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Association of *BDNF* rs6265 and *MC4R* rs17782313 with metabolic syndrome in Pakistanis

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The current case–control study sought the association of *BDNF* rs6265 and *MC4R* rs17782313 with metabolic syndrome (MetS), MetS components and other related metabolic parameters in a sample of Pakistani subjects. Fasting high-density lipoprotein cholesterol (HDL-C) and homeostatic model assessment of insulin sensitivity showed a significantly lower mean whereas body mass index (BMI), waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol, triglycerides (TG), cholesterol to HDL-C ratio, TG to HDL-C ratio, homeostatic model assessment of insulin resistance, visceral adiposity index, lipid accumulation product and the product of TG and glucose showed a significantly higher mean in the presence of MetS. Reduced HDL-C appeared as the most frequent and hypertriglyceridemia as the least frequent component of MetS whereas clustering of reduced HDL-C + abdominal obesity (AO) + hyperglycemia appeared as the most prevalent combination of MetS components. Moreover, *BDNF* rs6265 showed BMI and gender independent association with increased risk of MetS in Pakistani individuals whereas *MC4R* rs17782313 showed BMI and gender dependent association with increased risk of MetS in Pakistani females. In addition, *BDNF* rs6265 and *MC4R* rs17782313 showed gender-dependent associations with decreased risk of having low HDL-C in males and increased risk of having abdominal obesity in females, respectively. However, no association was observed for metabolic variables other than components of MetS across genotypes of both *BDNF* rs6265 and *MC4R* rs17782313.

Keywords. Abdominal obesity; *BDNF* rs6265; high-density lipoprotein cholesterol; hyperglycemia; hypertriglyceridemia; *MC4R* rs17782313

1. Introduction

Metabolic syndrome (MetS) is a clinical condition defined by the concurrence of multiple phenotypes in one individual leading to an increased risk of type-2 diabetes and cardiovascular disease, thus posing significant burdens on social and healthcare systems (Wilson *et al.* 2005; Lee and Sanders 2012). The extent of the increased risk can vary depending upon the combination of MetS components present along with the other non-MetS risk factors in a particular individual. A variety of definitions and criteria recommended by various authorities have been used to diagnose MetS (Alberti *et al.* 2005; Grundy *et al.* 2005; NCEP ATP III 2001). However, according to a recent consensus of various organizations, MetS is identified by the presence of at least three or more of the five anomalies including abdominal obesity (AO), hypertriglyceridemia, hyperglycemia, hypertension (HTN) and a decreased high-density lipoprotein cholesterol (HDL-C) level (Alberti *et al.* 2009). Many epidemiologic studies have revealed that MetS is becoming increasingly common worldwide. The global prevalence of MetS varies substantially from less than 10–84% depending upon the definition applied together with the geographical area, environment, gender, age, race and ethnicity of the population (Desroches and Lamarche 2007; Kolovou *et al.* 2007). Altogether, nearly one-quarter of the world's adult population has MetS as per the estimation of the International Diabetes Federation (IDF) (International Diabetes Federation IDF 2006). In particular, the prevalence of MetS in Pakistan is also escalating at an alarming rate. The prevalence of MetS in Pakistan according to the IDF definition and modified criteria of NCEP ATP III (National Cholesterol

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Education Program/Adult Treatment Panel III) has been reported as 34.8 and 49%, respectively (Hydrie *et al.* 2009).

The family and twin studies provide considerable evidence for a genetic component of MetS. It could be heritable in 10–30% of the cases (Carmelli *et al.* 1994; Edwards *et al.* 1997; Lin *et al.* 2005; Bellia *et al.* 2009). It is a polygenic disorder that could be a result from the interplay of genetic and environmental factors but its complete genetic background remains to be elucidated (Aguilera *et al.* 2013). Searching genetic determinants of MetS is challenging, attributable to the complexity of the MetS phenotype, indeterminate common pathogenetic mechanisms for clustering of metabolic abnormalities, multiple definitions and modified lifestyle factors. Common variants in candidate genes regulating energy balance along with environmental triggers may possibly increase predisposition to the MetS (Stancakova and Laakso 2014). Common variants in the Melanocortin-4 receptor (MC4R) and the brain-derived neurotrophic factor (BDNF) genes had been found from previous studies to be related to the disorders of energy balance such as obesity (Krashes *et al.* 2016; Xu and Xie 2016), however, their association with MetS is not fully elucidated. Hence, this study was aimed to investigate the association of *MC4R* rs17782313 and *BDNF* rs6265 polymorphisms with MetS in the Pakistani population.

2. Materials and methods

2.1 *Study design and subjects*

The study was performed at International Center for Chemical and Biological Sciences, University of Karachi, Pakistan, after recommendation of Independent Ethics Committee of the institute. It was a case–control study. The study involved 275 cases of MetS and 331 control subjects who did not have MetS. The study subjects were recruited from the general population of Karachi through random sampling without replacement. All the subjects signed written informed consent prior to their participation in the study. MetS cases were identified according to the NCEP ATP III criteria. According to this criteria, AO was taken as a waist circumference (WC) $\geq$102 cm for males and $\geq$88.9 cm for females; hyperglycemia as fasting blood glucose (FBG) $\geq$100 mg/dL; HTN as SBP $\geq$130 mmHg or DBP $\geq$85 mmHg or both; reduced HDL-C as <40 mg/dL in males and <50 mg/dL in females and hypertriglyceridemia as triglycerides (TG) $\geq$150 mg/dL. Thus, a subject was identified as a MetS case if he or she had three or more aforementioned anomalies with respect to NCEP ATP III criteria. Abnormal reference values for parameters of lipid profile other than MetS components were taken as follows: TC $\geq$200 mg/dL; low-density lipoprotein cholesterol (LDL-C) $\geq$100 mg/dL; very-low-density lipoprotein cholesterol (VLDL-C)>30 mg/dL; TC to HDL-C ratio (CHR) $\geq$4.5 (Gimeno-Orna *et al.* 2005); TG to HDL-C ratio (THR) $\geq$3.8 (Kohli *et al.* 2017). Similarly, abnormal reference values for clinical surrogate markers for predicting MetS were visceral adiposity index (VAI) >2.015 mmol/L, lipid accumulation product (LAP) >31.465 mmol/L and product of TG and glucose (TyG) >8.706 (Li *et al.* 2018). The subjects having history of endocrinopathies and medication such as phenothiazine, tricyclic antidepressants, anticonvulsants and steroids were omitted from the study.

