# Association of Dopamine β-Hydroxylase Polymorphism rs1611115 And Serum Levels with Psychiatric Disorders in Pakistani Population

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# Abstract

Dopamine β-hydroxylase (DBH) is a copper-containing enzyme that has an important role in maintaining the cellular homeostasis between the two neurotransmitters, dopamine (DA) and nor-adrenaline (NA). *DBH* functional polymorphisms are associated with multiple neuro-psychiatric conditions and are found to alter the DBH protein levels in serum, affecting DBH enzymatic activity. The current study was conducted to determine the genetic association of *DBH* functional polymorphism rs1611115 and its effect on DBH levels in serum in major depressive disorder (MDD), bipolar disorder (BPD) and schizophrenia (SHZ) in the Pakistani population. In total n = 1097 subjects including MDD (*n* = 427), BPD (*n* = 204), SHZ (*n* = 134) and healthy controls (*n* = 332), were screened for rs1611115 by polymerase chain reaction-restriction fragment length polymorphism. Univariate logistic regression was applied and results were adjusted for age and sex (multivariant analysis). The DBH levels in serum were determined through enzyme-linked immunosorbent assay (ELISA) and the Mann Whitney *U* test was applied. The results showed a significant association of minor allele (-1021C > T) with a higher risk of developing BPD and SHZ in both univariable and multivariable analyses. Moreover, the overall total serum concentration of DBH was comparatively raised in MDD, however, in cross-comparison DBH serum levels were found markedly higher in CC homozygotes compared to TT homozygotes within the BPD group. Thus the present study suggested a significant association of DBH rs1611115 with BPD and SHZ and also the effect of rs1611115 on DBH serum levels in MDD and BPD, for the first time in the Pakistani population.


# Introduction

Dopamine β-hydroxylase (DBH) is a copper-containing enzyme of the dopaminergic system, which catalyzes the dopamine (DA) conversion into nor-adrenaline (NA) (Weinshilboum, 1978, Preuss et al., 2013). The dopaminergic system regulates mood, attention, motivation, reward system, decision making and psychomotor activity of the brain and DA is one of the important stimulatory neurotransmitters in this pathway (Opmeer et al., 2010). The gene encoding DBH resides on chromosome 9q34, it has 12 exons and is approximately 23kb in size (Craig et al., 1988, Kobayashi et al., 1989). The DBH is expressed in the locus-coeruleus part of the brain (Barrie et al., 2014), and is localized in synaptic vesicles, of peripheral (noradrenergic) and central (noradrenergic and adrenergic) neurons and is also found in the neuro-secretory cells of the adrenal medulla (Myers et al., 2007, Weinshilboum, 1978). Upon adrenergic stimulation, the DBH and NA are released from vesicles through exocytosis from the sympathetic neurons and the adrenal medulla and then enters into the blood circulation, where DBH can be detected in plasma/serum (Cubells et al., 2011).

The DBH enzyme activity has been reported to vary widely in plasma and serum and is significantly influenced by genetics (Cubells et al., 2011, Tang et al., 2010). Genetic variations in *DBH* have been observed to affect the DBH expression and plasma activity in familial as well as twin-based studies (Ross et al., 1973, Fernandez et al., 2009, Cubells and Zabetian, 2004, Zabetian et al., 2003). The single nucleotide polymorphism (SNP) rs1611115 (-1021C > T) in *DBH* is a functional polymorphism, which has been reported previously to result in a 31 – 52% reduction in DBH plasma activity in the African American, East-Asian and Japanese populations (Zabetian et al., 2001, Kohnke et al., 2002). Numerous genetic studies have shown the clinical significance of rs1611115 for neuro-psychiatric disorders such as migraine (Sezer et al., 2016), addiction (Freire et al., 2006, Kalayasiri et al., 2007, Kosten et al., 2013), bipolar affective disorder (BPD) (Ates et al., 2013, Sidor et al., 2015, Chung et al., 2014), epilepsy (Depondt et al., 2004), schizophrenia (SHZ) (Windemuth et al., 2008, Bolton and Constantine-Paton, 2018), major depressive disorder (MDD) (Yadid and Friedman, 2008), attention deficit hyperactivity disorder (Bhaduri and Mukhopadhyay, 2006), Parkinson's disease (Shao et al., 2016), Huntington's chorea, Tourette syndrome (Cubells et al., 2011) and smoking (Freire et al., 2006) among different ethnicities worldwide.

However, the role of *DBH* SNPs in common psychiatric diseases including MDD, BPD and SHZ in individuals of Pakistani descent has not been established yet. The present study was, therefore, conducted to investigate the role of *DBH* rs1611115 functional polymorphism and the effect of risk allele on serum DBH levels in psychiatric disorder cohort (MDD, SHZ, and BPD) compared to healthy controls in the Pakistani population.

