A Functional Polymorphism (rs6265, G>A) of Brain-Derived Neurotrophic Factor Gene and Breast Cancer: An Association Study

Breast Cancer: Basic and Clinical Research
Volume 13: 1–5

DOI: 10.1177/1178223419844977

Mehir un Nisa Iqbal[1], Tahniyat Yaqoob[1], Syed Adnan Ali[2] and Taseer Ahmed Khan[1]

[1]Department of Physiology, University of Karachi, Karachi, Pakistan.
[2]Government Degree Science and Commerce College, Karachi, Pakistan.

ABSTRACT

PURPOSE: The objective of this study was to evaluate the relationship between brain-derived neurotrophic factor (BDNF) gene (Val66Met, rs6265, G>A) polymorphism and breast cancer (BC) among females of Southern Pakistan.

METHODS: This case-control study consisted of 300 females (BC cases [n = 100] and controls [n = 200]) with age range of 18 to 45 years. All participants were recruited during January to December 2014 and were screened for depression using Zung depression scale. Isolation of genomic DNA (gDNA) followed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was done. All statistical analysis was carried out on IBM-SPSS version 22 at *P*-value <.05. Hardy-Weinberg equilibrium (HWE), Pearson chi-square, and odds ratios (ORs) with 95% confidence interval (95% CI) were calculated.

RESULTS: Genotype distribution of BDNF gene polymorphism lies in the goodness-of-fit model among controls. The statistical analyses reveal a significant association between genotype frequencies ($\chi^2 = 12.709$, *P*-value = .002) of BDNF and BC among cases and controls. The AA genotype (OR = 5.2, 95%CI = 0.632-42.804) increases the risk of having BC.

CONCLUSIONS: Our results suggest that BDNF gene polymorphism may have an association with BC risk among Pakistani females. However, the present finding needs to be replicated with greater sample size with BC risk.

KEYWORDS: depression, BDNF, breast cancer, rs6265, Val66Met

RECEIVED: March 20, 2019. **ACCEPTED:** March 25, 2019.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Mehir un Nisa Iqbal, Department of Physiology, University of Karachi, 75270 Karachi, Pakistan. Email: mehirunisa@uok.edu.pk

Introduction

Breast cancer (BC) is an assorted group of the malignant disease having incidence more than 1.2 million/year worldwide.[1] It is a major health problem in developing countries including Pakistan.[2] It is aggravated by many factors including family history, nulliparity, being single, being older, early menarche, and late menopause.[3]

Cancers including BC has an association with depression.[4] Depression may exist with misery, harshness, lowered self-esteem, and bleakness, and suicidal mania also occurs in patients with cancer and confuses the findings of cancer. The severity of depression depends on the cancer types and reaction to its treatment.[5] An inverse correlation was found between social support and depressive disorder among Pakistani BC survivor (BCs).[6] Many factors including marital status,[7] early menarche,[8] nulliparity,[9] obesity,[10] and use of oral contraceptives (OC)[11] increase the depression which can modify BC risk.

Depression is one of the complex psychiatric disease phenotypes, with etiological factors which are under genetic control.[12] However, no particular risk genotype has been confirmed yet, but some minor genes and their mutations may act as an etiological factor for the depression. Among which brain-derived neurotrophic factor (BDNF) gene is one of them which is present at chromosome 11p14.1 along with various polymorphic markers. A 196 G/A variation causes Val-Met substitution in pro-BDNF molecule at codon 66 related to reduced BDNF activity.[13] However, depression can be more frequently found in patients with Met allele than Val allele.[14] BDNF is positively associated with depression among BCs undergoing mastectomy.[15] Although many studies have reported the association between the BDNF gene polymorphism and depression,[16,17] no study determined the relationship between BDNF gene polymorphism and BC risk. Breast cancer contributing factors increase the depression, which enhances the odds of developing BC. However, if we control the BC contributing factors, depression will be reduced which could prevent BC. So the rationale of this study was to carry out the association between BDNF gene polymorphism and BC risk.