2.2 *Anthropometric and blood pressure measurements*

WC was accurately measured with a measuring tape just above the hip bone in a relaxed (exhaled) abdomen. The body height (precision up to 0.1 cm) and weight (precision up to 0.1 kg) of each subject were assessed by using a portable stadiometer (Seca 214, Germany) and a mechanical column scale (Seca 755, Germany), respectively. Both measurements were taken in light clothing without shoes. The body mass index (BMI) was calculated by dividing the weight (kg) by height square (m^2). Blood pressure was measured in a sitting position by using a mercury column sphygmomanometer with a suitable cuff size covering $\sim$2/3 of the right-upper arm according to the standardized protocol. Two sets of measurements were taken to calculate the average value.

2.3 *Blood collection and biochemical assays*

Blood was taken from every subject after 12 h of fasting and FBG levels were assessed by using a blood glucose monitoring system (Abbott, UK). Serum was isolated for determining the fasting lipid profile including TC, TG, HDL-C, LDL-C and VLDL-C by an enzymatic colorimetric method using relevant commercial kits (Merck, Darmstadt, Germany) on an automatic chemistry analyzer (Roche Hitachi 902, Tokyo, Japan). Moreover, fasting serum insulin levels were determined by enzyme-linked immunosorbent assay on a Multiskan^{TM} FC Microplate Photometer (ThermoFisher Scientific, MA, USA) by using a DIA source INS-EASIA Kit (cat. no. KAP1251, Belgium) according to the manufacturer's protocol. The value of TC was divided by the value of HDL-C to calculate CHR. Similarly, the value of TG was divided by the value of HDL-C to calculate THR. Gender-specific formulae were used to calculate VAI (Amato *et al.* 2010) and LAP (Kahn 2005). VAI (males) = (WC/[39.68 + (1.88 × BMI)]) × (TG/1.03) × (1.31/HDL-C) and VAI (females) = (WC/[36.58 + (1.89 × BMI)]) × (TG/0.81) × (1.52/HDL-C). LAP (males) = (WC − 65) × TG and LAP (females) = (WC − 58) × TG. The index of TyG was calculated by using the formula: ln(TG [mg/dL] × FBG [mg/dL]/2) (Simental-Mendia *et al.* 2008). The values of TG and HDL-C were converted into mmol/L for computing in formulae used for calculating VAI and LAP. Homeostatic model assessment of insulin resistance

(HOMA-IR) = [fasting glucose [mg/dL] × fasting insulin uIU/mL]/405 and homeostatic model assessment of insulin sensitivity (HOMA-IS) = 1/[fasting insulin (mIU/L) × fasting glucose (mmol/L)] were calculated.

2.4 *DNA isolation and genotyping*

DNA was extracted from whole blood via an EZ-10 spin column genomic DNA isolation kit (cat. no. BS684, Bio Basic, ON, Canada). Following isolation, both *MC4R* rs17782313 and *BDNF* rs6265 variants were genotyped on an Applied Biosystems 7500 real-time polymerase chain reaction (PCR) machine (ABI, Foster City, CA, USA) by using TaqMan™ predesigned single-nucleotide polymorphism allelic discrimination assays (assay ID of variant rs17782313: C__32 667 060_10, cat. no. 4351376, ABI, Foster City, CA, USA; assay ID of variant rs6265: C__11592758_10, cat. no. 4351379, ABI, Foster City, CA, USA) and TaqMan® master mix (cat. no. 4381656, ABI, Foster City, CA, USA). In every batch of PCR, two negative controls (NTC or no template control) and a positive control (PC) for each genotype of both variants were also included. For reproducibility, 20% of the samples were genotyped in duplication.

2.5 *Statistical analysis*

The statistical analysis was mainly accomplished by using IBM SPSS statistical software version 21 (SPSS Inc., Chicago, IL, USA). The normality of quantitative variables was evaluated via Shapiro–Wilk test and normal quantile plots (Q–Q plots). All quantitative variables were expressed as means and mean ranks, and were compared by a simple non-parametric Mann–Whitney *U* test to observe the differences between cases and controls. On the other hand, all qualitative variables were expressed as counts and percentages, and were compared for differences between cases and controls by the χ^2 test. A Hardy–Weinberg equilibrium (HWE) test was performed to test the assumption whether the observed allelic and genotypic frequencies in controls conform to HWE. All genotypic frequencies were presented as counts and percentages, and were calculated through simple cross tabulations. Allelic frequencies were calculated by using an online calculator (*www.easycalculation.com/health/allele-frequency-calculator.php*), and the differences in allelic frequencies between MetS cases and control subjects were assessed via the χ^2 test. Logistic regression was performed to find the association of *BDNF* rs6265 and *MC4R* rs17782313 with the MetS assuming additive, dominant, recessive and over-dominant genetic models. Effect estimates (*B*), odds ratios (OR) and 95% confidence intervals (CI) were also calculated to determine the risk of MetS associated with the variant genotypes before and after adjustment for covariates (age and BMI). Confounders were determined by a simple formula: % change in effect estimates = actual change/unadjusted *B*, where the actual change was obtained by subtracting adjusted *B* from unadjusted *B*. Covariates that showed ≥10% change in effect estimate after adjustment were taken as potential confounders. The *h*-index (degree of dominance index) was calculated for choosing the appropriate genetic model (Zintzaras and Santos 2011). The association of lipid parameters across genotypes of variants *BDNF* rs6265 and *MC4R* rs17782313 was determined by a Kruskal–Wallis test. The p value <0.05 was considered significant for all statistical tests applied.