## Methodology

# Subject Recruitment

A total of 1097 participants were recruited for this study, including MDD (*n* = 427), BPD (*n* = 204), SHZ (*n* = 134) and healthy controls (*n* = 332). All the subjects participating in the study were recruited from the Pakistan Institute of Medical Sciences (PIMS) hospital, Islamabad, Pakistan. The participants were diagnosed by qualified registered psychiatrists of PIMS, according to the International Classification of Diseases, Tenth Revision (ICD-10) guidelines (F20-F29 schizophrenia, F30-F39 bipolar disorder and depression), (Janca et al., 1993).

Following the guidelines, any psychiatry patient with comorbidities such as neurological disorders (Parkinson's, Alzheimer's and stroke), thyroid disorder, malignancies or contagious infectious disease were excluded from the study. For healthy controls, if the participant had a history of any psychiatric disorder or had a positive family history of psychiatric disorders, or stroke, or had serious brain injuries, they were also excluded from the studied cohort. The clinical and demographic features of each participant were also documented.

# Sample Collection and Processing

5ml of blood sample was collected from each participant, of which 3ml blood was added in Ethylenediaminetetraacetic acid (EDTA) tube (1 Becton Drive, Franklin Lakes, NJ) for genomic DNA extraction according to the standard phenol-chloroform DNA extraction protocol (Sambrook and Russell, 2001). While 2ml blood was added in gel activator containing tubes (1 Becton Drive, Franklin Lakes, NJ), for serum separation. These tubes were subjected to centrifugation for 10 minutes at 2,000 RPM at 4°C. Extracted DNA and serum samples were stored at -20ºC till further use.

# Genotyping of rs1611115

Genotyping was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique using a previously reported set of primers (Sezer et al., 2016), with modified reaction and thermal profile. The 25µl PCR reaction mixture contained, 10X Taq buffer, 2mM dNTPs, 3mM $MgCl_2$, 0.5µM of each primer, 2.5U of Taq polymerase and 2.0µl (40-50ng/µl) of template DNA. The thermal profile for amplification consisted of initial denaturation at 95°C for 6 mins, followed by 35 cycles of 95°C for 35 sec, 56°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 mins. PCR products (131bp) were subjected to restriction enzyme digestion with 1U of *HhaI* at 37°C for 16 hrs. Fragments containing the C allele (wild type) were digested by *HhaI* giving two fragments of 109bp and 22bp, whereas fragments with the T allele (mutant) remained undigested. After digestion, the fragments were analyzed on a 3% agarose gel.

# Serum DBH Level Assessment

A subset from each case group (MDD, BPD and SHZ) and controls were selected based on genotype (with an almost equal number of males and females in each subset group) and were subjected to serum DBH level quantification. Human DBH (Dopamine beta Hydroxylase ELISA kit, ELAB science Cat.no. E- EL-H1226 (human serum), TX) kit was used as per the manufacturer's instructions for the detection of DBH levels in the serum samples.

# Statistical Analysis

All the statistical analyses were done using R studio version 4.00. To compare age, gender and demographic features between the cases and controls, the Fisher *t*-test was used for continuous variables and the $\chi^2$ test was applied for discrete data. The $\chi^2$ test was also used to determine whether the rs1611115 genotype distribution in the control group was in Hardy-Weinberg equilibrium (HWE). Power calculations for case-control association analysis were also performed via Quanto-v.1.2.4. Genotypes were coded additively (additive model) and logistic regression analysis was used to determine the differences in the distribution of the genotypes of the SNP in cases (MDD, BPD and SHZ) as compared to controls (univariate analysis) The results were adjusted for age, sex and tobacco use (multivariate analysis). Heterozygosity

analysis was also done to determine the risk allele effect. The SNP data were also analyzed in comparison to the 1000 genome project data of the Punjab population (n = 96) from Lahore (PJL) to determine the differences. The serum DBH levels were compared between the cases (MDD, BPD and SHZ) and controls using the Mann-Whitney *U* test. The level of significance was kept at a *P* value/α ≤ 0.05 in all statistical tests.

# Power Calculations

To attain 80% power, the sample size for each phenotype were calculated to determine the effect size. The selected parameters for the power calculation were, **a**: Outcome/design was set to disease, case-control unmatched, **b**: Hypothesis selected as Gene only, **c**: For Gene G, MAF of the controls were mentioned in allele frequency for all the three phenotype analysis, **d**: For the Outcome model the population risk ($K_0$) was taken as 6% for Major depressive disorder (MDD), 1.5% for Schizophrenia (SHZ) and 3% for Bipolar (BPD), based on the reported prevalence rate in Pakistan (Mirza et al., 2006), **e**: For power parameter, the sample size for each phenotype was taken equal to our cohort samples, 204: BPD, 134: SHZ, 427: MDD, 332: CON and the type I error (two-sided) was set to α:0.05. We observed our study to be underpowered and a larger sample size was required to attain the desired 80% power (Table S1).

