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ORIGINAL ARTICLE

Genetic Association of Butyrylcholinesterase with Major Depressive Disorder

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Abstract
Major depressive disorder (MDD) is characterized as clinical depression, which primarily affects the mood and behaviour of an individual. In the present study butyrylcholinesterase (BChE), a co-regulatory cholinergic neurotransmitter enzyme implicated in several putative neuronal and non-neuronal physiological roles was investigated for its role in MDD. Eighty MDD patients and sixty-one healthy controls were recruited for the study. BChE activity was measured by Ellman's method using serum while DNA samples of the patients were genotyped for *BCHE* polymorphisms rs3495 (c.*189G > A) and rs1803274 (c.1699G > A) by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and tetra-primer Amplification Refractory Mutation System- polymerase chain reaction (ARMS-PCR). The genotyping was further validated by Sanger Sequencing. Biochemical estimation of serum BChE levels revealed a statistically significant decrease of enzyme activity in MDD patients (69.96) as compared to healthy controls (90.97), which was independent of age and gender. *BCHE* single nucleotide polymorphism rs1803274 genotype GA was found to be associated with the disease under a dominant model (OR 2.32; 95% CI 1.09–4.96; p value = 0.025). Furthermore, risk

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allele-A frequency was higher in cases (p value = 0.013) than control. Carriers of rs1803274 GA genotype showed reduced mean BChE activity than wild-type allele GG homozygotes (p value = 0.040). Gender-based analysis revealed a protective role of rs3495 in females ($\chi^2 = 6.87$, p value = 0.032, RM: OR 0.173, CI = 0.043–0.699 (p value = 0.017). In addition, rs1803274 risk allele-A was observed to be significantly higher in males ($\chi^2 = 4.258$, p value = 0.039). In conclusion, the present study is indicative of a role of BChE in the pathophysiology of MDD where genetic polymorphisms were observed to effect BChE activity. Further replication studies in different ethnicities are recommended to validate the current observations.

Keywords Cholinergic neurotransmitter · Butyrylcholinesterase · Polymorphism association · Major depressive disorder

Introduction

Major depressive disorder (MDD), is a common psychiatric condition with lasting morbidity and mortality (Lohoff 2010). According to Tsuang et al. (2004), 10–15% of the overall global population may experience signs of clinical depression at least once in their lifetime. The prevalence of MDD has been observed to occur more commonly in females as compared to males, however, population between 25 and 44 years of age that include both the genders are more likely to develop MDD. (Albert 2015; Gold 1998; Scott and Collings 2010). The other risk factors for developing MDD are low socioeconomic status and compromised health (Gavin et al. 2010; Lorant 2003). The depressed individuals may not have any apparent cause for depression but still exhibit MDD symptoms, such individuals may have a family history of MDD and are most likely to possess genetic predisposition (Breslend et al. 2017; Monroe et al. 2014; Serretti et al., 2013). MDD is a multifactorial disease where genetics plays an important role in the disease onset and severity (Kendler et al. 1995; Kessing and Bukh 2013). Despite several reports on genetic susceptibility for MDD, a common susceptibility gene for MDD in different ethnicities is yet to be identified (Lohoff 2010). Psychiatric disorders are thought to be caused due to the abnormal transmission of neurotransmitters at neuronal synapses. Some examples of neuropsychiatric disorders in which alteration of neurotransmission occurs, include anxiety (Martin et al. 2009), schizophrenia (Berger 1981), MDD (aan het Rot et al. 2009), bipolar disorder (BP) (Manji et al. 2003; Salvadore et al. 2010), and Alzheimer disease (Chen et al. 2011).

Cholinergic neurons and its neurotransmitter acetylcholine belong to one of the major and evolutionary conserved neurotransmitter systems (Pezzementi et al. 2011). In human two different enzymes are present, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which hydrolyse acetylcholine. The role of AChE in cholinergic neurotransmission has been clearly defined, though it is considered as a co-regulator of cholinergic neurotransmission, the physiological role of butyrylcholinesterase is not yet clear (Pezzementi et al. 2011; Lockridge 2015).

There is a considerable evidence that confirms the presence of BChE in the neurons of the hippocampus, amygdala, and thalamus indicating its role in controlling memory and emotion through the neuronal pathways (Jacob et al. 2013). BChE has been reported to be altered in neuropathological conditions (Chen et al. 2015; Darvesh et al. 2003; Dhananjayan et al. 2012; Podoly et al. 2009; Dingova et al. 2016; Ezzaher et al. 2012; Josviak et al. 2017). In addition, the activity of BChE has been found to be significantly altered in psychiatric patients thus suggesting its role in aberrated cholinergic signalling in psychiatric patients (Deutsch and Campbell 1984). Thakar et al. (1985) observed reduced BChE activity in subjects with mania and depression (Thakar et al. 1985). Besides, BChE plays an effective role whenever there is a reduction or absence of AChE, a primary ACh catabolizing enzyme (Adler et al. 2011; Johnson and Moore 2012).