3. Results

3.1 *Quantitative variables differ significantly between MetS cases and non-MetS controls*

All quantitative clinical variables were found significantly different ($p<0.05$) in MetS cases as compared to those of non-MetS controls (table 1). BMI, WC, SBP, DBP, FBG, insulin, HOMA-IR, TC, TG, LDL-C, VLDL-C, CHR, THR, VAI, LAP and TyG showed a significantly higher mean whereas HDL-C and HOMA-IS showed a significantly lower mean in the presence of MetS.

3.2 *Reduced (↓) HDL-C appeared as the most prevalent component of MetS*

The prevalence of individual components of MetS in cases was 98.9% ↓HDL-C, 78.1% AO, 76.7% hyperglycemia, 52.0% HTN and 41.8% hypertriglyceridemia. Similarly, the prevalence of MetS components in female cases was 99.2% ↓HDL-C, 91.9% AO, 88.9% hyperglycemia, 36.0% HTN and 27.2% hypertriglyceridemia whereas in male cases it was 98.5% ↓HDL-C, 67.6% HTN, 64.7% AO, 64.7% hyperglycemia and 56.1% hypertriglyceridemia. Nevertheless, ↓HDL-C remained the most prevalent component while hypertriglyceridemia remained the least prevalent component of MetS in overall case group as well as in female and male cases (figure 1).

3.3 *Most prevalent combination of MetS components*

Among all combinations of MetS components, ↓HDL-C + AO + hyperglycemia appeared to be the most frequent combination of MetS components in overall cases (58.18%), and the same combination remained most prevalent in female cases (80.88%). On the other hand, ↓HDL-C + hyperglycemia + HTN (38.84%) was the most commonly found combination of MetS components in male cases closely followed by ↓HDL-C + AO + HTN (38.13%) and ↓HDL-C + AO + hyperglycemia (38.13%). Similarly, the

Table 1. Comparison of quantitative variables between MetS cases and non-MetS controls

	Overall (N = 606)					Females (N = 270)				Males (N = 336)			
	MetS (cases) = 275					MetS (cases) = 136				MetS (cases) = 139			
	Non-MetS (controls) = 331					Non-MetS (controls) = 134				Non-MetS (controls) = 197			
Parameters	Groups	Mean	SEM	Mean rank	p-value	Mean	SEM	Mean rank	p-value	Mean	SEM	Mean rank	p-value
TC (mg/dL)	MetS	159.45	2.46	337.62	<0.001*	152.13	3.27	138.69	<0.001*	166.62	3.57	201.21	<0.001*
	Controls	145.58	2.04	275.15		150.93	2.98	132.26		141.94	2.75	145.42	
TG (mg/dL)	MetS	145.39	5.00	375.64	<0.001*	118.15	4.40	157.49	<0.001*	172.05	8.32	225.27	<0.001*
	Controls	99.20	2.48	243.57		90.38	2.61	113.18		105.20	3.72	128.45	
HDL-C (mg/dL)	MetS	28.48	0.43	272.79	<0.001*	29.44	0.66	114.69	<0.001*	27.53	0.55	156.45	<0.001*
	Controls	32.00	0.57	329.02		34.75	0.89	156.62		30.13	0.72	177.01	
LDL-C (mg/dL)	MetS	97.78	2.32	333.28	<0.001*	87.51	2.99	137.06	<0.001*	107.83	3.34	200.06	<0.001*
	Controls	86.42	1.85	278.76		86.44	2.83	133.91		86.41	2.45	146.23	
VLDL-C (mg/dL)	MetS	29.00	0.99	373.81	<0.001*	23.64	0.86	156.92	<0.001*	34.24	1.66	223.82	<0.001*
	Controls	20.06	0.49	245.08		18.16	0.52	112.59		21.35	0.73	129.47	
CHR	MetS	5.92	0.12	363.73	<0.001*	5.45	0.15	156.78	<0.001*	6.39	0.17	212.75	<0.001*
	Controls	4.78	0.06	253.46		4.56	0.09	113.91		4.94	0.09	137.28	
THR	MetS	5.49	0.24	386.58	<0.001*	4.26	0.19	170.04	<0.001*	6.71	0.41	227.72	<0.001*
	Controls	3.31	0.08	240.29		2.81	0.11	106.96		3.66	0.12	126.72	
FBG (mg/dL)	MetS	109.17	1.52	385.93	<0.001*	111.18	1.79	176.58	<0.001*	107.22	2.44	206.60	<0.001*
	Controls	96.66	0.57	235.02		97.52	0.86	93.70		96.07	0.76	141.61	
BMI (kg/m^2)	MetS	31.43	0.45	403.01	<0.001*	31.82	0.70	176.51	0.001*	31.049	0.59	227.19	<0.001*
	Controls	23.96	0.25	220.83		23.91	0.41	93.88		23.99	0.61	127.09	
WC (cm)	MetS	108.58	1.01	418.22	<0.001*	109.55	1.45	185.26	<0.001*	107.64	1.41	232.87	<0.001*
	Controls	87.33	0.70	208.19		84.93	1.22	84.99		88.96	0.82	123.08	
SBP (mmHg)	MetS	122.63	0.93	370.87	<0.001*	118.42	1.43	164.57	<0.001*	126.75	1.09	216.73	<0.001*
	Controls	111.73	0.64	247.53		105.96	0.94	105.99		115.66	0.74	134.47	
DBP (mmHg)	MetS	81.91	0.65	376.37	<0.001*	79.04	0.92	164.08	<0.001*	84.71	0.85	219.73	<0.001*
	Controls	73.25	0.48	242.96		70.57	0.72	106.50		75.07	0.61	132.36	
VAI	MetS	4.06	0.15	403.00	<0.001*	3.95	0.18	172.35	<0.001*	4.08	0.24	231.06	<0.001*
	Control	2.23	0.06	220.84		2.38	0.10	98.10		2.13	0.07	124.36	
LAP	MetS	75.37	2.76	422.08	<0.001*	70.3	3.56	181.92	<0.001*	80.33	4.24	241.89	<0.001*
	Control	29.57	1.24	204.98		28.6	1.73	88.39		30.25	1.71	116.72	
TyG	MetS	8.84	0.03	390.52	<0.001*	8.68	0.04	166.10	<0.001*	8.99	0.04	230.05	<0.001*
	Control	8.38	0.02	231.20		8.33	0.03	104.44		8.42	0.03	125.07	
HOMA-IR	MetS	7.45	0.21	381.77	<0.001*	7.25	0.35	161.38	<0.001*	7.64	0.48	221.46	<0.001*
	Control	4.59	0.14	238.47		4.86	0.22	109.24		4.41	0.19	131.13	
HOMA-IS	MetS	0.01	0.00	225.23	<0.001*	0.01	0.00	109.63	<0.001*	0.01	0.00	115.54	<0.001*
	Control	0.02	0.00	368.53		0.01	0.00	161.76		0.01	0.00	205.87	