## Results

# Descriptive Analysis of Cohort

Descriptive features of the studied cohort are given in Table 1. Most of the participants in the current study were inhabitants of the Punjab province of Pakistan and their average age (years) ± SD were 33.09 ± 11.52 in MDD, 30.7 ± 11.70 in BPD, and 29.63 ± 9.88 in SHZ and 23.79 ± 5.72 in controls. The cohort had 38.2% males in MDD, 58.3% males in BPD, 70.9% males in SHZ and 53.0% males in controls. The studied case-control cohort were not age and sex-matched ($P < 0.05$). It was also observed in the cohort that more tobacco users were in cases, SHZ ($P = 8.38E-14$), BPD ($P = 8.904E-10$), MDD ($P = 5.15E-03$) as compared to the controls.

Table 1
Demographic characteristics of the cohort (cases and controls)

| | CON | BPD | SHZ | MDD | BPD vs CON (*P*-value) | SHZ vs CON (*P*-value) | MDD vs CON (*P*-value) |
|---|---|---|---|---|---|---|---|
| Total (*n*) | 332 | 204 | 134 | 427 | - | - | - |
| Sex (Male) | 176(53.0%) | 119(58.3%) | 95(70.9%) | 163(38.2%) | 0.27* | 5.80E-04* | 6.19E-05* |
| Age (Years) (mean ± SD) | 23.79 ± 5.72 | 30.70 ± 11.70 | 29.63 ± 9.88 | 33.09 ± 11.52 | 9.02E-14** | 1.34E-09** | 2.20E-16** |
| Tobacco Users | 25(7.5%) | 56(27.5%) | 48(35.8%) | 65(15.2%) | 8.904E-10* | 8.38E-14* | 5.15E-03* |
| Ethnicity (Punjabis) | 243(73.2%) | 153(75.0%) | 105(78.4%) | 287(67.2%) | 0.72 | 0.30 | 0.09 |

Values are *n* (%) or Mean (SD), **P*-value $\chi^2$ test, ***P*-value from Welch Two Sample t-test.

# Genetic analysis of rs1611115

The SNP rs1611115 was found to be in Hardy-Weinberg equilibrium (HWE), $P = 0.074$. The univariate logistic regression analysis showed the minor allele of the SNP rs1611115 was significantly associated with a higher risk of BPD: [Odds Ratio (OR) (95% Confidence Interval (CI)) = 1.16(1.08–1.24), $P = 5.18E-05$] and SHZ [OR (95% CI ) = 1.24(1.08–1.44), $P = 2.37E-03$].

This association remained significant when results were adjusted for age and sex (multivariate analysis), BPD: [OR (95% CI) = 1.17(1.10–1.25), *P* = 4.02E-06] and SHZ: [OR (95% CI) = 1.24(1.09–1.42), *P* = 1.14E-03].

The multivariable analysis was also performed with an additional environmental factor 'tobacco use' along with age and sex and found consistent results, BPD: [OR(95%CI) = 1.16(1.09–1.24), *P* = 6.96E-06***] and SHZ: [OR (95% CI) = 1.19( 1.05–1.36), *P* = 0.006**].

Heterozygosity analysis was performed to determine the effect of the T allele in disease manifestation, and found a significant association with BPD: univariate analysis [OR (95% CI) = 1.28(1.18–1.39), *P* = 5.85E-09], and SHZ: [OR (95% CI) = 1.26(01.08–1.44), *P* = 1.01E-09]. These results were remained significant when adjusted for age and sex, BPD: [OR (95% CI) = 1.26(1.17–1.37), *P* = 2.57E-09] and SHZ : [OR (95% CI) = 1.26(1.08–1.48), *P* = 0.001], Table 2.

# Comparison with 1000 Genome Project PJL Data

The genotype count and allele frequencies of SNP rs1611115 from the 1000 Genomes Project PJL data (Punjabi descent from Pakistan), were also retrieved and compared with the current study data. Marked differences in allele [$\chi^2$ (*P*) = 12.85 (0.0003)] and genotype count [$\chi^2$ (*P*) = 18.82 (< 0.001)] were observed despite the same ethnicity of the present study cohort and 1000 Genome PJL data (Table 2).

