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Association of G1691A Mutation in Women with Breast Cancer

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Abstract

Objectives: More evidence indicates that the G1691A mutation in the factor V Leiden (*FVL*) gene might be associated with susceptibility to breast cancer in humans being. Breast cancer is gradually becoming the most common cancer in Indian women, pathogenesis can be influenced by single nucleotide polymorphisms and several studies in the past have identified different genetic variants in the human genome that showed strong or moderate evidence of associations. *FVL* is a missense mutation, result of an amino acid substitution of glutamine for arginine at 506 position in the factor V molecule at nucleotide 1691 substitutes G for A, resulting in a mutant protein resistant to the anticoagulant action of activated protein C.

Methods: We aimed to investigate the role of G1691A mutation in *FVL* gene and breast cancer women from south Indian population. One hundred cases and 100 controls were included in this study. DNA was separated and PCR-RFLP was performed followed by 2% electrophoresis.

Results: The results of this study indicates that *FVL* mutation is associated with breast cancer patients ($p<0.05$).

Conclusion: Our results concluded that *FVL* mutation has a role in breast cancer women and these results are supported by prior studies.

Keywords: Breast cancer; *FVL* gene; G1691 mutation; Tamoxifen

Introduction

Cancer is a complex disease resulting from genetics, environmental factors and their interactions, effects the public health problem worldwide [1]. Epidemiological studies have indicated that traditional risk factors including diet rich in fat, less physical activity, smoking, and alcohol consumption contributed to the susceptibility to cancer. As is known, comparing with the traditional factors, genetic variants are not associated with potential confounders, can be measured objectively and precisely, and may act as a lifelong marker of diseases [2]. Breast cancer is a multifactorial disease, leading cause of morbidity and 4% of mortality in women worldwide [3]. Breast cancer is gradually becoming the most common cancer in Indian women, mainly in urban areas such as Mumbai, Delhi, Ahmadabad, and Bangalore [4]. Multiple environmental factors for breast cancer have been identified, including age at first birth, menarche and menopause, and family history, but the underlying genetic bias remained largely unknown [5]. Breast cancer risk and pathogenesis can be influenced by single nucleotide polymorphisms and several studies in the past have identified different genetic variants in the human genome that showed strong or moderate evidence of associations [6-7]. Coagulation markers in predicting thrombosis during chemotherapy and/or hormonal treatment in patients with cancer is not established, it is well known that some genetically based abnormalities of coagulation are generally associated with an increased risk for venous thromboembolism. Inherited resistance to activated protein C is a prothrombotic condition resulting from a gain-of-function mutation of coagulation factor V, commonly referred to as factor V Leiden (*FVL*) [8]. *FVL* mutation is a result of an amino acid substitution of glutamine for arginine at 506 position in the factor V molecule. This missense mutation at in the *FVL* gene at nucleotide 1691 substitutes G for A, resulting in a mutant protein resistant to the anticoagulant action of activated protein C [9]. We hypothesized a role of G1691A mutation in *FVL* gene and breast cancer women from cosmopolitan city, Hyderabad in south Indian population.

Materials and Methods

Study population

This is a case-control study carried out in South Indian population. Breast carcinoma women were evaluated based on clinical and pathological investigations. The details of the selection of cases and controls, and inclusion and exclusion criteria were described in our prior publication [10]. Ethical committee has approved the study and informed consent was obtained from each participant. Participants were asked about their family history of cancer, and the clinical information for these cases was obtained from medical records like tumor size, stage, and whether they were receiving chemotherapy, and radiotherapy.

Sample collection

The tumor (tissue) specimen samples were collected from the breast cancer women and who has undergone for breast conservative therapy or radical mastectomy. The tumor samples were obtained of various

tumor sizes and diagnosed mainly as invasive ductal carcinoma or invasive lobular carcinoma. Peripheral blood sample were obtained from healthy women without any complications in an ethylenediaminetetraaceticacid (EDTA) tube.

DNA and amino acid substitution mutation

Genomic DNA was isolated from tissue samples from the breast cancer patients and blood samples collected in an EDTA tube by salting out technique, which was routinely used [10]. The quality and quantity of the DNA was quantified by spectrophotometer. The purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm (A_{260}/A_{280}). Non-annealed DNA should have an A_{260}/A_{280} ratio of 1.7–1.9. Genotyping for the rs6020 (G1691A) was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) followed by agarose gel electrophoresis. Amplification of the fragment was performed with forward primer 5'-TCAGGCAGGAAC AACACCAT-3' and reverse primer 5'- GGTTACTTCAAGGACAAAATACCTGTAA-3' which has been published Alharbi et al. [11]. Primers were synthesized by Bioserve biotechnology (Hyderabad, India) for PCR analysis. DNA was denatured at 95°C for 5 min, amplified by 35 cycles of 95°C for 30s, 56°C for 30s, 72°C for 45s and the final extension with 72°C for 5 min. PCR products were digested with *HIndIII* (A↓AGCTT) (Fermentas, USA) at 37°C and electrophoresed on ethidium bromide added 2% agarose gel (Figure 1).