Data represented as mean, SEM and mean ranks. *p<0.05 was considered significant and analysis was done using the Mann–Whitney U test. Classification of MetS was done according to NCEP ATP III criteria.

N, sample size; MetS, metabolic syndrome; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; CHR, total cholesterol-to-HDL-C ratio; THR, triglycerides-to-HDL-C ratio; FBG, fasting blood glucose; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; VAI, visceral adiposity index; LAP, lipid accumulation product; TyG, the product of triglycerides and glucose; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-IS; homeostatic model assessment of insulin sensitivity; SEM, standard error of mean.

most common combination of four MetS components in both total (20.36%) and female cases (22.06%) was ↓HDL-C + AO + HTN + hyperglycemia whereas in males ↓HDL-C + AO + HTN + hyperglycemia and ↓HDL-C + AO + HTN + hypertriglyceridemia were most common with equal prevalence (18.70%). Furthermore, five component-based combination of MetS (↓HDL-C + AO + hyperglycemia + HTN + hypertriglyceridemia) was most prevalent in male cases (10.79%) followed by total (8.0%) and female cases (5.14%).

3.4 *Genotype and allele frequencies of BDNF rs6265 and MC4R rs17782313*

The comparison of genotype and allele frequencies of *BDNF* rs6265 and *MC4R* rs17782313 between MetS cases and non-MetS controls is indicated in tables 2 and 3, respectively. The genotype frequencies of *MC4R* rs17782313 and *BDNF* rs6265 for the control group were in HWE (p>0.05). The observed minor allele frequency (MAF) of the BDNF risk allele A in MetS cases and non-MetS controls was 19.6 and

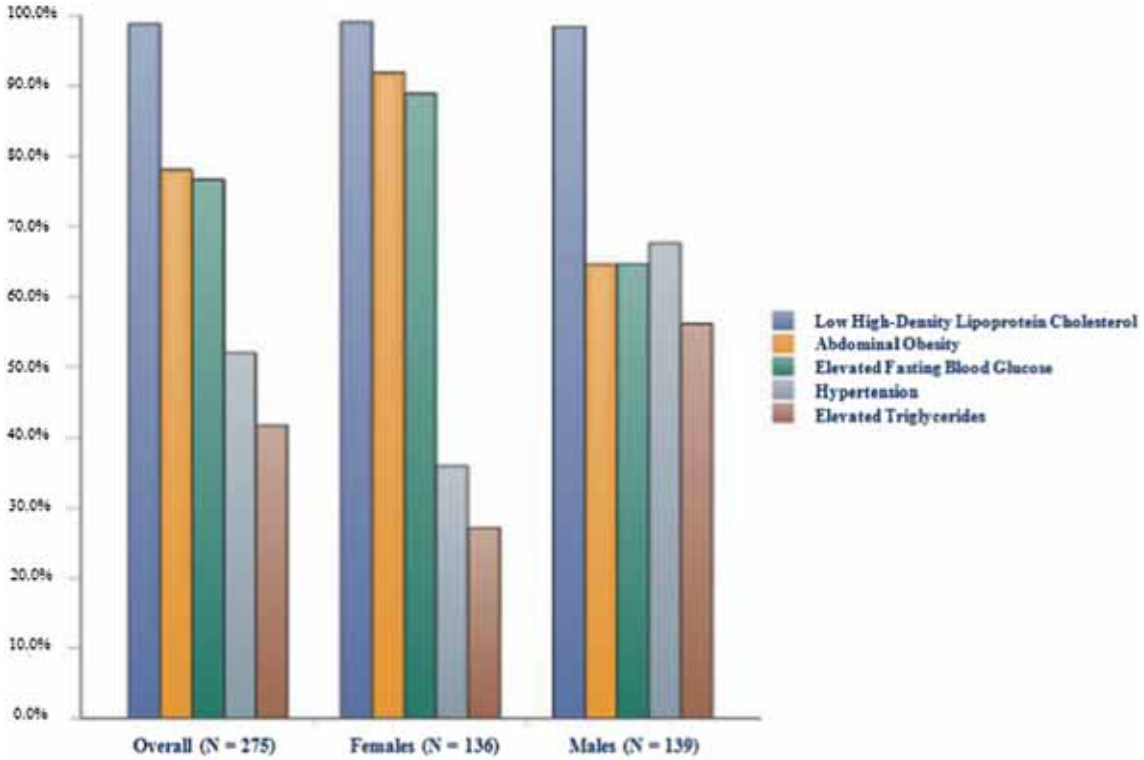

Figure 1. Order of occurrence from most prevalent to least prevalent components of MetS in cases according to NCEP ATP III criteria.