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Table 2
Association of *DBH* rs1611115 with BPD, SHZ and MDD

| ***DBH* rs1611115 (C > T)** | | | **Univariable analysis** | **Multivariable analysis** | |
|---|---|---|---|---|---|
| **Phenotype** | **Control (n)** (CC/CT/TT) | **Cases(n)** (CC/CT/TT) | **OR(95%C.I)**P | **OR(95%C.I)** P* | **OR(95%C.I)**P** |
| **BPD** | 332 (135, 173, 24) | 204 (37,157,10) | 1.16(1.08–1.24)5.18E-05 | 1.17(1.10–1.25)4.02E-06 | 1.16(1.09–1.24)6.96E-06*** |
| **SHZ** | 332 (135, 173, 24) | 134 (26,103,5) | 1.24(1.08–1.44)2.37E-03 | 1.24(1.09–1.42)1.14E-03 | 1.19( 1.05–1.36)0.006** |
| **MDD** | 332 (135, 173, 24) | 427 (178,223,26) | 0.96(0.80–1.14)0.62 | 0.99(0.85–1.17)0.99 | 0.99( 0.85–1.17)0.98 |

| **Heterozygosity Analysis** | | |
|---|---|---|
| | **Univariable Analysis** | **Multivariable Analysis** |
| **Phenotype** | **OR(95%C.I)**P | **OR(95% C.I)**P* |
| **BPD** | 1.28(1.18–1.39)5.85E-09 | 1.26(1.17–1.37)2.57E-09 |
| **SHZ** | 1.25(1.08–1.44)2.37E-03 | 1.26(1.08–1.48)1.14E-03 |

| **Comparison of Genotype and Allele frequencies from 1000 genomes PJL data and the Control data from our study** | | | | | | |
|---|---|---|---|---|---|---|
| | **Genotype Count** (CC/TC/TT) | **Genotype Frequency** (CC/TC/TT) | $\chi^2$ ( P **value)** | **Allele Count** (C/T) | **Allele Frequency** (C/T) | $\chi^2$ ( P **value**) |
| **Our data** (n **= 332)** | 135/173/24 | 0.407/0.521/0.072 | 18.82 (< 1.00E-04) | 443/221 | 0.667/0.333 | 12.85 (3.00E-04) |
| **1000 Genome data (**n **= 96)** | 63/28/5 | 0.656/0.292/0.052 | | 154/38 | 0.802/0.198 | |

*Adjusted for age and sex, **adjusted for age, sex and tobacco users, the *P*, *P**, *P*** values from Pearson's Chi-squared $\chi^2$ test with Yates' continuity correction. (*P*-value from univariate analysis), (*P** and *P***-value from multivariate analysis), OR: Odd Ratios, C.I: Confidence Interval.

# Serum analysis of DBH concentration

Descriptive features and the total serum concentrations of the selected samples (cases and controls) from the studied cohort are given in Table 3. The descriptive statistics of all the genotype-based studied sub-cohorts are represented in Tables 4 & 5. The serum levels of Dopamine β hydroxylase (DBH) were found not to be normally distributed (Fig. S1), therefore, a non-parametric Mann-Whitney *U* test was applied. In the initial analysis, we investigated the association of the DBH levels with the phenotypes compared to controls. The total DBH serum levels were found elevated ($P = 0.008$) in the MDD group as compared to the control group (Fig. 1). In the second analysis, the DBH serum levels were also analyzed within each group concerning the genotypes (CC, TC and TT) to determine the association of DBH serum levels with the

SNP. Significant differences in DBH concentrations were observed in the BPD group. Serum DBH levels were observed significantly higher in BPD ($P = 0.008$) as compared to the controls, in CC homozygotes. The lower serum DBH levels were found in TT homozygotes in BPD as compared to the reference CC homozygotes of the BPD group ($P = 0.014$) and also compared to the TT homozygotes of the MDD group ($P = 0.038$). However, after multiple testing Bonferroni's corrections (adjusted $P = 0.003$), the $P$ valve did not remain significant for the BPD (CC) vs CON (CC) and BPD (TT) vs MDD(TT) findings, (Table 6 & Table S2).

Table 3
Descriptive features of subset-cohort for ELISA

| Parameters | CON | BPD | SHZ | MDD | BPD vs CON | SHZ vs CON | MDD vs CON |
|---|---|---|---|---|---|---|---|
| n | 21 | 23 | 21 | 23 | -- | -- | -- |
| **Sex (Male)*** | 10 | 11 | 13 | 12 | 1.00* | 0.53* | 1.00* |
| **Age (Years)** (Mean ± SD)** | 22.4(2.18) | 28.0(10.85) | 29.6(8.70) | 34.4(10.10) | 0.02** | 1.3E-03** | 9.72E-06** |
| **Age of onset (years) (Mean ± SD)** | -- | 22.37(8.64) | 23.90(7.58) | 30.48(10.81) | -- | -- | -- |
| **Tobacco Users** | 2.0(9.52%) | 3.0(13.0%) | 8.0(38.1%) | 5.0(21.74%) | 1.00* | 0.13* | 0.30* |
| **Ethnicity (Punjabis)*** | 10.0(47.62%) | 19.0(82.61%) | 11.0(52.38%) | 13.0(56.52%) | 1.00* | 0.76* | 1.00* |
| **Total Serum DBH levels ng/µl (Mean ± SD)** | 80.06 ± 17.94 | 92.19 ± 27.67 | 82.74 ± 25.12 | 94.88 ± 19.82 | 0.06# | 0.41# | 0.008# |

Values are *n* (%) or Mean (SD), *P** value from Pearson's Chi-squared $\chi^2$ test with Yates' continuity correction. "*P*** value from Welch Two Sample T-test", *P*#-valve from non-parametric Mann-Whitney *U* test. ng/µl: nanogram per microliter.