Currently, 75 mutations have been identified in *BCHE* that result in abnormal enzyme structure and function (Lushchekina et al. 2015). However, the loss of function mutations namely K-variant, fluoride-resistant variant, silent variant, atypical variant, J-variant, H-variant, and Cynthiana variant, have been associated with numerous pathological conditions (Darvesh et al. 2003). Amongst them, the extensively studied *BCHE* variant is the K-variant (*BCHE*-K; c.1699G > A; Ala567Thr; rs1803274) located at exon 4 and given the name in honour of Werner Kalow. The *BCHE*-K variant carriers show reduced BChE activity, approximately 30% less relative to its wildtype (*BCHE*-U) and thus it fails to hydrolyse its respective substrate completely (Bono et al. 2015; Podoly et al. 2009; Wang et al. 2015). *BCHE*-K variant has been found to be linked with several neurological disorders including Alzheimer's (McIlroy, 2000; Wang et al., 2015), mild cognitive impairment (MCI) (Pongthanaracht et al. 2017), neurodegeneration (Aeinehband et al. 2015), ischaemic stroke (Oguri et al. 2009), and stress (Fiocco et al. 2009). Another variant rs3495 (c.*189G > A), in the 3′UTR region, which is 189 bp downstream of the stop codon has also been reported to cause reduced enzyme activity (Lima et al. 2013). Furtado-Alle et al. (2008) have shown that the K-variant and rs3495 have a *cis* regulatory effect on gene expression. As both the variants display a *cis* effect, therefore, their role in neurological disorders development might be overlapping. The current study was therefore conducted to determine the role of rs1803274 and rs3495 in the onset of MDD. To the best of our knowledge, such investigation has not been carried out for any psychiatric condition in Pakistan.

Materials and Methods

Sampling of Study Subjects

Eighty MDD patients (male = 45 and female = 35) and sixty-one healthy volunteers participated in the study. The average age of the male and female participants was ≈ 34 ± 13 and ≈ 37 ± 15 years, respectively. Inclusion and exclusion criteria used to specify subjects for participation and diagnostic evaluation of MDD subjects were according to the International Classification of Diseases (ICD-10). Inclusion criteria

were depressed mood, agitation, insomnia or hypersomnia, feeling guilt or worthlessness, indecisiveness, and suicidal thoughts. Exclusion criteria were substance abuse, comorbidity, other psychiatric disorders, and bereavement. The control samples were collected from mentally and physically healthy individuals with no signs of depression according to the Beck depression inventory (BDI). The age, gender, and ethnicity of healthy controls were matched with the cases. Four millilitres of venous blood was obtained from the subjects and divided into plain vacutainer tubes for serum extraction (Becton Dickinson, Plymouth, UK) and EDTA-k vacutainer (Becton Dickinson, Plymouth, UK) for DNA extraction.

Ethical Approval of the Study

The study was approved by the Ethics Review Board of The Department of Biosciences, COMSATS University Islamabad and conformed to the Helsinki declaration. Each study participants was informed about the purpose of the research and written consent was obtained from all the subjects before sample collection.

BChE Activity Determination by Ellman's Method

Blood obtained in plain vacutainer tubes were centrifuged at 3500 rpm (1507 g) for 10 min to separate the serum. Clear serum was then transferred in labelled eppendorf tubes and stored at − 20 °C till further use. Butyrylcholinesterase activity was measured according to Worek et al. (1999) that had been derived from Ellman's 1961 principal (Ellman et al. 1961) with few changes, which included the use of serum instead of plasma. 3.0 mL of 0.1 mM phosphate buffer, Ph. 7.4, 100 µL Dithiobis nitrobenzoic acid (DTNB) (10 mM), and 10 µL of serum was mixed in polystyrene cuvettes and equilibrated at 37 °C for 20 min. 50 µL of butyrylthiocholine (63.2 mM) was then added and absorbance was recorded at 436 nm for five minutes with a one-minute interval on a Specord 50 plus spectrophotometer (Analytic Jena, Germany). The coefficient of absorbance of TNB- at 436 nm ($\varepsilon = 10.6\ mM^{-1}\ cm^{-1}$) was computed from the value at 436 nm set at $\varepsilon = 13.6\ mM^{-1}\ cm^{-1}$ (Worek et al. 1999).

Extraction of DNA and Genotyping

Extraction of genomic DNA from the blood sample was done by an organic Phenol/Chloroform protocol (Green and Sambrook 2017). DNA quantity was determined using a nanodrop (Thomas Scientific, Implen Pearl Nano-Photomete, Swedesboro, New Jersey). The chemicals and consumables used in the PCR were purchased from Sigma-Aldrich, St. Louis, MO, and Thermo Scientific, Miami, OK.