19.9%, respectively. Similarly, MAF of the *MC4R* risk allele C in cases and controls was 44.2 and 40.3%, respectively. The observed genotype frequencies for *BDNF* rs6265 in cases were 66.9% GG, 26.9% GA and 6.2% AA while in controls they were 62.8% GG, 34.4% GA and 2.7% AA. Similarly, the observed genotype frequencies for *MC4R* rs17782313 in cases were 30.9% TT, 49.8% CT and 19.3% CC while in controls these were 36.6% TT, 46.2% CT and 17.2% CC. The *BDNF* gene variant rs6265 showed unequal distribution ($\chi^2 = 7.329$, $p = 0.026$) between MetS cases and non-MetS controls, with the AA genotype occurring more frequently in MetS cases. On the other hand, genotypic frequencies of *MC4R* rs17782313 did not differ significantly between cases and controls ($\chi^2 = 2.163$, $p = 0.339$). However, after gender-based stratification, genotypic frequencies of *MC4R* rs17782313 differed significantly between female MetS cases and controls ($\chi^2 = 6.158$, $p = 0.046$), with the CT genotype occurring more frequently in MetS cases.

3.5 *Association of BDNF rs6265 and MC4R rs17782313 with MetS*

BDNF rs6265 was found to be associated with MetS according to a recessive-genetic model (table 2) and this association remained significant after adjustment with the BMI (table 4). The relative percentage change for effect estimates after adjustment with the BMI was 23.42%. The AA genotype of *BDNF* rs6265 increased ~2.8 times the risk of having MetS. On the other hand, association of *MC4R* rs17782313 with MetS was observed in females only ($p = 0.038$, OR = 1.15) according to the over-dominant model (table 3). However, this association was lost ($p_{adj} = 0.141$) after adjustment with the BMI (table 4). The relative percentage change for effect estimates in females after adjustment with the BMI was 16.3%.

3.6 *Association of BDNF rs6265 and MC4R rs17782313 with individual components of MetS*

Association of *BDNF* rs6265 with ↓HDL-C in males and *MC4R* rs17782313 with AO in females was seen (supplementary table 1). Further pair-wise analysis by logistic regression revealed that the GA genotype of *BDNF* rs6265 was protective against low HDL-C in males ($p = 0.022$, OR = 0.492 and CI = 0.268–0.903) while the CC genotype of *MC4R* rs17782313 increased the risk of AO in females ($p = 0.023$, OR = 2.33 and CI = 1.122–4.854).

3.7 *No association of BDNF rs6265 and MC4R rs17782313 with metabolic variables other than MetS components*

A lack of association ($p>0.05$) was observed for metabolic variables other than components of MetS including LDL-C,

Table 2. Comparison of genotypic and allelic frequencies of *BDNF* rs6265 variant between MetS cases and non-MetS controls

	Overall ($N = 606$)			Females ($N = 270$)			Males ($N = 336$)		
	MetS $N = 275$	Control $N = 331$	*p*-value	MetS $N = 136$	Control $N = 134$	*p*-value	MetS $N = 139$	Control $N = 197$	*p*-value
				BDNF rs6265					
HWE (χ^2/*p*-value)	5.97/0.0146*	2.05/0.1522		3.79/0.0516	0.01/0.9203		2.3/0.1294	3.08/0.0793	
				Co-dominant model					
GG	184 (66.9%)	208 (62.8%)		94 (69.1%)	91 (67.9%)		90 (64.7%)	117 (59.4%)	
GA	74 (26.9%)	114 (34.4%)	0.026[a]	34 (25.0%)	39 (29.1%)	0.425[a]	40 (28.8%)	75 (38.1%)	0.065[a]
AA	17 (6.2%)	9 (2.7%)		8 (5.9%)	4 (3%)		9 (6.5%)	5 (2.5%)	
OR (CI) GG *vs* GA	0.734 (0.515–1.045)		0.086	0.844 (0.490–1.452)		0.540	0.693 (0.432–1.112)		0.128
OR (CI) GG *vs* AA	2.135 (0.929–4.906)		0.073	1.936 (0.563–6.653)		0.294	2.340 (0.758–7.224)		0.139
				Dominant model					
AA + GA	91 (33.1%)	123 (37.2%)	0.297	42 (30.9%)	43 (32.1%)	0.831	49 (35.3%)	80 (40.6%)	0.320
GG	184 (66.9%)	208 (62.8%)		94 (69.1%)	91 (67.9%)		90 (64.7%)	117 (59.4%)	
OR (CI)	0.836 (0.598–1.170)			0.946 (0.566–1.580)			0.796 (0.508–1.248)		
				Recessive model					
AA	17 (6.2%)	9 (2.7%)	0.041*	8 (5.9%)	4 (3%)	0.257	9 (6.5%)	5 (2.5%)	0.086
GA + GG	258 (93.8%)	322 (97.3%)		128 (94.1%)	130 (97%)		130 (93.5%)	192 (97.5%)	
OR (CI)	2.357 (1.034–5.376)			2.031 (0.597–6.913)			2.658 (0.871–8.112)		
				Over-dominant model					
GA	74 (26.9%)	114 (34.4%)	0.046*	34 (25.0%)	39 (29.1%)	0.448	40 (28.8%)	75 (38.1%)	0.078
GG + AA	201 (73.1%)	217 (65.6%)		102 (75.0%)	95 (70.9%)		99 (71.2%)	122 (61.9%)	
OR (CI)	0.701 (0.494–0.994)			0.812 (0.474–1.391)			0.657 (0.412–1.048)		
				Allelic model					
G Allele	442 (80.4%)	530 (80.1%)	0.895[a]	222 (81.6%)	221 (82.5%)	0.798[a]	220 (79.1%)	309 (78.4%)	0.824[a]
A Allele	108 (19.6%)	132 (19.9%)		50 (18.4%)	47 (17.5%)		58 (20.9%)	85 (21.6%)	
h-index	− 0.468			− 0.315			− 0.490		

