident that there is a clear over-representation of the 'G' allele in BC patients in contrast to the controls. It may be hypothesized from the current investigation that, along with other internal and external causative factors of BC, presence of *ETS2* rs461155 'G' allele may hinder splicing of *ETS2* by

**Table 3. Comparison of Allelic and Genotypic Frequencies for rs461155 in Different Populations**

| Populations | Allele | | $\chi^2$, p Value | Genotype | | | $\chi^2$, p Value |
|---|---|---|---|---|---|---|---|
| | A | G | | AA | AG | GG | |
| INDIAN | 0.618 | 0.382 | - | 0.361 | 0.514 | 0.125 | - |
| HCB | 0.611 | 0.389 | 0.925, 0.336 | 0.422 | 0.378 | 0.20 | 4.12, 0.127 |
| JPT | 0.636 | 0.364 | 0.858E-01, 0.771 | 0.364 | 0.545 | 0.091 | 0.806, 0.668 |
| YRI | 0.475 | 0.525 | 4.54, 0.030 | 0.20 | 0.55 | 0.25 | 8.44, 0.015 |
| CEU | 0.158 | 0.842 | 44.5, <0.0001 | 0.00 | 0.317 | 0.683 | 78.1, <0.0001 |

SRp40, thereby resulting in down regulation of *ETS2*. Further research investigating the role of *ETS2* as a candidate for BC is warranted in the light of the present investigation.

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