Table 4
Descriptive statistics of serum DBH concentration in each group.

| Phenotype | CON | MDD | BPD | SHZ |
|---|---|---|---|---|
| Number of samples | 21 | 23 | 23 | 21 |
| Minimum | 52.35 | 48.25 | 32.67 | 31.23 |
| .25% Percentile | 64.24 | 82.59 | 74.34 | 71.04 |
| Median | 80.08 | 97.01 | 92.09 | 84.52 |
| 75% Percentile | 90.98 | 109.5 | 107.8 | 99.01 |
| Maximum | 115.7 | 124.2 | 153.2 | 124.2 |
| Mean | 80.06 | 94.88 | 92.19 | 82.74 |
| Std. Deviation | 17.94 | 19.82 | 27.67 | 25.12 |
| Std. Error of Mean | 3.916 | 4.134 | 5.77 | 5.481 |
| Lower 95% CI | 71.89 | 86.31 | 80.23 | 71.31 |
| Upper 95% CI | 88.23 | 103.5 | 104.2 | 94.18 |
| Mean ranks | 33.81 | 53.46 | 49.28 | 40.14 |

CI: confidence Interval, CON: Controls, BPD: Bipolar Disorder, SHZ: Schizophrenia, MDD: Major Depressive Disorder

Table 5
Descriptive statistics of serum DBH concentration in each group with respect to genotype

| Phenotype | CON | | | MDD | | | BPD | | | SHZ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | CC | TC | TT | CC | TC | TT | CC | TC | TT | CC | TC | TT |
| Number of Samples | 6 | 7 | 8 | 8 | 8 | 7 | 8 | 8 | 7 | 8 | 8 | 5 |
| Minimum | 64.94 | 61.08 | 52.35 | 68.6 | 61.66 | 48.25 | 71.54 | 53.94 | 32.67 | 42.22 | 35.57 | 31.23 |
| 25% Percentile | 67.83 | 62.62 | 60.79 | 72.86 | 79.37 | 93.29 | 94.49 | 77.48 | 42.17 | 70.18 | 67.38 | 56.38 |
| Median | 74.05 | 80.08 | 88.25 | 92.5 | 99.08 | 101 | 107.6 | 88.98 | 81.82 | 87.7 | 80.69 | 86.06 |
| 75% Percentile | 83.68 | 104.1 | 93.7 | 108.3 | 104.6 | 118.6 | 124.9 | 111.1 | 92.09 | 108.9 | 95.78 | 100.7 |
| Maximum | 90.98 | 115.7 | 115.7 | 124.2 | 109.6 | 122.3 | 153.2 | 130.2 | 102.1 | 124.2 | 115.7 | 112.4 |
| Mean | 75.68 | 81.16 | 82.39 | 93.0 | 92.64 | 99.58 | 108.8 | 92.37 | 73.04 | 87.05 | 80.14 | 80.02 |
| Std. Deviation | 9.346 | 21.51 | 20.91 | 19.45 | 16.42 | 25.46 | 24.44 | 23.45 | 25.97 | 26.35 | 23.81 | 29.78 |
| Std. Error of Mean | 3.816 | 8.13 | 7.392 | 6.875 | 5.804 | 9.623 | 8.64 | 8.291 | 9.816 | 9.318 | 8.417 | 13.32 |
| Lower 95% CI | 65.87 | 61.26 | 64.91 | 76.74 | 78.92 | 76.04 | 88.35 | 72.76 | 49.02 | 65.02 | 60.23 | 43.04 |
| Upper 95% CI | 85.48 | 101 | 99.87 | 109.3 | 106.4 | 123.1 | 129.2 | 112 | 97.06 | 109.1 | 100 | 117 |
| Mean ranks | 26.92 | 34.86 | 38.06 | 49.56 | 51.38 | 60.29 | 65.06 | 49.0 | 31.57 | 44.25 | 37.0 | 38.6 |

CI: confidence Interval, CON: Controls, BPD: Bipolar Disorder, SHZ: Schizophrenia, MDD: Major Depressive Disorder

Table 6
Comparative analysis of serum DBH protein concentration w.r.t genotype in each group.

| Phenotype | Serum DBH Levels ng/µl (Mean ± SD) | | | Mann-Whitney *U* test (non-parametric) (*P*) | | |
|---|---|---|---|---|---|---|
| | CC | TC | TT | CC vs TC | CC vs TT | TC vs TT |
| CON | 75.67 ± 9.35 | 81.16 ± 21.51 | 82.39 ± 20.91 | 0.92 | 0.51 | 0.89 |
| MDD | 93 ± 19.45 | 92.64 ± 16.42 | 99.58 ± 25.46 | 0.93 | 0.46 | 0.53 |
| BPD | 108.98 ± 24.44 | 92.36 ± 24.45 | 73.04 ± 25.97 | 0.19 | 0.01 | 0.18 |
| SHZ | 87.05 ± 26.35 | 79.59 ± 24.99 | 80.89 ± 28.02 | 0.63 | 0.88 | 0.93 |

Mean (SD) standard Deviation, CC ancestor allele, TT risk allele, ng/µ: nanogram per microliter.