Genotyping of *BCHE* rs3495 polymorphism was carried out by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method from a previously published data (Oliveira et al. 2017) (Table 1). The PCR was performed in a total volume of 25 µl with 2 µl of 20-40 ng/µl genomic DNA, 0.5 µl (0.002 mM)

Table 1 Primer sequences and amplified product size for the single nucleotide polymorphisms rs3495 and rs1803274 of *BCHE*

Primers sequence	Amplicon size
BCHE- rs3495-F: 5′ CACTAGCAAGAAAGAAAGTTGTGTG-3′	369 bp
BCHE- rs3495-R: 5′ -AATACACGTGACTAAAAGCAGAGC-3′	
**BCHE*-OF: 5′-CTT TTC AGG CAA AGC GAGCTA ATA AC-3′	556 bp
**BCHE*-OR: 5′- GAA AGA AAT TGA ACCAGG CCA T-3′	
Allele G: *BCHE*-IF: 5′-CCA TAT TTT ACA GGA AATATT GAT GCA G-3′	407 bp
Allele A: *BCHE*-IR: 5′-ATC CTG CTT TCC ACT CCCATT CGG T-3′	201 bp

F = forward primer, IF = inner forward primer, IR = inner reverse, OF = outer forward primer, OR = outer reverse primer, R = reverse primer, *OF and OR are internal control primers for allele-specific polymerase chain reaction

deoxynucleoside triphosphate (dNTP's) mix (10 mM) (MBI Fermentas Life Sciences, UK), 2.5 µl 10X Taq buffer (500 mM KCl, 100 mM Tris-HCl pH 8.8), 2.5 µl (2.5 mM) $MgCl_2$ (25 mM) (MBI Fermentas Life Sciences, UK), 1 µl (0.004 mM) of each (forward and reverse) primers (10 mM), 0.5 µl (2.5U) of Taq Polymerase 5U/µl (MBI Fermentas Life Sciences, UK), and 15 µl water (DNase/RNase free). The PCR was performed in a thermal cycler (Thermo Electron Corporation) using the following steps: denaturation (initial) at 95 °C/10 min, followed by 35 cycles of 95 °C/30 s (denaturation), 56 °C/30 s (annealing), 72 °C/45 s (extension), and the final extension was performed for 10 min at 72 °C. Restriction digestion of PCR product was done using 0.2ul (2U) *NSP1* (XceI) (10 U/µl) restriction enzyme (ThermoFisher Scientific, Miami, OK) by overnight incubation at 37 °C. *NSP1* (XceI) recognizes the sequence R_CATG^Y thus it cleaves in the presence of c.*189A-allele. Digested PCR products were resolved on 3.5% agarose gel and observed under UV transilluminator (Alpha imager Mini Bucher Biotech, Basel, Switzerland) (Fig. 1A).

Genotyping of rs1803274 single nucleotide polymorphism (SNP) was performed using a Tetra-ARMS-PCR method as performed by Ye et al. (1992). The outer primers acting as control primers yields a fragment of 556 bp (Internal Control), whereas two inner primers in the presence of their respective allele yield fragments for G- and A-alleles i.e. 407 bp and 201 bp, respectively (Fig. 1B). The genotyping of *BCHE* rs1803274 was carried out in a total volume of 25 µl containing 2 µl of 20 ng-40 ng/µl genomic DNA, 0.5 µl (0.002 mM) deoxyribonucleotide triphosphate (dNTPs) mix (10 mM) (MBI Fermentas Life Sciences, UK), 2.5 µl 10X Taq buffer (500 mM KCl, 100 mM Tris-HCl pH 8.8), 3.0 µl (3 mM) $MgCl_2$ (25 mM) (MBI Fermentas Life Sciences, UK), 0.5 µl (0.002 mM) of two 10 mM allele-specific primers (inner primers), and 0.75 µL (0.003 mM) for the 10 mM internal control (outer primers), 0.75ul (3.75U) of Taq Polymerase 5U/µl (MBI Fermentas Life Sciences, UK), and 13.75 µl of water (DNase/RNase free (Invitrogen®). The thermal profile was as follows: denaturation (initial) at 95 °C/5 min followed by 35 cycles of 95 °C/30 s (denaturation), 56 °C/30 s (primer annealing), and 72 °C/45 s (extension) with a final extension at 72 °C/7 min.

