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Association of Phosphatase and Tensin Homolog rs3830675 Gene Polymorphisms and Breast Cancer

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Background: Phosphatase and tensin homolog gene (PTEN) activity is pathologically lost in many human cancers. Germ-line mutations of PTEN have been found in several cancers.
Objectives: The purpose of this study was to determine the possible association of PTEN rs3830675 polymorphism with the development of breast cancer in Iranian women.
Patients and Methods: Genomic DNA was extracted from peripheral blood lymphocytes of 49 patients and 43 healthy women, using the standard Salting out/Proteinase K method. PTEN rs3830675 (IVS4) polymorphism was detected using the PCR-RFLP method.
Results: The allele frequency of rs3830675 polymorphism were 37% and 27%, in patients and healthy subjects, respectively; showing that the polymorphism was 10% more prevalent in patients with breast cancer.
Conclusions: PTEN rs3830675 polymorphism was associated with breast cancer, which might suggest the involvement of PTEN polymorphism in the molecular pathways related to the development of breast cancer. However, more studies with larger sample sizes are needed to address this question.

Keywords: Breast Neoplasms; Polymorphism, Genetic; PTEN Phosphohydrolase

1. Background

Breast cancer is one of the most common cancers in women. The early diagnosis increases survival of patients with breast cancer. Critical role of genetic factors involved in breast cancer development has been demonstrated before, which is generally considered as an imbalance between the activities of tumor suppressor genes and growth factors and oncogenes. Oncogene and tumor suppressor genes have a key role in the cancer regulatory pathways, tumor growth, and cellular proliferation (1). Oncogene activation and/or inactivation of tumor suppressors cause abnormal cell growth. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a tumor suppressor gene, which is located on 10q23.31, spans 105 kb and contains 9 exons (2, 3).This genomic area is frequently deleted in many types of cancers, including brain, prostate, and breast (4, 5). PTEN is a dual lipid and protein phosphatase playing an important role in cell proliferation, cell cycle progression, and apoptosis of cancer cells (3, 6, 7). The growth suppression activity of PTEN is mediated by blocking the cell cycle progression in the G1phase through deregulation of PI3K/AKT signaling pathway (8). It dephosphorylates the PI3K molecule and suppresses the mitogen-activated protein kinase (MAPK) signaling pathway (9). This enzyme can also dephosphorylate the PIP3 (phosphatidylinositol 3, 4, 5-trisphosphate) molecule which has lipid transporter activity (10). Germ line mutations in the PTEN gene are associated with multiple autosomal dominant neoplastic syndromes such as the Cowden disease, which may predispose the formation of several malignancies, including breast cancer. Studies in patients with breast cancer indicated a correlation between the defective PTEN and the factors predictive of poor prognosis as well as resistance to hormone therapy (5, 11, 12). The involvement of PTEN somatic mutations has also been reported in the pathogenesis of various cancers such as breast cancer (13, 14). One of the common PTEN gene polymorphisms is IVS4 (rs3830675) which is caused by the insertion of 5 nucleotides (ACTAA) in 109 bp downstream of the exon 4 of the intron 4. Presence of this polymorphism is associated with the early diagnosis of breast cancer (15).

Implication for health policy/practice/research/medical education:
Breast cancer is the first cancer affecting women both in developed and developing countries. The incidence of breast cancer is on the rise in the developing counties. We focused on the genetic assessment in our study, because it can help the early detection of breast cancer.

2. Objectives

The aim of this study was to investigate the possible role of germ line PTEN rs3830675 (IVS4) polymorphism on breast cancer development in Iranian women.

3. Patients and Methods

Study was designed as a case-control study. Inclusion criteria were: age 35-65 years, no history of thyroid cancer/adenoma, uterine fibroid, fibrocystic disease of the breast, endometrial cancer and renal carcinoma. We finally enrolled 50 patients with pathologically proven breast cancer, but one patient was excluded because of thyroid disorder (multi nodular goiter), and thus 49 patients were entered the study. In the control group, first 45 volunteer women were considered, but finally 43 were enrolled because of missing data on some of them. All participants were enrolled in the study after obtaining an informed written consent. Also, the study was approved by the Institutional Review Board and the Ethics Committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences (approval code:96-811030). DNA was extracted from peripheral blood samples according to the standard Salting-out/Proteinase K method and was stored at-20˚C. For genotyping, the PCR -RFLP method was used to detect the IVS4 polymorphism in DNA samples. For this purpose, genomic DNA was amplified using the following primers: (F5´CTTTATGCAATACTTTTTCCTA3´) and (R5´GGGGGTGATAACAGTATCTA3´). The PCR amplification was performed in a volume of 25 microliters containing 1.5 microliters 10 × buffer, 1 microliter template DNA(50ng/L), 0.3 microliters of each dNTPs (10mM) (Boehringer Mannheim Co., Germany), 0.8 microliters of each primer (10M) (TIB MOLBIOL Synthesalabor Co., Germany, www.tib-molbiol.de), 0.25 microliters $MgCl_2$ (50mM) and 1 unit Taq polymerase (Boehringer Mannheim Co., Germany). PCR reaction was repeated for 30 cycles in an automatic thermocycler (Omnigene & Hybaid Co.) under the following conditions: denaturation at 95˚C for 7 min, annealing at 55.4˚C for 1 min, and extension at 72˚C for 45 sec, and final extension at 72˚C for 5 min. The amplified PCR products were digested with MspCI (Roche, Germany) restriction enzyme according to the manufacturer's instructions. The digested samples were separated by electrophoresis through a 2% agarose gel electrophoresis (Cambrex, Denmark) and detected by UV illumination after ethidium bromide staining. The RFLP patterns produced by this restriction enzyme in the presence and absence of IVS4 gene polymorphism in the samples are shown in Table. In normal subjects with homozygous gene, a 420bp DNA fragment was detected, whereas in subjects with polymorphic homozygous allele, a 320bp DNA fragment was detected. Data was analyzed by SPSS program. Quantitative variables including demographic data (age, weight, height) were checked for normal distribution by Kolmogorov–Smirnov test, and were described as mean ± SD and qualitative variables were shown as percentages. For all statistical tests, the significance level was considered as $P < 0.05$. To compare two groups of data, the independent t-test and for the allele frequency determination the Mann-Whitney test was used.