# Discussion

In the current study the association of the *DBH*-rs1611115 and its effect on the DBH serum levels in different psychiatric phenotypes MDD, BPD and SHZ was investigated in comparison to controls in the Pakistani population. In genetic association analysis, we observed a significant association of the risk allele (T) of rs1611115 with a higher risk in BPD and SHZ and the heterozygosity testing suggested that a single copy of the T allele would be sufficient to contribute to BPD and SHZ susceptibility in Pakistani population. In comparison to our study, there are contradictory findings regarding the role of rs1611115 in psychiatric patients in different populations worldwide. A study on the Turkish population reported no association of rs1611115 with BPD (Ates et al., 2013), while the same SNP revealed an association with SHZ susceptibility in the North Indian population (Punchaichira et al., 2020). In one of the studies on Chinese MDD patients, rs1611115 disease risk association was observed, while in the present study no significant association of rs1611115 was observed in the MDD cohort, which complies with another Chinese study on West Chinese MDD patients (Zhou et al., 2015, Hess et al., 2009).

The minor allele (T) frequency of the studied controls was also compared to the 1000 Genome Project PJL ethnic group from Pakistan and it was found to be significantly different. It might suggest geographical differences to be a possible reason (Khan et al., 2020). As the 1000 genome project has represented data of only the Lahore-Punjab population (PJL) which is geographically separated about 400–450 km apart from our sample's geographical location which is mostly from Islamabad and Rawalpindi. Secondly, 1000 genome have a better quality of genotyping data, and nonetheless, 1000 genome had a smaller sample size (n = 96), as compared to the current study sample (controls) size (n = 332), which was larger than the 1000 genome PJL data set.

As in the current study, no comparative ELISA-based serum estimation of the DBH levels has been conducted before for MDD, BDP and SHZ. However, the determination of DBH activity in serum and plasma of psychiatric patients through different biochemical techniques have revealed reduced plasma DBH activity that appeared to be due to diminished DBH levels in the circulation in carriers of the T allele in different psychiatric conditions (Dunnette and Weinshilboum, 1976, Zabetian et al., 2001, Kohnke et al., 2002, Bhaduri and Mukhopadhyay, 2008). In the present study, irrespective of the genotypes (CC/CT/TT), the serum-based expression profiling of DBH determined higher serum DBH levels only in the MDD cohort. DBH being an important regulatory enzyme in the dopaminergic system that converts DA into NA and the higher concentrations of serum DBH can thus potentially disturb the homeostasis between DA and NA, which then alter the sympathetic neuronal responses (Hussain et al., 2021, Borodovitsyna et al., 2017). Thus the elevated DBH levels result in reduced DA in the circulation leading to altered neuronal activity, which is a hallmark feature in psychiatric disorders and therefore considered to be one of the contributing factors in MDD aetiology as reported previously (Zheng et al., 2016). When the serum DBH levels in the sub-cohort were grouped by genotype, significant differences in expression were observed. The TT homozygotes showed significantly lower serum DBH levels, while CC homozygotes had comparatively higher DBH levels within the BPD group. However, in cross-comparison between the groups, apparently, the serum-DBH levels were seemed lower in the TT homozygotes in BPD than in MDD (TT homozygotes) and seemed higher than controls (CC homozygotes), but these findings were not statistically remained significant after multiple testing Bonferroni's corrections. In an earlier study, Zabetian et al. (2001) observed reduced DBH serum expression in the TT homozygotes that resulted in diminished DBH activity, in turn, disrupts the DA to NA homeostasis and subsequently raises the levels of DA in the circulation (Barrie et al., 2014), thus making the risk allele carriers susceptible to various neurological and psychiatric conditions (Zabetian et al., 2001).

Various functional studies of *DBH* promoter concerning the influence of genetic changes and promoter activity, with the subsequent effect on DBH expression, have reported the role of *cis*-regulatory motifs, containing CREB and additional Phox2a/2b binding sites present in domain II of the DBH promoter. The genetic changes in domain II resulted in inactivated promoter indicating its crucial role in the DBH promoter activity along with the main domain IV of the DBH promoter (Hoyle et al., 1994, Kim et al., 1998). The studied polymorphism rs1611115 is among the functional SNPs residing in domain II and is also a part of the haplotype block (between − 600bp to -1100bp) of the *DBH* promoter, located at position − 1021 up-stream from the start codon, thus significantly affecting the DBH gene expression (Tang et al., 2007).