Genotypic and allelic frequencies are represented in counts (percentages within parentheses). The *p*-value and OR with 95% CI were calculated by logistic regression. *$p < 0.05$ was considered significant.

MetS, metabolic syndrome; HWE, Hardy–Weinberg equilibrium; *h*-index; degree of dominance index; OR, odds ratios; CI, confidence interval.

[a] Performed by Pearson's χ^2 test.

VLDL-C, TC, CHR, THR, VAI, LAP and TyG across the genotypes of *BDNF* rs6265 (supplementary table 2) and *MC4R* rs17782313 (supplementary table 3) in overall as well as in gender-stratified study population.

4. Discussion

The manifestation of MetS can be attributable to the interplay of various environmental and genetic factors (Elder *et al.* 2009); however, the full extent of its genetic components remains to be elucidated. The complete understanding of its genetic background would help to decipher the mechanistic pathways that can ultimately serve as new targets for therapeutic intervention. Variants of genes encoding proteins of the leptin–melanocortin pathway including MC4R and BDNF have been associated with obesity (van der Klaauw and Farooqi 2015). The association of these variants with alterations in the BMI and other obesity-related anthropometric measures also indicate their possible involvement in engendering the risk of MetS and other related metabolic anomalies. To our knowledge, this is the first study that examined the association of genetic variants *BDNF* rs6265 (G>A) and *MC4R* rs17782313 (T>C) with MetS in Pakistani individuals.

MetS is a group of interrelated physiological, biochemical, clinical and metabolic factors that directly enhances the risk of cardiovascular disease, type-2 diabetes mellitus and all-cause mortality (Zafar *et al.* 2018). In other words, MetS can be viewed as a concurrence of various cardio-metabolic risk factors in one individual. In addition to components of MetS namely AO, hypertriglyceridemia, hyperglycemia, HTN and reduced HDL-C, dyslipidemia involving other lipid parameters such as TC, LDL-C, VLDL-C, CHR and THR may also coexist in patients with MetS (Millan *et al.* 2009). Furthermore, surrogate markers including VAI, LAP and TyG may also be considered reliable for identifying MetS (Li *et al.* 2018). Congruently, all the aforementioned quantitative clinical variables were found significantly anomalous in MetS cases as compared to non-MetS control subjects in the current study.

Many case–control and prospective observational studies from different racial and ethnic groups worldwide have confirmed that reduced HDL-C is a strong, consistent and independent predictor of cardiovascular risk such as myocardial infarction and ischemic stroke. Thus, reduced HDL-C may serve as a key contributing factor in the

Table 3. Comparison of genotypic and allelic frequencies of *MC4R* rs17782313 variants between MetS cases and non-MetS controls

	Overall ($N = 606$)			Females ($N = 270$)			Males ($N = 336$)		
	MetS $N = 275$	Control $N = 331$	p-value	MetS $N = 136$	Control $N = 134$	p-value	MetS $N = 139$	Control $N = 197$	p-value
				MC4R rs17782313					
HWE (χ^2/p-value)	0.03/0.8625	0.52/0.4708		1.21/0.2713	1.93/0.1648		0.69/0.4062	0.04/0.8415	
				Co-dominant model					
CT	137 (49.8%)	153 (46.2%)		74 (54.4%)	56 (41.8%)		63 (45.3%)	97 (49.2%)	
CC	53 (19.3%)	57 (17.2%)	0.339[a]	26 (19.1%)	24 (17.9%)	0.046[a]	27 (19.4%)	33 (16.8%)	0.732[a]
TT	85 (30.9%)	121 (36.6%)		36 (26.5%)	54 (40.3%)		49 (35.3%)	67 (34.0%)	
OR (CI) CT *vs* TT	1.275 (0.889–1.828)		0.187	1.982 (1.148–3.423)		0.014*	0.888 (0.546–1.444)		0.632
OR (CI) TT *vs* CC	1.324 (0.831–2.109)		0.237	1.625 (0.809–3.262)		0.172	1.119 (0.597–2.096)		0.726
				Dominant model					
CT + CC	210 (63.4%)	190 (69.1%)	0.144	100 (73.5%)	80 (59.7%)	0.017*	90 (64.7%)	130 (66.0%)	0.814
TT	85 (30.9%)	121 (36.6%)		36 (26.5%)	54 (40.3%)		49 (35.3%)	67 (34.0%)	
OR (CI)	1.288 (0.917–1.809)			1.875 (1.121–3.135)			0.947 (0.600–1.494)		
				Recessive model					
CC	53 (19.3%)	57 (17.2%)	0.514	26 (19.1%)	24 (17.9%)	0.798	27 (19.4%)	33 (16.8%)	0.529
CT + TT	222 (80.7%)	274 (82.8%)		110 (80.9%)	110 (82.1%)		112 (80.6%)	164 (83.2%)	
OR (CI)	1.148 (0.759–1.736)			1.083 (0.586–2.003)			1.198 (0.683–2.103)		
				Over-dominant model					
CT	137 (49.8%)	153 (46.2%)	0.378	74 (54.4%)	56 (41.8%)	0.038*	63 (45.3%)	97 (49.2%)	0.479
CC + TT	138 (50.2%)	178 (53.8%)		62 (45.6%)	78 (58.2%)		76 (54.7%)	100 (50.8%)	
OR (CI)	1.155 (0.838–1.591)			1.662 (1.027–2.690)			0.855 (0.553–1.321)		
				Allelic model					
C Allele	243 (44.2%)	267 (40.3%)	0.176[a]	126 (46.3%)	104 (38.8%)	0.077[a]	63 (45.3%)	97 (49.2%)	0.853[a]
T Allele	307 (55.8%)	395 (59.7%)		146 (53.7%)	164 (61.2%)		76 (54.7%)	100 (50.8%)	
h-index	0.513			1.046			− 1.393		