Materials and Methods

Ethical approval

This study was approved by the Board of Advanced Studies and Research (Approval#10; dated March 28, 2012), University of Karachi. This research was conducted in accordance with Helsinki declaration, and all participants have given their informed consent for participation in the study.

Study participants

In this case-control study, we enrolled premenopausal women (100 cases and 200 controls) randomly from a tertiary care hospital located in Karachi, Pakistan, from January to December 2014. Cases were histopathologically diagnosed with BC with age range of 18 to 45 years, whereas controls were age matched with cases. The sample size was calculated using online sample size calculator (http://www.openepi.com/SampleSize/SSCC.htm) with 95% confidence interval (CI) and 80% of statistical power. A self-structured questionnaire was also provided to collect the information about the person's demographics and other parameters.

Zung Self-Rating Depression Scale

All study subjects were screened for depression using a qualitative scale known as *Zung Self-Rating Depression Scale* (ZSRDS).[18] The response of each subject was interpreted using SDS Index formula: SDS Index = (Raw Score/80 total points) × 100 or SDS Index = Raw Score × 1.25. The scores then determined the severity of depression. Score <50 is normal whereas >50 indicates depression.

gDNA isolation

The isolation of genomic DNA (gDNA) performed from the venous blood using kit method (ReliaPrep Blood gDNA Miniprep System, Promega) is in accordance with the manufacturer's instruction.

BDNF genotyping

The primer-specific polymorphism was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The gene-specific forward primer (FP)-5′ACTCTGGAGAGCGTGAATGG3′ and reverse primer (RP)-5′ACTACTGAGCATCACCCTGGA3′[19] were used. The reaction mixture for PCR contained 25 µL of 2× DNA polymerase ready mix (Kapa Biosystem, USA), 0.3 µL of both primers (10 pmol), 4 µL of gDNA, and 20.2 µL of nuclease free water in a total reaction volume of 50 µL. Amplification was performed in an automated thermal cycler (Veriti, Applied Biosystem, USA) under PCR conditions: initial denaturation at 94°C (5 minutes), 40 cycles of 94°C (30 seconds), 62°C (2 minutes), and 72°C (1 minute), followed by 7 minutes of final extension at 72°C. The products were then analyzed using gel electrophoresis on 1.5% agarose gel at 80 V run in 0.5× TBE buffer for 30 minutes staining with 0.5 µg/mL of ethidium bromide followed by the gel visualization using ChemiDoc-It2 (UVP, UK) Vision works LS software (ver-7.1).

The amplified products were then digested with 5 U of the enzyme Eco72I (Fermentas, USA) at 37°C followed by 2.5% agarose gel electrophoresis and visualized gel images using the same imager. The size of amplified product is 171 bp denoted as A-allele (Met-allele), whereas the size of post digestion product is 99 and 72 bp denoted as G allele (Val-allele).

Table 1. Clinical characteristics of BC.

CLINICAL CHARACTERISTICS	FREQUENCY (N)
BC types	
Infiltrating ductal carcinoma (IDC)	64
Infiltrating papillary carcinoma (IPC)	6
Ductal carcinoma in situ (DCIS)	4
Others	8
BC Grades	
I	6
II	42
III	30
IV	4
Estrogen receptor (ER) status	
Estrogen receptor +ve (from 0 to +9)	14
Estrogen receptor –ve (from –1 to –2)	2
Progesterone receptor (PR) status	
Progesterone receptor +ve (from +0 to +8)	10
Progesterone receptor –ve (from –1 to –2)	6
Her 2 neu oncoprotein	
Her2neu +ve (from 0 to +3)	6
Her2neu –ve (from –1 to –2)	8

Abbreviation: BC, breast cancer.

Statistical analysis

The association between BDNF genotyping and BC was calculated by Pearson chi-square. The deviation of allele frequencies was calculated by Hardy-Weinberg equilibrium (HWE). Risk of depression-associated BC was evaluated using odds ratios (ORs) and 95% CI via conditional logistic regression analysis. All analysis was performed using IBM-SPSS version 22 at a significance level <0.05.