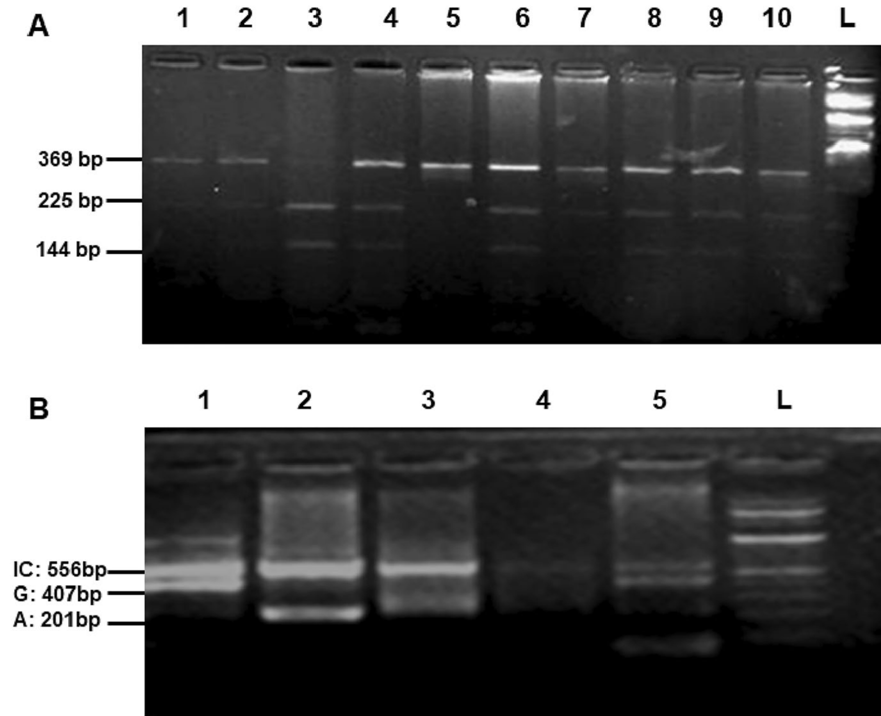

Fig. 1 **A** Agarose gel electrophoresis of the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of of *BCHE* rs3495 polymorphism, digestion with *NSP1* restriction enzyme. Agarose gel shows the product size after digestion: normal homozygote GG band is of 369 bp (Lane 1, 2, 5); variant homozygote AA shows two fragments of 225 bp and 144 bp length (Lane 3); and the heterozygote GA shows bands at 369 bp, 225 bp, and 144 bp (Lane 4, 6, 7, 8, 9, 10). Size of fragments is compared with DNA ladder (L) at the right side. **B** Genotyping of rs1803274. Gel image showing ARMS-PCR amplification of *BCHE* SNP rs1803274. The genotyping is based on presence or absence of allele-specific bands, while internal control (IC) is present in all (556 bp). G-allele-specific band (407 bp), A-allele-specific band (201 bp). Size of fragments is compared with 100 bp DNA ladder (L) at the right side. Lane 1 (GG) genotype consisting of two fragments; 556 bp and 407 bp. Lanes 2 and 3 (AC) genotype consisting of fragments 556 bp and 201 bp. Lane 5 GA genotype consisting of three fragments; 556 bp, 407 bp, and 201 bp

Validation of Genotyping by Sanger Sequence Analysis

Validation of the results of genotyping was done by Sanger sequencing of 35% of the study subjects (Fig. 2A, B). BioEdit software was used for analysing Sanger sequencing data to detect the respective variations. 100% concordance of the sequencing data with the genotyped data in the study subjects was obtained.

Statistical Analysis

Mann–Whitney test (Non-parametric) was used to find the statistical significance at $\alpha \leq 0.05$ for butyrylcholinesterase. Genotype data were evaluated using the Chi-square

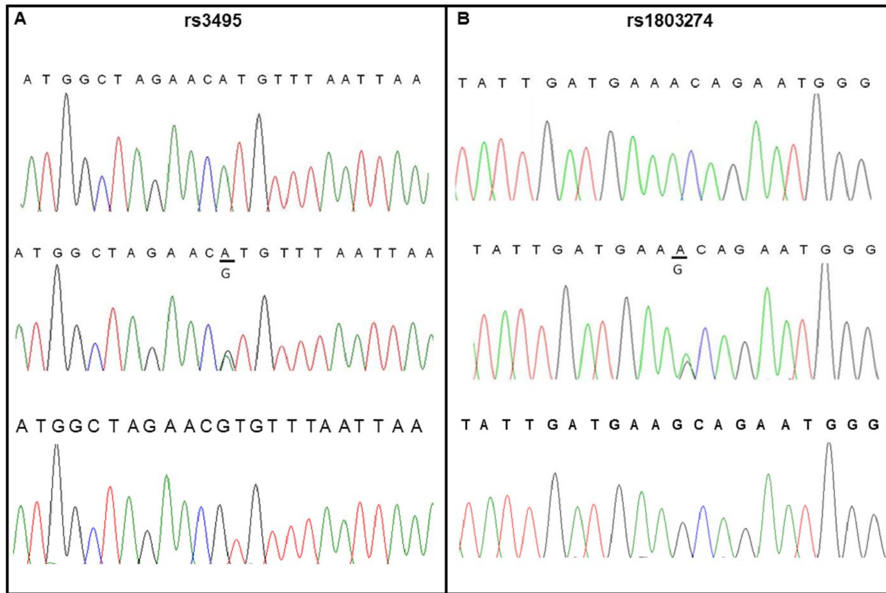

Fig. 2 **A** Sanger sequencing chromatogram for rs3495 containing the SNP homozygous AA (**A**), heterozygous GA. **B** Sanger sequencing chromatogram for rs1803274 homozygous AA, heterozygous AG