Genotypic and allelic frequencies are represented in counts (percentages within parentheses). The p-value and OR with 95% CI were calculated by logistic regression. *$p<0.05$ was considered significant.

MetS, metabolic syndrome; HWE, Hardy–Weinberg equilibrium; h index; degree of dominance index; OR, odds ratios; CI, confidence interval.

[a] Performed by Pearson's χ^2 test.

Table 4. Association of *BDNF* rs6265 and *MC4R* rs17782313 with MetS after adjustment with BMI

	Overall ($N = 606$)			Females ($N = 270$)			Males ($N = 336$)		
	OR	95% CI	p_{adj}	OR	95% CI	p_{adj}	OR	95% CI	p_{adj}
				BDNF rs6265					
Co-dominant model									
GA *vs* GG	0.649	0.424–0.994	0.047*	0.720	0.374–1.388	0.327	0.631	0.359–1.11	0.110
AA *vs* GG	2.530	0.973–6.579	0.057	2.009	0.503–8.023	0.324	3.130	0.833–11.76	0.091
Dominant model	0.774	0.519–1.156	0.211	0.841	0.456–1.553	0.580	0.760	0.445–1.296	0.313
Recessive model	2.884	1.119–7.432	0.028*	2.192	0.555–8.654	0.263	3.647	0.982–13.53	0.053
Over-dominant model	0.614	0.403–0.936	0.023*	0.691	0.361–1.323	0.264	0.590	0.338–1.031	0.064
h-index		− 0.525			− 0.529			− 0.462	
				MC4R rs17782313					
Co-dominant model									
CT *vs* TT	1.113	0.728–1.701	0.621	1.476	0.784–2.781	0.228	0.892	0.501–1.586	0.696
CC *vs* TT	1.060	0.618–1.819	0.832	0.903	0.398–2.050	0.808	1.213	0.589–2.500	0.601
Dominant model	1.097	0.738–1.632	0.647	1.285	0.710–2.328	0.407	0.977	0.571–1.671	0.932
Recessive model	0.997	0.616–1.614	0.990	0.721	0.346–1.501	0.382	1.295	0.679–2.469	0.433
Over-dominant model	1.090	0.747–1.591	0.655	1.529	0.868–2.694	0.141	0.832	0.498–1.391	0.484
h-index		1.478			4.161			− 0.952	

Odds ratios with 95% confidence interval were calculated by logistic regression. *$p<0.05$ was considered significant and p_{adj} shows p value by adjusting BMI.

p_{adj}, adjusted p value; OR; odds ratios; CI: confidence interval; h index: degree of dominance index.

pathogenesis of MetS (Rader and Hovingh 2014). Low HDL-C also appeared as the most frequent component of MetS in the current study followed by AO, hyperglycemia, HTN and hypertriglyceridemia. Thus, hypertriglyceridemia was found to be the least frequent component of MetS in our study. In a somewhat similar manner, reduced HDL-C, central obesity and HTN most often led to the diagnosis of MetS in West Africans and African Americans with hypertriglyceridemia as the least common component (Sumner *et al.* 2010). The possible non-genetic causes for the emergence of reduced HDL-C as the most common component of MetS in our population could be excess weight, sedentary lifestyle, poor diet or smoking (in males).

The most prevalent combination of MetS components in overall and female cases was ↓HDL-C + hyperglycemia + AO while in male cases it was ↓HDL-C + hyperglycemia + HTN. In comparison, ↓HDL-C + HTN + hypertriglyceridemia and ↓HDL-C + AO + hypertriglyceridemia were the most common combinations in Thai men and women, respectively (Aekplakorn *et al.* 2011). Similarly, the most prevalent combination in overall, female and male elderly Korean population was hypertriglyceridemia + HTN + hyperglycemia (Lee *et al.* 2016). Moreover, hypertriglyceridemia + ↓HDL-C + HTN and AO + ↓HDL-C + HTN were the two most common combinations of MetS components seen in rural Brazilian population with hypertriglyceridemia + ↓HDL-C + HTN combination being most common in males and AO + ↓HDL-C + HTN being most common in females (Pimenta *et al.* 2013). Thus, most prevalent combination of MetS components differs across various populations and also between males and females within the same population.