Results

This study consisted of 100 BC cases and 200 controls. Out of 100 cases and 200 controls, 82 and 198 were positively amplified, respectively. All selected females were in the age group 18 to 45 years and were premenopausal. The clinical characteristics of patients with BC are summarized in Table 1. Demographic, anthropometric profile, and other parameters are described in Table 2.

Table 2. Demographics, anthropometry profile, depression, and other parameters.

PARAMETERS	BC (82)	CONTROLS (198)	χ^2	*P*-VALUE	OR (95% CI)[a]
Marital status					
Single	6	146	103	**.000**	1
Married	76	52			**35.564 (14.614-86.54)**
Menarcheal age					
12-14	60	170	15.267	**.000**	1
>14	0	8			0.000 (0.000-NC)
<12	22	20			**3.117 (1.59-6.111)**
Cycle length					
Normal (3-7)	46	162	26.29	**.000**	1
Short (1-2)	32	24			**4.696 (2.52-8.749)**
Long (>7)	4	12			1.174 (0.361-3.813)
Parity					
Parous	74	48	102.737	**.000**	1
Nulliparous	8	150			0.035 (0.016-0.077)
Age at first birth					
<20	24	4	9.6	**.022**	1
20-24	30	26			**0.192 (0.059-0.627)**
25-29	16	14			**0.19 (0.053-0.684)**
>29	4	4			**0.167 (0.029-0.953)**
Basal metabolic index (BMI)					
Normal (18-25)	6	100	84.084	**.000**	1
Underweight (<18)	2	42			0.714 (0.138-3.688)
Overweight (25-30)	12	24			**7.5 (2.551-22.051)**
Obesity (>30)	62	32			**22.143 (8.873-55.259)**
Waist-to-hip ratio (WHR)					
Acceptable (<0.85)	2	58	24.836	**.000**	1
Unacceptable (>0.85)	80	140			**16.571 (3.942-69.672)**
Family history of BC					
No	62	184	16.304	**.000**	1
Yes	20	14			**4.24 (2.02-8.896)**
Family history of other cancer					
No	50	166	17.189	**.000**	1
Yes	32	32			**3.32 (1.853-5.948)**
OC					

(Continued)

Table 2. (Continued)

PARAMETERS	BC (82)	CONTROLS (198)	χ^2	*P*-VALUE	OR (95% CI)[a]
No	72	186	3.014	.083	1
Yes	10	12			2.153 (0.891-5.202)
Depression					
No	6	98	44.181	**.000**	1
Yes	76	100			**12.413 (5.166-29.825)**

Abbreviations: BC, breast cancer; CI, confidence interval; OC, oral contraceptives; OR, odds ratio.
All significant values are presented in bold.
[a]Binary logistic regression analysis, all the controls matched with age.

Table 3. Relationship between BDNF gene polymorphism and BC.

GENOTYPE	BC	CONTROLS	χ^2	*P*-VALUE	OR[a] (95% CI)
GG (Val/Val)	1	8	12.709	**.002**	1
GA (Val/Met)	52	80			2.109 (0.253-17.549)
AA (Met/Met)	29	110			5.2 (0.632-42.804)
Total	82	198			

Abbreviations: BC, breast cancer; BDNF, brain-derived neurotrophic factor; CI, confidence interval; OR, odds ratio.
[a]Binary logistic regression analysis, all the controls were matched with age.
P-value $< .05$ considered as significant.

Hardy-Weinberg equilibrium was calculated for the whole sample and genotype distribution of BDNF polymorphism lies under goodness-of-fit model among controls ($\chi^2 = 1.98$; $P = .159$), not in cases ($\chi^2 = 15.565$; $P < .001$). Our study found a significant association between BDNF gene polymorphism and BC. However, AA and GA genotypes were at risk of having BC (Table 3).