(χ^2) test/Fisher exact Test. Association analysis for both the SNPs was determined by calculating the odds ratio (OR) with 95% confidence interval. Results were appraised for deviations from Hardy–Weinberg equilibrium (HWE) in MDD and healthy controls using the goodness-of-fit chi-square test (http://www.had2know.com/academics/hardy-weinberg-equillibrium-calculator-2-alleles.html) of genotype frequencies. IBM SPSS Statistics (IBM Incorporation, USA, version 21) and GraphPad (Prism 7.0 La Jolla, CA), were used for the statistical data analysis, considering p value ≤ 0.05 to be statistically significant. The association of the polymorphisms with the disease was determined using logistic regression analysis under dominant, recessive, and multiplicative models. For both the studied SNPs, the change in nucleotide from G to A, the A-allele of both SNPs were considered risk alleles (based on frequency distribution in cases and controls). Under multiplicative analysis the association of A-alleles for both the SNPs with the disease was assessed taking wild-type G-allele as reference. While genotype association under the dominant and recessive models were done taking the following combination: dominant model: GA + AA vs GG; recessive model: AA vs. GA + GG.

Results

Determination of BChE Activity

BChE activity was observed to be notably reduced ($p = \mathbf{0.000}$) in MDD patients 69.96 ± 0.001 µmol/l/min. (mean ± SE) as compared to healthy individuals

Table 2 BChE activity in the overall cohort of MDD and healthy controls and gender-based analysis of the data

	$N=$	BChE %activity mean	SD	SE	95% CI for mean	Significance $p \leq 0.05$
BChE activity in MDD and healthy controls						
Healthy controls	53	90.97	0.015	0.002	0.024–0.032	***0.000***
MDD cases	76	69.96	0.006	0.001	0.018–0.021	
BChE activity in MDD males and females						
Male (mean age 34.18)	27	70.56	19.81	3.813	62.72–78.40	0.1443
Female (mean age 34.10)	49	69.63	23.02	3.288	63.01–76.24	

95% confidence interval (CI) of mean, also shown in the table is significant at $p \leq 0.05$ and below. The significant values are bold and italicized

90.97 ± 0.002 µmol/l/min. (mean ± SE; Table 2), whereas comparison between the two genders revealed no substantial difference in BChE activity (Table 2).

BChE Activity in Different Genotypes of Healthy Controls and MDD Patients

For rs3495 SNP, carriers of GG genotype homozygotes showed significantly decreased enzyme activity in the MDD cohort as compared to the healthy control (*p* value = 0.015), whereas the AA genotype revealed no significant difference amongst the groups (*p* value = 0.07; Table 3). However, when the overall effect of the genotypes was determined regardless of the MDD and control (MDD + Controls), heterozygote carriers GA of rs1803274 SNP revealed reduced BChE activity (*p* value = 0.04; Table 4).

rs3495 and rs1803274 Genotype and Allele Association

The genotype analysis of the two SNPs was also carried out independently of the BChE activity. No association of rs3495 was observed for MDD ($\chi^2 = 2.69$; *p* value = 0.260; (dominant model (DM): odds ratio (OR) 1.491, 95% confidence interval (CI) 0.558–3.985, $p = 0.466$; recessive model (RM): OR 0.460, 95% CI 0.160–1.301, $p = 0.157$ and allele-based analysis: OR 0.681, 95% CI 0.321–1.186, *p* value = 0.127; Table 5). The significant genotype and allele association was found for MDD predisposition for rs1803274 SNP (DM; OR 2.324, 95% CI 1.095–4.964, *p* value = 0.025; allele-based analysis OR 2.07, 95% CI 1.13–3.81, *p* value = 0.013; Table 6).

Gender-Based Genotype and Allele Association

The gender-based analysis of the data revealed significant differences in genotypes and allele frequency distribution for rs3495 between female controls and MDD

Table 3 BChE activity in genotypes of rs3495 and rs1803274

rs3495				rs1803274			
Genotypes	*N*	BChE activity %mean ± SE (95% CI)	*p* value	Genotypes	*N*	BChE activity %mean ± SE (95% CI)	*p* value
MDD-GG	7	63.41 ± 1.47 (59.81–67.01)	***0.015***	MDD-GG	25	79.20 ± 5.66 (67.53–90.88)	0.08
Control-GG	6	92.20 ± 10.83 (64.35–120.0)		Control-GG	6	97.51 ± 8.93 (74.56–120.46)	
MDD-GA	12	61.41 ± 5.776 (48.70–74.13)	0.67	MDD-GA	18	64.03 ± 4.40 (54.75–73.32)	0.26
Control-GA	10	94.42 ± 6.88 (78.86–109.90)		Control-GA	4	70.17 ± 24.11 (0–146.90)	
MDD-AA	10	84.11 ± 11.06 (59.08–109.1)	0.07	MDD-AA	8	70.26 ± 6.51 (54.85–85.66)	0.29
Control-AA	07	104.85 ± 9.11 (82.55–127.10)		Control-AA	2	97.94 ± 34.26 (0–533.25)	