The current study revealed the association of *BDNF* rs6265 with the risk of MetS that remained significant even after adjustment with BMI. This result indicates the direct influence of *BDNF* rs6265 on MetS that is independent of BMI. Zhao *et al.* (2014) also found association of *BDNF* rs6265 with MetS in Chinese children but it disappeared after adjustment with BMI (Zhao *et al.* 2014). On the other hand, Zeman *et al.* (2010) and Suriyaprom *et al.* (2014) did not find any significant association between *BDNF* rs6265 and risk of MetS in studies conducted on Caucasians and Thai subjects, respectively. However, BMI was not adjusted in these studies (Zeman *et al.* 2010; Suriyaprom *et al.* 2014). In addition, Zhang *et al.* (2013) observed a weak association between the *BDNF* rs6265 polymorphism and clozapine (an atypical antipsychotic drug)-induced MetS in a sex-specific manner as association was evident in male schizophrenic patients (taking clozapine) only (Zhang *et al.* 2013). A recent meta-analysis study reported that MetS can be associated with reduced levels of circulating BDNF (Kavyaa *et al.* 2015). *BDNF* rs6265 variant disturbs intracellular trafficking of pro-BDNF, and also reduces secretion and production of the mature BDNF with the A allele (Chen *et al.* 2004). Thus, it can be said that the presence of the BDNF variants such as rs6265 may lead to reduced levels of circulating BDNF that in turn may play a role in the manifestation of MetS phenotype. However, the BDNF levels were not measured in the current study. The association of *MC4R* rs17782313 with MetS in females was also observed in the current study but disappeared after adjustment with BMI. It implies that the observed association of *MC4R* rs17782313 with MetS may be BMI dependent. Since obesity may act as a master trigger to turn on the gene expression modifications obligatory for the expression of MetS (Taylor *et al.* 2013), it is possible that *MC4R* rs17782313 can influence the MetS phenotype via enhancing BMI in a gender-specific manner. Here, it is important to note that we adjusted BMI while seeking association of *MC4R* rs17782313 with MetS but we did not adjust AO as it is one of the components of the MetS phenotype. A number of other studies also reported association between variant rs17782313 and MetS but BMI was not adjusted in these studies (Heid *et al.* 2008; Dusatkova *et al.* 2013; Yang *et al.* 2013). However, Povel *et al.* in a cohort of Dutch population observed a significant association between *MC4R* rs1778213 and MetS that remained significant even after adjustment with BMI (Povel *et al.* 2012). Here, it is worth mentioning that the disparities observed among studies (including our study) for the association of *BDNF* rs6265 and *MC4R* rs17782313 with MetS may be attributable to differences in study design, sample size, age of the participants, geographic and ethnic background of the study population and consideration for potential confounding factors.

We also sought the association of *MC4R* rs17782313 and *BDNF* rs6265 with MetS individual components. Only a protective association of *BDNF* rs6265 (GA genotype) against the risk of having reduced HDL-C levels (a component of MetS) was seen in males of our study population. In contrast, Suriyaprom *et al.* in Thais and Zhang *et al.* in Han Chinese (schizophrenia patients being treated with clozapine) did not find any association of rs6265 with HDL-C levels (Zhang *et al.* 2013; Suriyaprom *et al.* 2014). Moreover, Peng *et al.* reported a significant association of A allele (GA/AA genotype) of the *BDNF* rs6265 with the reduced level of HDL-C in a long-lived population of Guangxi Province (China) (Peng *et al.* 2017). The current study also demonstrated significant association of *MC4R* rs17782313 (CC genotype) with AO in females. AO appeared as the second most prevalent component of MetS and also the part of most prevalent combination of MetS components (↓HDL-C + AO + hyperglycemia) in females of the current study. On the contrary, Brodowski *et al.* found no association of *MC4R* rs17782313 with AO in postmenopausal Polish women while IDF criteria were used for defining MetS in their study (Brodowski *et al.* 2017). However, 98.1% females of our study sample were premenopausal and not postmenopausal and we used NCEP III criteria for the definition of MetS.

The current study failed to find the association of *BDNF* rs6265 and *MC4R* rs17782313 with any metabolic variable other than components of MetS including lipid parameters

(TC, LDL-C, VLDL-C, CHR and THR) and surrogate markers for predicting MetS (VAI, LAP and TyG). Likewise, Kalenda *et al.* in Leipzig childhood cohort observed no association of *BDNF* rs6265 (Kalenda *et al.* 2018) while Rotter *et al.* in middle-aged and elderly Caucasian men found no association of *MC4R* rs17782313 (Rotter *et al.* 2016) with TC, LDL-C, HDL-C and TG. On the contrary, Brodowski *et al.* reported association of *MC4R* rs17782313 with TC, LDL-C and TG in postmenopausal Polish women (Brodowski *et al.* 2017). Moreover, *MC4R* rs17782313 was reported to be associated with hypertriglyceridemia in obese Brazilian pediatric cohort (Fernandes *et al.* 2015), older Chinese women (Yang *et al.* 2016) and general Japanese population (Katsuura-Kamano *et al.* 2014).

Since MetS is defined by a cluster of at least three or more of the five altered components, it is possible that a genetic variant that is associated with MetS as a whole (defined by sum of all the combinations of its components) may or may not show association with individual component/s or combination/s of MetS or other related metabolic trait/s (Povel *et al.* 2012). Similarly, if a genetic variant shows association with MetS individual component/s or combination/s or other related metabolic trait/s, it may or may not associate with the syndrome as a whole (Lee *et al.* 2018). Even the same allele may associate with an increased risk of having one component and decreased risk of having another component (Carty *et al.* 2014).

In conclusion, significantly aberrant metabolic parameters can be found in the presence of MetS with low HDL-C as the most frequent component of MetS and AO + hyperglycemia + ↓HDL-C as the most frequent combination of MetS components in Pakistanis. Moreover, *BDNF* rs6265 may be associated with increased risk of having MetS in Pakistani individuals independent of BMI and gender whereas *MC4R* rs17782313 may be associated in a gender and BMI dependent manner with increased risk of having MetS in Pakistani females. In addition, *BDNF* rs6265 may also be associated with decreased risk of having low HDL-C in Pakistani individuals independent of gender whereas *MC4R* rs17782313 may be associated in a gender dependent manner with abdominal obesity in Pakistani females. However, both variants *BDNF* rs6265 and *MC4R* rs17782313 may not be associated with any related metabolic variable other than MetS components. The current study is a step further in understanding the genetic background of MetS phenotype and may aid in identifying the mechanistic pathways that can eventually serve as new targets for therapeutic intervention.

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