Discussion

This investigation was performed on 100 BC cases and 200 controls. Out of 300, 280 samples were positive. The findings of this study showed that early-onset menarche, short and long cycle length, age at first birth, overweight, obesity, positive family history of BC or any other cancers, use of OC, and depression were significantly related to the BC. Previous studies showed the relationship between BC contributing factors like marital status,[7] early-onset menarche,[8] nulliparity,[9] increased basal metabolic index (BMI),[10] increase waist-to-hip ratio (WHR),[10] and use of OC[11] with depression.

This study also reports that the functional BDNF "AA" genotype was associated with BC. A study showed that Met allele of BDNF increases the risk of new depressive episode following a severe life event.[20] Among Chinese BC population, BDNF Met allele was found to be prevalent in patients with cognitive impairment induced by chemotherapy.[21] Furthermore, a report on Han Chinese population documented that BDNF Met allele was related to bipolar disorders (BPD) among non-cancerous depressive patient,[14] whereas the Met allele did not show an association with major depressive disorders among Asians.[22]

Brain-derived neurotrophic factor is a peptide usually synthesized by the posttranscriptional modification as a precursor protein called prepro-BDNF present in rough endoplasmic reticulum (RER). Mammalian pro-BDNF molecule proteolytically cleaved to form truncated-BDNF also called pro-BDNF which is a 28-kDa BDNF followed by the second cleavage to form mature 14-kDa BDNF (mBDNF). The formation of pro-BDNF is by Ca^{2++}-dependent serine proteinase which is also called as membrane-bound transcription factor site-1 protease (MBTFS-1) or Subtilisin/kexinisozyme 1 (SKI-1).[23] pro-BDNF is not an intermediate product for this pathway, which is further not involved in the conversion of mBDNF molecule and seems to be a true final product[24] whose biologic importance remains elusive. It was thought that only free mBDNF in plasma is active biologically than pro-BDNF which was inactive and located inside the cell. The biological functions of mBDNF and pro-BDNF elicited opposite effects.[25] mBDNF is specifically bound with the low-affinity cell surface tyrosine kinase B (Trk-B) receptor[26] and plays a vital role in controlling various signaling pathways such as mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and phospho-lipase C (PLC)[27] to stimulate formation of dendritic bristles. Conversely, it also has a suppressing effect on dopamine release by down-regulating GABA (A) receptor's expression,[28] whereas pro-BDNF performed its activity via binding on p75 receptor that triggers apoptosis and prevents BC.

This investigation has the following limitation. The sample size was modest, and the association might be stronger by enrolling a large number of subjects in both cases and controls.

Conclusions

The results from this study suggest that BDNF Val66Met gene polymorphism may be associated with BC and that the Met allele increases the risk of BC.

Author Contributions

MI and TAK did the conception and design of the study.
TY, MI and SAA did extraction, analysis and interpretation of data.
MI did the drafting of article or critical revision for important intellectual content; and
TAK did final approval of the version to be published.