95% confidence interval (CI) of mean, also shown in the table is significant at $p \leq 0.05$ and below. The significant values are bold and italicized

Table 4 Genotype-based mean BChE activity in the overall study cohort (MDD + controls; significance at $p \le 0.05$)

rs3495				rs1803274			
Genotypes	*N*	BChE activity %mean ± SE (95% CI)	*p* value	Genotypes	*N*	BChE activity %mean ± SE (95% CI)	*p* value
GG	31	82.75 ± 4.99 72.54–92.95	0.198	GG	13	76.70 ± 6.349 62.86–90.53	***0.040***
GA	22	65.15 ± 5.31 54.11–76.19		GA	22	76.42 ± 5.621 64.72–88.11	
GG	31	82.75 ± 4.99 72.54–92.95	0.107	GG	13	76.70 ± 6.349 62.86–90.53	0.427
AA	10	75.80 ± 8.13 57.40–94.19		AA	17	92.65 ± 7.735 76.25–109.0	

95% confidence interval (CI) of mean, also shown in the table is significant at $p \le 0.05$ and below that are bold and italicized

Table 5 Statistical analysis of genotype and allele frequencies of *BCHE* SNP rs3495

Genotype	Controls *N* = 41	MDD *N* = 44	MDD vs. Controls		
			z test (*p* value)	χ^2 (*p* value)	OR (95% CI) *p* value
GG	9 (21.96%)	13 (29.5%)	0.7988 (0.4237)	2.69 (0.260)	**DM:** 1.491 (0.558–3.985) (0.466) **RM:** 0.460 (0.160–1.301) (0.157)
GA	16 (39.02%)	21 (48.7%)	0.808 (0.4179)		
AA	16 (39.02%)	10 (22.8%)	− 1.629 (0.103)		
Allele	*N* = 82	*N* = 88	MDD vs. Controls		
			χ^2 (*p* value)	OR (95% CI) *p* value	
G	34 (41.46%)	47 (53.40%)	2.428 (0.1191)	OR 0.681 (0.321–1.186) (0.127)	
A	48 (58.54%)	41 (46.60%)			

CI confidence interval; *DM* dominant model; *MDD* major depressive disorders; *N* number of individuals; *OR* odds ratio; *RM* recessive model

($\chi^2 = 6.87$, *p* value 0.032). The AA genotype of the SNP revealed a protective association with females in the control group under a recessive model (genotype analysis: RM: $p = 0.017$, OR 0.173; 95% CI 0.043–0.699; allele-based analysis: χ^2 4.989 (*p* values = 0.025, OR 2.326, 95% CI (0.103–4.905), *p* value = 0.040; Table 7).

The rs1803274 SNP did not reveal any association with male or female gender in the studied groups (Table 8). However, allele-A frequency distribution between MMD males as compared to males of the control group was significantly different ($\chi^2 = 4.258$, *p* value = 0.039; OR 2.462, 95% CI 0.957–6.239, *p* value = 0.058; Table 8) pointing towards the association of rs1803274 with disease susceptibility in males.

Table 6 Statistical analysis of genotype and allele frequencies of *BCHE* SNP rs1803274

Genotype	Controls $N=61$	MDD $N=80$	MDD vs. Controls		
			z test (p value)	χ^2 (p value)	OR (95% CI) p value
GG	42 (68.87%)	39 (48.75%)	− 2.3919 (0.016)	***5.993 (0.049)***	**DM** = ***2.324 (1.095–4.964) (0.025)***
GA	16 (26.22%)	32 (40%)	1.7096 (0.087)		**RM** = 2.45 (0.570–12.031) (0.232)
AA	3 (4.91%)	9 (11.25%)	1.335 (0.1852)		

Allele Frequency	Controls $N=122$	MDD $N=160$	MDD vs. Controls	
			χ^2 (p value)	OR (95% CI) p value
G	100 (81.97%)	110 (68.75%)	***6.36 (0.012)***	***2.07 (1.13–3.81) (0.013)***
A	22 (18.03%)	50 (31.25%)		

Significance at $p \leq 0.05$ are bold and italicized

CI confidence interval; *DM* dominant model; *MDD* major depressive disorders; *N* number of individuals, *OR* odds ratio; *RM* recessive model