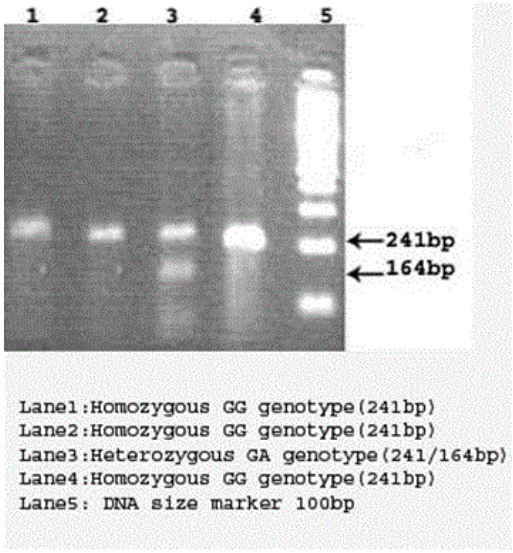

Figure 1: 2% agarose gel picture represent the digested fragments for G1691 mutation in *FVL* gene.

Statistical analysis

Statistical analysis was carried out using SPSS (Chicago, IL, USA) software version 16.0 for Microsoft windows®. Continuous variables were compared between the groups using two-tailed student's *t* test. Significant cutoff was set at 0.05. Crude ORs with 95% CIs were used to evaluate the strength of the association between G1691 mutation and breast cancer patients. Pooled ORs were calculated for allelic contrast (G vs. A) genetic model. Z-test was used to determine the significance of the pooled ORs, and p value < 0.05 was considered significant. Yates correction was also performed. A level of $p<0.05$ was considered statistically significant.

Results

There was a deviation from hardy Weinberg equilibrium in the cases or controls for G1691A mutation ($p<0.05$). The distribution of *FVL* (G1691A) mutation among breast cancer patients are tabulated in Table 1. The frequency of *FVL* GG and GA genotypes among breast cancer patients were 95% and 5% respectively. The percentage of G allele was 0.95% and that of A allele was 0.05%. In controls, the allele frequency was 100% in G allele. None of the mutations were observed in the AA genotype in both the cases and controls. When we compared the frequency G191A mutation in cases and controls, we found a statistically significant difference between them and found to be significant (GA vs GG: OR-1.1, 95%CI (1.0-1.8); $p=0.03$ and G vs A: OR-1.1, 95%CI (1.0-1.8); $p=0.03$).

Genotypes/Alleles	Patients (n=100)	Controls (n=100)	OR (95% CI)	*p* value
GG	95 (95%)	100 (100%)	Reference	Reference
GA	5 (5%)	0* (0)	1.1 (1.0-1.8)	0.03
AA	0 (0)	0 (0)	-	-
G	195 (97.5)	200 (100)	1.1 (1.0-1.9)	0.03
A	05 (2.5)	0 (0)	Reference	Reference

Table 1: Genotype and allele frequency of FVL (G1691A) gene mutations.

Discussion

In this prospective case-control study, we scrutinized the association of common and widely studied mutation of *FVL* (1691G>A) gene with breast cancer cases and control subjects in south Indian population. Till date, there are limited studies on breast cancer patients in the south Indian population is available. To our knowledge, this is the first study evaluating the role of the gain-of-function mutations of the *FVL* in breast cancer womens. We found that *FVL* mutation has a role in breast cancer patients and control subjects ($p<0.05$).

Our results are similar with the prior studies carried out in the different ethnicities [8,12,13]. In this study, five mutations were appeared in the *FVL* gene in the breast cancer women and none of the mutations were found in the control subjects. All our selected breast cancer cases ($n=100$) were treated with Tamoxifen therapy. We have carried out the prognostic significance of tamoxifen therapy in breast cancer patients with 5 years of survival study.

The potential interaction between the *FVL* mutation and tamoxifen, a serum estrogen receptor modulator with known thrombogenic effects that have been attributed to its estrogen agonist activity, has been systematically explored in the large tamoxifen risk-reduction trials, which should provide the setting in which the effects are easiest to discern. The International Breast Cancer Intervention Study was a nested case-control study that assessed intrinsic and acquired risk factors for thromboembolic event, in which women with elevated breast cancer risk based on family history were randomized to 5 years of tamoxifen or placebo use. Tamoxifen has been an important and

effective agent in the treatment of patients with hormone receptor–positive breast cancer for more than three decades. Its ability to substantially reduce the incidence of new hormone receptor–positive invasive and in situ breast cancers has been established for women who are at increased risk for breast cancer based on family history and other risk factors, including prior breast cancer [12]. The oral selective estrogen receptor modulator, tamoxifen, taken daily for 5 years, substantially reduces breast cancer risk for women who are at increased risk owing to their family cancer history, reproductive risk factors, or personal history of atypical hyperplasia or lobular carcinoma *in situ* [14].