The studies on haplotype block analyses of promoter region SNPs of *DBH* have reported a significant role of rs1611115 in the variation in DBH plasma levels and activity as compared to any other SNP in the same haplotype block (Tang et al., 2006, Zabetian et al., 2003). These authors also reported the individual SNP effect, as well as haplotype block effect, where rs1611115 accounted for the largest proportion of variance in DBH plasma activity in populations from diverse geographic origins including African-Americans, East-Asian, American-Europeans, Germans and Japanese (Zabetian et al., 2001, Kohnke et al., 2002). A study similar to these author's work on genotype as well as haplotype association of the *DBH* SNPs with plasma DBH activity has been reported in unrelated individuals from the East-Indian population (Bhaduri and Mukhopadhyay, 2008). These reported studies, therefore, suggest a population-specific role, of the haplotype block containing rs1611115, in *DBH* promoter regulation and its protein serum levels and therefore explains the differential expression of DBH in different psychiatric phenotypes in the current study.

There are also few studies (Nelson and Davis, 1997, Mustapic et al., 2007) that have reported the contribution of non-genetic risk factors on the serum DBH levels, where Nelson and Davis (1997) reported the association of dysregulation of the hypothalamic-pituitary axis (HPA) with variations in DBH plasma levels, thus indicating the role of HPA in the regulation of DBH protein in plasma. While Mustapic et al., (2007) on the other hand conducted a related study on war veterans with or without posttraumatic stress disorder (PTSD) in Croatian Caucasians and found variation in plasma DBH levels, depending upon stress exposure irrespective of which allele (C/T) of rs1611115 was present. The contribution of non-genetic risk factors might be the reason that no significant differences were observed in DBH serum levels within MDD and SHZ cohorts with respect to the genotype (CC/CT/TT) in the current study.

The present and previous findings of the association of rs1611115 genetic susceptibility with psychiatric conditions and DBH serum levels thus suggest a significant population-based variation of this polymorphic locus. The GWAS and Meta-GWAS studies data on DBH polymorphisms have also revealed the role of rs1611115 in phenotypes from different ethnicities (Table S3). The meta-analyses showed the rs1611115 significant association with Parkinson's disease (PD) in southern China ethnic group and Chinese population (Tang et al., 2018), while another meta-GWAS study (Kang et al., 2018), on larger data set of European and Asian (including Indian and Chinese) population, determined no association with PD. However the GWAS study (Mustapic et al., 2014) on the population of European ancestry and Native Americans revealed the significant association of rs1611115 in PTSD patients, moreover, this study also includes the first GWAS data on plasma DBH activity showing the influence of rs1611115 on plasma DBH activity. These paradoxical results therefore highlight and suggest the complex nature of the psychiatric conditions and also a significant inter-ethnic variation of this polymorphic locus in different psychiatric phenotypes. Therefore, there is a need for thorough investigations in comparison to overlapping phenotypes as investigated by Wu et al. 2020 and Xu & Lin 2011(Wu et al., 2020, Xu and Lin, 2011), involving larger cohorts, while, the current study has smaller psychiatric phenotype cohorts and was also underpowered, which are the limitations of this study. However, the rs1611115 was found in HWE in our study which suggests very low chances of genotyping error in the current study. Furthermore, *in-vitro* and *in-vivo* functional characterization and Mendelian randomization analysis as described by (Fuquan Zhang 2021, Wang et al., 2021), are also needed, to infer whether the risk factors causally affect a health outcome and to determine the population-specific role of the studied SNP in the Pakistani population. To predict disease risk, a machine-learning model analysis by Liu et al., 2020 (Liu et al., 2020), can also be applied by genotyping other *DBH* SNPs on the Pakistani population. Despite the above limitations, our study gives information about the role of rs1611115 in psychiatric conditions and its effects on serum DBH levels in the Pakistani population.

# Conclusion

In conclusion, the present study on the role of DBH polymorphism rs1611115 revealed the genetic association of *DBH* rs1611115 risk allele T with BPD and SHZ phenotypes and influence of T allele on DBH serum levels in BPD and, in MDD phenotype irrespective of the allele (C/T) in Pakistani population. The current study also explains that divergent genetic

backgrounds, as well as different environmental niches of the different ethnic populations, could not be ruled out in causing greater genetic as well as clinical heterogeneity in psychiatric illnesses.

# Declarations

### Ethics approval and consent to participate

The current study conforms to the Helsinki Declaration and was approved by the Ethics Review Board of the Department of Biosciences, COMSATS University Islamabad, Pakistan. All the participants were informed about the purpose of the study and informed consent was attained from all the participants. The privacy rights of all the participants were preserved by anonymizing the relevant data.