Discussion

MDD is a multifactorial disorder with genetics, environmental, and psychological factors being involved in the progress of this disease (Corvin et al. 2011; Kendler et al. 2004; Lohoff 2010; McLaughlin et al. 2010; Shapero et al. 2014; Uher 2014). Imbalances of neurotransmitter systems are thought to be connected to depression, anxiety, and other mood disorders. Although the cholinergic hypothesis of depression did not previously get attention, maybe due to lack of evidence in the past but some recent studies have pointed towards a possible role of the cholinergic system in depression (Dilsaver and Coffman 1989; Janowsky et al. 1972; Riemann et al. 1994). In the current study, BChE activity was noted to be lower in the MDD cohort with the possible genetic association of SNP rs3495, while rs1803274 genetic change modulate enzyme activity regardless of the phenotype, i.e. cases and controls. The genetic association independent of BChE activity was found for rs1803274 with the disease while rs3495 revealed a protective association in the female gender. It is to be noted that in general, depression is more likely to affect females than males (Albert 2015; Gold 1998; Kendler et al. 2004), however, based on present study it can be predicted that *BCHE* genetic changes provide protection in females towards depression instead of disease development.

The aberrated BChE activity for MDD has not been extensively studied and reported previously in literature. Some studies on the subject reported reduced levels of BChE in MDD, bipolar (BP), and in other affective disorders (Thakar et al. 1985). In contrast, a higher level of serum BChE has also been reported in psychiatric patients including patients with depression (Modai et al. 1987). Results from successive studies generated controversial conclusions about the relationship of depression with BChE activity. Few studies suggest high BChE levels favours susceptibility towards stress, anxiety, and depression and some suggests it's lower

Table 7 Gender-based statistical analysis of genotype and allele data of *BCHE* SNP rs3495

Genotype	Controls $N=+31$	MDD $N=27$	MDD vs. Controls		
			z test (p value)	χ^2 (p value)	OR (95% CI) (p value)
Females					
GG	7 (22.58%)	9 (33.33%)	0.913 (0.362)	***6.87 (0.032)***	**DM:** 1.714 (0.537–5.477) (0.393) ***RM: 0.173 (0.043–0.699) (0.017)***
GA	11 (35.48%)	15 (55.55%)	1.533 (0.126)		
AA	13 (41.94%)	3 (11.12%)	***2.619 (0.008)***		

Allele	Controls $N=62$	MDD $N=54$	MDD vs. Controls	
			χ^2 (p value)	OR (95% CI) p value
Females				
G	25(40.32%)	33(61.11%)	***4.989 (0.025)***	0.43 (0.204–0.907) (0.040)
A	37(59.68%)	21(38.89%)		

Genotype	Controls $N=10$	MDD $N=17$	MDD vs. Controls		
			z test (p value)	χ^2 (p value)	OR (95% CI) p value
Males					
GG	2(20%)	4(23.53%)	0.213(0.833)	0.582 (0.748)	DM: 1.231 (0.135–12.69) (1.000) RM: 0.612 (0.08–4.20) (0.692)
GA	5(50%)	6(35.30%)	− 0.751(0.4532)		
AA	3(30%)	7(41.17%)	0.580(0.5619)		

Allele	Controls $N=20$	MDD $N=34$	MDD vs. Controls	
			χ^2 (p value)	OR (95% CI) p value
Males				
G	9(45%)	14(41.18%)	0.075(0.783)	0.856 (0.281–2.608) (1.000)
A	11(55%)	20(58.82%)		

Significance at $p \leq 0.05$ are bold and italicized

CI confidence interval; *DM* dominant model; *MDD* major depressive disorders; *N* number of individuals, *OR* odds ratio; *RM* recessive model

activity to be associated with these symptoms (Brimijoin and Tye 2017; Brimijoin et al. 2016; Ezzaher et al. 2012; Modai et al. 1987). All the studies mentioned above have failed to find any conclusive connection between enzyme activity and the psychopathological conditions. The results of the present study showed statistically significant lower activity of BChE in MDD cohort as compared to the control group. It is noteworthy that some of the BCHE gene polymorphisms, for instance, H, J, and K variants exhibit 60%, 66%, and 30% reduction in enzyme activity, respectively (Lockridge et al. 2016). Study by Bretlau et al. (2013) found that the succinylcholine action was extended in patients who were heterozygous for the K-variant, unlike the

Table 8 Gender-based statistical analysis of genotype and allele data of *BCHE* SNP rs1803274