The mutant Factor V is referred to as *FVL* because Dutch investigators from the city of Leiden were the first to report the mutation [15]. *FVL* is a genetic disorder characterized by a poor anticoagulant response to APC. APC is a natural anticoagulant protein that cleaves and inactivates procoagulant Factors Va and VIIIa, thereby down regulating further thrombin generation. APC inactivates Factor Va by cleavage at three different amino acid positions: R (arginine) 306, R 506, and R 679 [16]. *FVL* heterozygotes have a 2- to 3-fold increased risk for central venous catheter-related thrombosis [17]. The mutation increases the risk for catheter-related thrombosis in patients with advanced or metastatic breast cancer and those undergoing allogeneic bone marrow transplantation [8,18]. A limitation of the current study includes a comparatively small number of selected subjects diagnosed with breast cancer (n=100). Because previous studies have attempted to evaluate and analyze other SNPs in the same gene, our selection of a single specific SNP may present another limitation of our study. Until better targeted therapies are available for *FVL* mutation carriers, the option of tamoxifen for breast cancer prevention should be discussed along with the evidence of benefits and potential side effects, thereby enabling an informed choice for women who wish to consider prevention therapy.

Conclusion

In conclusion, *FVL* mutations found to be a role in breast cancer women, associated in our study.

References

1. Jia ZM, Liu Y, Cui SY (2014) Lack of association between cyclin-dependent kinase inhibitor 1B rs2066827 polymorphism and breast cancer susceptibility. Tumour Biol 35: 5527-5531.
2. Benson JR, Jatoi I (2012) The global breast cancer burden. Future Oncol 8:697–702.
3. Alhomidi MA, Vedicherla B, Movva S, Rao PK, Ahuja YR, et al. (2013) Mitochondrial D310 instability in Asian Indian breast cancer patients. Tumour Biol 34: 2427-2432.
4. Dey S, Boffetta P, Mathews A, Brennan P, Soliman A, et al. (2009) Risk factors according to estrogen receptor status of breast cancer patients in Trivandrum, South India. Int J Cancer 125: 1663–1670.
5. Bu T, Liu L, Sun Y, Zhao L, Peng Y,et al. (2014) XRCC1 Arg399Gln polymorphism confers risk of breast cancer in American population:a metaanalysis of10846 cases and 11723 controls. PLoS One 28: e86086.
6. Zhang B, Beeghly-Fadiel A, Long J (2011) Genetic variants associated with breast-cancer risk: comprehensive research synopsis, meta-analysis, and epidemiological evidence. Lancet Oncol 12: 477-488.
7. Minhas S, Bhalla S, Shokeen Y, Jauhri M, Saxena R,et al. (2014) Lack of any Association of the CTLA-4+49 G/A polymorphism with Breast cancer risk in a North Indian population. Asian Pac J Cancer Prev 15: 2035-2038.
8. Mandala M, Curigliano G, Bucciarelli P, Ferretti G, Mannucci PM,et al. (2004) Factor V Leiden and G20210A prothrombin mutation and the risk of subclavin vein thrombosis in patients with breast cancer and a central venous catheter. Ann Oncol 15: 590-593.
9. Parveen F, Shukla A, Agrawal S (2013) Should factor V Leiden mutation and prothrombin gene polymorphism testing be done in women with recurrent miscarriage from North India. Arch Gynecol Obstet 287: 375-381.
10. Kalyan KC, Mohan RN, Laxmi A, Adhitya V, Tabassum SN,et al. (2013) Clinical Impact of CYP2D6 and SULT1A1 Polymorphisms and Tamoxifen with Breast Cancer. Open Access scientific reports 2:1-4.
11. Alharbi KK, Khan IA, Syed R (2012) Thrombophilic gene polymorphism studies in G6PD deficient individuals from Saudi population. Bioinformation 8: 1255-1259.
12. Garber JE, Halabi S, Tolaney SM, Kaplan E, Archer L, et al. (2010) Factor V Leiden mutation and thromboembolism risk in women receiving adjuvant tamoxifen for breast cancer. J Natl Cancer Inst 102: 942-949.
13. Curigliano G, Mandala M, Sbanotto A, Colleoni M, Ferretti G, et al. (2006) Factor v Leiden mutation in patients with breast cancer with a central venous catheter: risk of deep vein thrombosis. Support Cancer Ther 3: 98-102.
14. Phillips KA, Lindeman GJ (2014) Breast cancer prevention for BRCA1 and BRCA2 mutation carriers: is there a role for tamoxifen. Future Oncol 10: 499-502.
15. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ,et al. (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 369: 64–67.
16. Dahlback B (2008) Advances in understanding pathogenic mechanisms of thrombophilic disorders. Blood 112: 19 –27.
17. Van Rooden CJ, Rosendaal FR, Meinders AE, Van Oostayen JA, Van Der Meer FJ, et al. (2004) The contribution of factor V Leiden and prothrombin G20210A mutation to the risk of central venous catheter-related thrombosis. Haematologica 89: 201–206.
18. Fijnheer R, Paijmans B, Verdonck LF, Nieuwenhuis HK, Roest M, et al. (2002) Factor V Leiden in central venous catheter-associated thrombosis. Br J Haematol 118: 267–270.