### Conflict of Interest

The authors declare that they have no conflict of interest.


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### Authors' contributions

**Aisha Nasir Hashmi (A.N.H), Maleeha Azam (M.A):** Conceptualization, formulation or evolution of overarching research goals and aims, design of methodology.

**Aisha Nasir Hashmi (A.N.H):** Investigation and conducting research, specifically performing the experiments, or data/evidence collection. Data curation, formal analysis, application of statistical, mathematical, techniques to analyze or synthesize study data. Preparation, creation and/or presentation of the published work, specifically visualization/ data presentation, Writing, preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation)

**Maleeha Azam (M.A), Raheel Qamar (R.Q):** Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/experiments and other research outputs. Provision reagents, materials, instrumentation, computing resources, or other analysis tools, Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team. Funding acquisition for the project leading to this publication

**Maleeha Azam (M.A), Raheel Qamar (R.Q), Muhammad Ajmal (M.A):** Supervision, management and coordination responsibility for the research activity planning and execution

**Aisha Nasir Hashmi (A.N.H), Maleeha Azam (M.A), Raheel Qamar (R.Q):** Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or post-publication stages

**Raees Ahmed Dharejo (R.A.D), Usama Bin Zubair (U.Z), Rizwan Taj (R.T):** Disease diagnosis, Provision of study materials, Provision of blood samples

**Netasha Khan (N.K):** Primary data curation

**Iqra Kashif (I.K):** Assisted in data collection

**All authors: Read and approved the final version of the manuscript.**

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# Figures

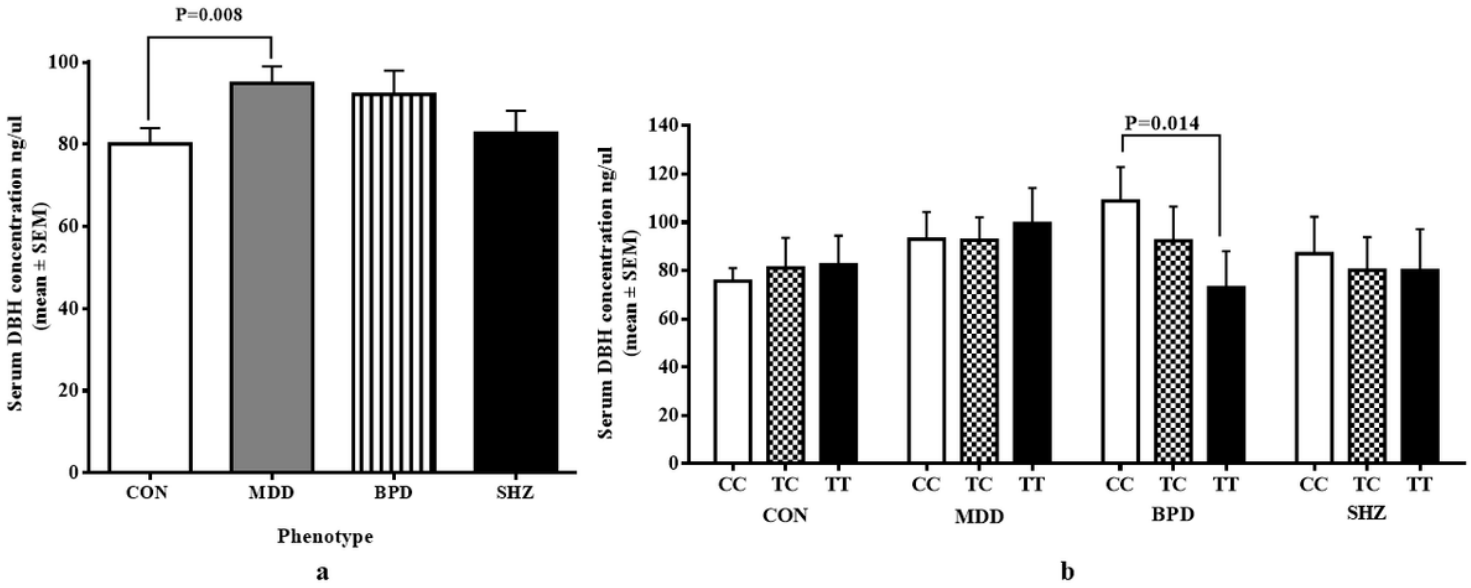


**Figure 1**

Bar charts representing a comparison of serum DBH levels in cases and controls.

**Legends:** (a) Bar chart representing overall serum DBH concentration in each group, (b) bar chart representing serum DBH levels in each group, with respect to alleles (CC, TC, TT). *P* valves are from non-parametric Mann-Whitney *U* test. CON = Controls, MDD = Major depressive disorder, BPD = Bipolar disorder, SHZ = Schizophrenia).

# Supplementary Files

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