4. Results

The demographic data of the studied population and genotypes, and allele frequency results are shown in Tables 1, 2, and 3, respectively. The frequency of the wild type and mutant alleles were 63% and 37% in patients with breast cancer, respectively. However, the frequency of these alleles in the control group was 73% and 27%, respectively. So, the frequency of IVS4 allele in patients with breast cancer was 10% higher compared to the control group. . In addition, the genotype frequency was determined to be in the Hardy-Weinberg equilibrium. In patients with this polymorphism, the mean tumor size was 5 ± 2.2 cm, whereas in patients without this, it was 3 ± 2.1 cm. However, there was no significant association between the tumor size and allele type, using t-test analysis. The "metastatic lymph node ratio" was defined as the number of metastasis harboring lymph nodes divided by the total number of studied lymph nodes. The ratio was 0.453 ± 0.341 and 0.311 ± 0.019, in patients with this polymorphism and those with the wild type allele, respectively. However, the difference was not statistically significant using Mann-Whitney test ($P = 0.35$). In addition, there was no significant association between the age and wild type or polymorphic alleles.

Table 1. The Demographic Features of Participants

	Control (n= 43)	Case (n = 49)	P value
Age, y, mean ± SD	48 ± 10	49 ± 11	0.949
Weight, kg, mean ± SD	73 ± 14	67 ± 11	0.188
Height, cm, mean ± SD	156 ± 5	154 ± 5	0.394

Table 2. The Genotype Frequency of Homozygote Wild Type or Mutant (WW or MM) and Heterozygote (MW) Individuals

Genotype	Control (n= 43)	Case (n= 49)	P value
MM, No. (%)	2 (4.7)	6 (12.2)	0.046
MW, No. (%)	19 (44.2)	24 (49)	0.052
WW, No. (%)	22 (51.1)	19 (38)	0.048

Table 3. The Allele Frequency of Wild Type (W) and Mutant (M) Alleles

	Control (n = 83)	Case (n = 98)	P value
M, No. (%)	23 (26.7%)	36 (36.7%)	0.055
W, No. (%)	63 (73.2%)	62 (63.2%)	0.059

5. Discussion

In this study, the polymorphic allele frequency of PTEN IVS4 in women with breast cancer was higher than the healthy women (37% vs. 27%). In 2001, Depowski et al. suggested a role for PTEN gene in breast cancer development (16). As the PTEN gene product has a protein phosphatase activity which has a key role in tumor inhibition, IVS4 polymorphism might be associated with breast cancer development. In 2002, Bose et al, reported that PTEN mutation increases the probability of lymph node metastasis in patients with breast cancer, however, they could not find a significant association between lymph node metastasis and the IVS4 allele (17). PTEN mutation was rare in the French-Canadian population with breast cancer (18), but some other investigators reported high prevalence of PTEN mutation in patients with breast cancer (19). Altogether, allelic loss, mutations and polymorphisms of the tumor suppressor gene PTEN have been reported in several cancers such as gastrointestinal, prostate, ovarian, and endometrial cancer (12, 20-22).Whether the IVS4 polymorphism disrupts the splicing or expression of the PTEN is currently unknown. The chromosomal position of this genetic variant, which is next to exon 5, a hot spot for the Cowden disease mutations, suggests that a splicing error causing a deletion in the exon 5 can have a potentially significant effect. However, Bryan et al. could not identify alternative splice forms of this gene using RT-PCR method (15). Additionally, this polymorphism may be in genetic linkage disequilibrium with a functional mutation in another gene, and thus its functional effects remain speculative (15).