Genotype	Controls $N=26$	MDD $N=47$	MDD vs. Controls		
			z test (*p* value)	χ^2 (*p* value)	OR (95% CI) *p* value
Females					
GG	17 (65.38%)	23 (48.93%)	− 1.352 (0.177)	1.87 (0.392)	**DM:** 1.971 (0.659–5.988) (0.223) **RM:** 1.342 (0.271–7.342) (1.000)
GA	06 (23.09%)	17 (36.18%)	0.177 (0.250)		
AA	3 (11.53%)	7 (14.89%)	0.399 (0.689)		
Allele	**Controls $N=52$**	**MDD $N=94$**	**MDD vs. Controls**		
			χ^2 (*p* value)	OR (95% CI) *p* value	
Females					
G	40 (76.92%)	63 (67.02%)	1.579 (0.208)	0.610 (0.261–1.410) (0.257)	
A	12 (23.08%)	31 (32.98%)			
Genotype	**Controls $N=35$**	**MDD $N=33$**	**MDD vs. Controls**		
			z test (*p* value)	χ^2 (*p* value)	OR (95% CI) *p* value
Males					
GG	25 (71.42%)	16 (48.48%)	− 1.932 (0.053)	3.21 *p* value (0.200)	**DM:** 2.656 (0.873–8.223) (0.082) **RM:** *p* value (0.232)
GA	10 (28.58%)	15 (45.45%)	1.443 (0.149)		
AA	0 (0%)	2 (6.07%)	1.478 (0.138)		
Allele	**Controls $N=70$**	**MDD $N=66$**	**MDD vs. Controls**		
			χ^2 (*p* value)	OR (95% CI) *p* value	
Males					
G	60 (85.71%)	47 (71.22%)	4.258 (***0.039***)	2.462 (0.957–6.239) 0.058	
A	10 (14.2%)	19 (28.78%)			

Significance at $p \leq 0.05$ are bold and italicized

CI confidence interval; *DM* dominant model; *MDD* major depressive disorders; *N* number of individuals, *OR* odds ratio; *RM* recessive model

carriers of wild-type genotype. In another study, the prolonged action of mivacurium in patients was observed to occur in the presence of a heterozygous form of the K-variant (Gätke et al. 2005). The present study observed reduced BChE activity in MDD thus supporting the previous findings that the BCHE gene polymorphisms (K-variant (rs1803274)) had an association with neuronal pathophysiology.

In the current study, the individuals who were heterozygous carriers (AG) of the K-variant showed an association with reduced enzyme activity. In contrast, a case–control study of coronary artery disease revealed that *BCHE*-K allele in both heterozygous and homozygous form induces a higher risk of developing CAD (Vaisi-Raygani et al., 2008), same is the case for neurological disease AD in which the K-variant is associated with pathological effects (McIlroy 2000; Podoly et al.

2009; Wang et al. 2015). Since gender is considered as an important risk factor for the development of depression, in the present study the evaluation of gender-based analysis for the K-variant did not reveal any association despite the significant difference in A-allele frequency distribution between MDD and control males. The lack of association of K-variant in the present study can be attributed to a limited sample size in each group. Thus, replicating the study on a bigger cohort might reveal the gender-based association of the SNP with MDD. A study conducted in Western Iran has shown a protective role of the *BCHE*-K variant, which was limited to the male gender, here the prevalence of mild cognitive impairment (MCI) was observed to be more frequent in females than males and the homozygous form of *BCHE*-K was not observed in females but only in the male patients (Pongthanaracht et al. 2017).On the contrary, the opposite effect of the K-variant was found in the Spanish population, where the protective effect was limited to females (Alvarez-Arcaya et al. 2000). Another study showed lower susceptibility to Alzheimer's disease (AD) for women carrying the K-variant. From the previous and present findings, it may be hypothesized that the K-variant is dependent on gender with an additional role of gender and ethnicity towards disease susceptibility (Alvarez-Arcaya et al. 2000).

The results of the present study are in agreement with the previous findings where rs3495 variant was found to be associated with lower BChE activity, with reduced plasma BChE activity in GG genotype of MDD patients as compared to the healthy controls (Lima et al. 2013; Oliveira et al. 2017). Moreover, in the present study, the protective association of rs3495 was observed with female gender where the A-allele was more pronounced homozygously in healthy controls than females with depression.

The lack of association of *BCHE* SNPs and its activity with MDD may be due to some limitations of the current study. For instance, sample size was small. In addition, the patients were undergoing treatment with antidepressant medications which were not disclosed by the clinicians. Nevertheless, some reports suggest no influence on BChE activity when using antipsychotics (Modai et al. 1987). Furthermore, there was uncertainty about the period of any recent drug administration.

Conclusion

In conclusion, in the present study, reduced BChE activity was found to be associated with MDD which was observed to be affected by genetic variation in *BCHE*. Carriers of risk allele and genotype of SNP rs1803274 showed disease susceptibility, in addition to the higher frequency of the risk allele in male gender. The outcome of the present study and replication studies on a larger sample size may lead to the development of new approaches to cholinergic enzyme-based treatment for MDD patients.

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data analysis, manuscript editing. SMN, RQ, MA: study design, provision of funds, data analysis, and manuscript finalization.

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Data Availability Available on request.

Code Availability Available on request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The Ethics Review Board of The Department of Biosciences, COMSATS University Islamabad, approved the study, the study conformed to the Helsinki declaration.

Consent to Participate Each of the study participants was informed about the purpose of the research and written consent was obtained from all the subjects before sample collection.

Consent for Publication Each of the authors mentioned in the manuscript have consented for authorship, read and accepted the manuscript, also provided permission for data sharing and publication of the manuscript.

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