ORCID iD

Mehir un Nisa Iqbal https://orcid.org/0000-0002-8987-8877

REFERENCES

1. Zahra F, Humayoun F, Yousaf T, Khan NA. Evaluation of risk factors for carcinoma breast in Pakistani women. *J Fatima Jinnah Med Assoc*. 2013;1:34-38.
2. Shaukat U, Ismail M, Mehmood N. Epidemiology, major risk factors and genetic predisposition for breast cancer in the Pakistani population. *Asian Pac J Cancer Prev*. 2013;14:5625-5629.
3. Shamsi U, Khan S, Usman S, Soomro S, Azam I. A multicenter matched case control study of breast cancer risk factors among women in Karachi, Pakistan. *Asian Pac J Cancer Prev*. 2013;14:183-188.
4. Patel-Kerai G, Harcourt D, Rumsey N, Naqvi H, White P. The psychosocial experiences of breast cancer amongst Black, South Asian and White survivors: do differences exist between ethnic groups? *Psychooncology*. 2017; 24: 515-522.
5. Pandey M, Sarita GP, Devi N, Thomas BC, Hussain BM, Krishnan R. Distress, anxiety, and depression in cancer patients undergoing chemotherapy. *World J Surg Oncol*. 2006;4:68.
6. Nausheen B, Kamal A. Familial social support and depression in breast cancer: an exploratory study on a Pakistani sample. *Psychooncology*. 2007;16:859-862.
7. Whisman MA, Robustelli BL, Beach SRH, Snyder DK, Harper JM. Marital discord and depression in middle-aged and older couples: is taxon status associated with depression? *J Soc Pers Relat*. 2014;32:967-973.
8. Joinson C, Heron J, Lewis G, Croudace T, Araya R. Timing of menarche and depressive symptoms in adolescent girls from a UK cohort. *Br J Psychiatry*. 2011;198:17-23, sup. 1-2.
9. Ramezanzadeh F, Aghssa MM, Abedinia N, et al. A survey of relationship between anxiety, depression and duration of infertility. *BMC Womens Health*. 2004;4:9.
10. Wiltink J, Michal M, Wild PS, et al. Associations between depression and different measures of obesity (BMI, WC, WHtR, WHR). *BMC Psychiatry*. 2013;13:223.
11. Skovlund C, Mørch L, Kessing L, Lidegaard Ø. Association of hormonal contraception with depression. *JAMA Psychiatry*. 2016;73:1154-1162.
12. Hyde CL, Nagle MW, Tian C, et al. Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. *Nat Genet*. 2016;48:1031-1036.
13. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*. 2003;112:257-269.
14. Xu J, Liu Y, Wang P, et al. Positive association between the brain-derived neurotrophic factor (BDNF) gene and bipolar disorder in the Han Chinese population. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B:275-279.
15. Kim JM, Jang JE, Stewart R, et al. Determinants of suicidal ideation in patients with breast cancer. *Psychooncology*. 2013;22:2848-2856.
16. Zhao M, Chen L, Yang J, et al. BDNF Val66Met polymorphism, life stress and depression: a meta-analysis of gene-environment interaction. *J Affect Disord*. 2018;227:226-235.
17. He SC, Wu S, Wang C, et al. Interaction between job stress and the BDNF Val-66Met polymorphism affects depressive symptoms in Chinese healthcare workers. *J Affect Disord*. 2018;236:157-163.
18. Zung WW. A self-rating depression scale. *Arch Gen Psychiatry*. 1965;12:63-70.
19. Kim JM, Stewart R, Park MS, et al. Associations of BDNF genotype and promoter methylation with acute and long-term stroke outcomes in an east Asian cohort. *PLoS ONE*. 2012;7:e51280.
20. Brown GW, Craig TK, Harris TO, et al. Functional polymorphism in the brain-derived neurotrophic factor gene interacts with stressful life events but not childhood maltreatment in the etiology of depression. *Depress Anxiety*. 2014;31:326-334.
21. Ng T, Teo SM, Yeo HL, et al. Brain-derived neurotrophic factor genetic polymorphism (rs6265) is protective against chemotherapy-associated cognitive impairment in patients with early-stage breast cancer. *Neuro Oncol*. 2016;18:244-251.
22. Verhagen M, van der Meij A, van Deurzen PA, et al. Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity. *Mol Psychiatry*. 2010;15:260-271.
23. Seidah NG, Mowla SJ, Hamelin J, et al. Mammalian subtilisin/kexin isozyme SKI-1: a widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. *Proc Natl Acad Sci U S A*. 1999;96:1321-1326.
24. Garcia KL, Yu G, Nicolini C, et al. Altered balance of proteolytic isoforms of pro-brain-derived neurotrophic factor in autism. *J Neuropathol Exp Neurol*. 2012;71:289-297.
25. Teng HK, Teng KK, Lee R, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci*. 2005;25:5455-5463.
26. Petrella JR, Mattay VS, Doraiswamy PM. Imaging genetics of brain longevity and mental wellness: the next frontier? *Radiology*. 2008;246:20-32.
27. Nemoto K, Ohnishi T, Mori T, et al. The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. *Neurosci Lett*. 2006;397:25-29.
28. Brunig I, Penschuck S, Berninger B, Benson J, Fritschy JM. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA(A) receptor surface expression. *Eur J Neurosci*. 2001;13:1320-1328.