In conclusion, in our study, the allele frequency of rs3830675 polymorphism was higher in patients with breast cancer than the healthy ones, but the association was not statistically significant probably because of the small sample size. Finally, other polymorphisms in the PTEN gene might be associated with the development of breast cancer, which may warrant additional investigations in the future. In addition, functional effects of these polymorphisms remain to be identified. Identification of the genes involved in breast cancer development can potentially lead earlier diagnosis of breast cancer and more effective treatment of these patients.

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Authors' Contribution

Study concept and design: Dr. Baharam Mofid, Dr. Mehdi Hedayati; Data acquisition: Dr. Mehdi Hedayati, Parisa Eshraghi, Laleh Hoghooghi Rad; Analysis and interpretation of the data: Dr. Mehdi Hedayati, Marjan ZarifYeganeh; Drafting the manuscript: Dr. Mehdi Hedayati, Marjan Zarif Yeganeh; Critical revision of the manuscript for important intellectual content: Dr. Mehdi Hedayati and Dr. Maryam Daneshpour; Statistical analysis: Dr. Mehdi Hedayati; Administrative, technical, and material support: Laleh Hoghooghi Rad; Study supervision: Dr. Bahram Mofid, Dr. Mehdi Hedayati.

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References

1. Bishop JM. Molecular themes in oncogenesis. *Cell.* 1991;**64**(2):235–48.
2. Tamura M, Gu J, Tran H, Yamada KM. PTEN gene and integrin signaling in cancer. *J Natl Cancer Inst.* 1999;**91**(21):1820–8.
3. Li DM, Sun H. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. *Cancer Res.* 1997;**57**(11):2124–9.
4. Petrocelli T, Slingerland JM. PTEN deficiency: a role in mammary carcinogenesis. *Breast Cancer Res.* 2001;**3**(6):356–60.
5. DeGraffenried LA, Fulcher L, Friedrichs WE, Grunwald V, Ray RB, Hidalgo M. Reduced PTEN expression in breast cancer cells confers susceptibility to inhibitors of the PI3 kinase/Akt pathway. *Ann Oncol.* 2004;**15**(10):1510–6.
6. Fine B, Hodakoski C, Koujak S, Su T, Saal LH, Maurer M, et al. Activation of the PI3K pathway in cancer through inhibition of PTEN by exchange factor P-REX2a. *Science.* 2009;**325**(5945):1261–5.
7. Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, et al. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc Natl Acad Sci U S A.* 1997;**94**(17):9052–7.
8. Macarthur AM, Fountain SW. Intracavity suction and drainage in the treatment of emphysematous bullae. *Thorax.* 1977;**32**(6):668–72.
9. Perks CM, Vernon EG, Rosendahl AH, Tonge D, Holly JM. IGF-II and IGFBP-2 differentially regulate PTEN in human breast cancer cells. *Oncogene.* 2007;**26**(40):5966–72.
10. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem.* 1998;**273**(22):13375–8.
11. Nelen MR, Padberg GW, Peeters EA, Lin AY, van den Helm B, Frants RR, et al. Localization of the gene for Cowden disease to chromosome 10q22-23. *Nat Genet.* 1996;**13**(1):114–6.
12. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet.* 1997;**16**(1):64–7.
13. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science.* 1997;**275**(5308):1943–7.
14. Kong D, Suzuki A, Zou TT, Sakurada A, Kemp LW, Wakatsuki S, et al. PTEN1 is frequently mutated in primary endometrial carcinomas. *Nat Genet.* 1997;**17**(2):143–4.
15. Carroll BT, Couch FJ, Rebbeck TR, Weber BL. Polymorphisms in PTEN in breast cancer families. *J Med Genet.* 1999;**36**(2):94–6.
16. Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. *Mod Pathol.* 2001;**14**(7):672–6.
17. Bose S, Crane A, Hibshoosh H, Mansukhani M, Sandweis L, Parsons R. Reduced expression of PTEN correlates with breast cancer progression. *Hum Pathol.* 2002;**33**(4):405–9.
18. Guenard F, Labrie Y, Ouellette G, Beauparlant CJ, Bessette P, Chi-

quette J, et al. Germline mutations in the breast cancer susceptibility gene PTEN are rare in high-risk non-BRCA1/2 French Canadian breast cancer families. *Fam Cancer.* 2007;**6**(4):483–90.
19. Chung MJ, Jung SH, Lee BJ, Kang MJ, Lee DG. Inactivation of the PTEN gene protein product is associated with the invasiveness and metastasis, but not angiogenesis, of breast cancer. *Pathol Int.* 2004;**54**(1):10–5.
20. Sakai A, Thieblemont C, Wellmann A, Jaffe ES, Raffeld M. PTEN gene alterations in lymphoid neoplasms. *Blood.* 1998;**92**(9):3410–5.
21. Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, et al. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. *Cancer Res.* 1997;**57**(18):3935–40.
22. Okami K, Wu L, Riggins G, Cairns P, Goggins M, Evron E, et al. Analysis of PTEN/MMAC1 alterations in aerodigestive tract tumors. *Cancer Res.* 1998;**58**(3):509–11.