Original Article

D2S2944 marker: A common marker for the obesity-depression associations

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Abstract : *Associations have previously been reported between a 124-bp allele at D2S2944 and recurrent, early-onset depression. These e lier reports also noted but did not emphasize a possible association between this lele and obesity-depression. The present study attempts to establish a link between the symptoms of depression, metabolic disorders and obesity, to unravel the underlying association/s. This study was carried out using a case-control design on 400 subjects comprising of 150 cases and 260 controls, matched for mean age and sex. The study tested potential D2S2944 allelic association between anthropometric mar s and depression using odds ratios and chi square test. Frequency of 124-bp genotype was found to be 0.26 in cases and 0.21 in controls. The allelic distribution for 124 bp allele at D2S2944 locus differed significantly among cases and controls 38 vs 0.30; OR=1.43, 95% CI: 1.057-1.944, p=0.02). When adjusted for the gender the allele frequency for the 124-bp allele was found to differ significantly among the male subjects (0.38 vs 0.26, OR=1.5485, 95% CI=1.0249-2.3395) while no such d rence was observed among the females (0.30 vs 0.35, OR=1.4801, 95 % CI = 0.9217- 2.3766). No significant associations were found in depressive smokers for the presence or absence of 124 bp allele but there were definitely more number of smokers homozygous for 124 bp allele (48% vs 67%). This exploratory study sets the stage for further in-depth, larger study for molecular understanding of the metabolic relationship between obesity and depression and the role of D2S2944 marker on chromosome 2.*

Keywords: *Depression, Obesity, D2S2944, 124bp allele, anthropome markers Chromosome 2*

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INTRODUCTION

Epidemiologic data suggest an association between obesity and depression however findings vary considerably across different studies. The high prevalence of depression (10%) and overweight (65%), worldwide, indicates that there is a high probability that these will co-occur.[1] Though it has not been established that the main cause of depression is obesity likewise the sole contributing factor of obesity is depression is yet to be proven. Obesity may cause depression, for example through the negative body image which is a squeal to obesity.[2] But it is also possible that depression may cause obesity, most likely through changing eating patterns or reduced physical activity or both.[3-5] Depressed people are more likely to become obese because of physiological changes in their neuroendocrine and immune systems that occur during depression. Moreover, the symptoms and consequences of

depression, such as difficulty in adhering to fitness regimens, overeating and negative thoughts make it difficult for depressed individuals to take good care of themselves.[6] Depressive symptoms can exert influence on the genotype–obesity association through both unique genetic and environmental pathways.[7] Before causal pathways can be explored further, it is necessary to establish the exact mode in which depression and obesity are associated with each other. Since depressive symptoms have been linked with obesity and disregulation in eating (e.g. both hyperphasia or appetite loss),[1,8] the genetic loci which are associated with both disorders can serve as a useful marker.

The Chromosome 2 (2q34-q37) has been found to be linked with obesity. [9] Li et al ,[10] studied obese subjects and found significant linkage on the 2q34 region for total cholesterol (TC) and suggestive linkage on the marker D2S2944 (210.4 cM). At the same time linkage and association of the D2S2944 tetranucleotide repeat region with major depression has been reported by Zubenko et al, (2002b, c) and Philbert et al.[11-13] Using logistic regression to analyze adjusted and interactive D2S2944 associations with depression, controlling for all other risk factors Langbehn et al, (2006),[14] reported a strong association with DSM-IV major depression and the 124 bp allele, specifically in those with histories of alcohol abuse/dependence and/or antisocial personality disorder (ASPD). Beem et al, [15] tested for association of 124 bp allele to continuous measures of anxiety, depression and neuroticism and found significant associations with anxiety and anxious depression in males only. Therefore, it can be envisaged that 124 bp allele may impact obesity indices in dependent subjects.

Both obesity and depression are risk factors for other chronic diseases such as coronary heart disease;[16] thus, it is an important public health endeavor to understand the depression–obesity link. Most studies till date have focused on obesity causing depression and not the reverse.[8] The purpose of this study is to examine whether subjects with depression are genetically predisposed to obesity as measured by anthropometric markers. Findings of present study will help understanding the population specific effects of this marker and its relation to depression obesity epidemic.

MATERIALS AND METHODS

Blood samples were collected from 150 depressed individuals visiting the Psychiatry unit of BDK Hospital, Jhunjhunu after obtaining an informed consent from them or their guardians (in cases of severe depression). Control group comprised of 260 unrelated healthy volunteers without any history of psychiatric disorders. Depression was diagnosed by an experienced psychiatrist using the ICD-10 criteria.[17] The study was conducted within the norms of Declaration of Helsinki for human experimentation and with prior approval by the Institutional Human Ethics Committee at BITS, Pilani. All participants completed a structured questionnaire about their socio-demographic background, personal history of disease and other lifestyle characteristics. The anthropometric markers like height, weight, hip and waist circumference were recorded for each subject. Genomic DNA was isolated from peripheral white blood cells by the method described elsewhere.[18] All DNA samples were amplified as described by Zubenko et al[12] using Polymerase Chain Reaction (PCR). Amplified PCR products were separated on a 12% non-denaturing polyacrylamide gel. Two independent observers assigned the genotypes and unambiguous genotypes could be assigned to 143 cases and 252 controls only. X^2 test and Odds Ratios (OR) with 95% Confidence Interval (CI) were used to test differences between the cases and controls. Student's t test was applied

for test of significance between means. Tests of statistical significance were two sided. All statistics were performed using SPSS v 16.0 (SPSS Inc, Chicago, III).

RESULTS

The present study was carried out using a case-control design on 400 subjects comprising of 150 cases and 260 controls, matched for mean age and sex. The socio-demographic characteristics of the 150 patients and 252 controls are presented in Table 1. The cases and controls did not differ significantly for any of the studied anthropometric or clinical marker (p>0.05). The genotypic and allelic distribution at D2S2944 locus among cases (143) and controls (252) is given in Table 2. Frequency of 124-bp genotype was found to be 0.26 in cases and 0.21 in controls. The allelic distribution for 124 bp allele at D2S2944 locus differed significantly among cases and controls (0.38 vs 0.30; OR=1.43, 95% CI: 1.057-1.944, p=0.02). When adjusted for the gender the allele frequency for the 124-bp allele was found to differ significantly among the male subjects (0.38 vs 0.26, OR=1.5485, 95% CI=1.0249-2.3395) while no such difference was observed among the females (0.30 vs 0.35, OR=1.4801, 95 % CI = 0.9217- 2.3766) (Table 3).

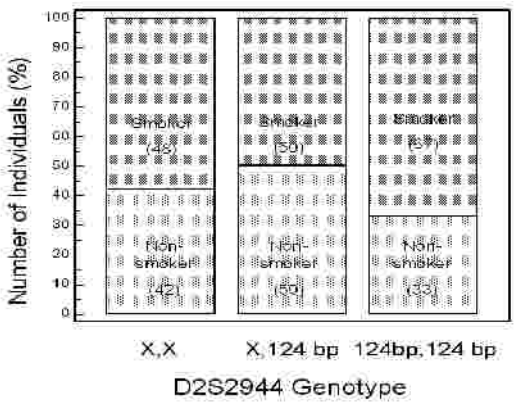

Fig 1: Association of 124 bp allele with nicotine dependence in depressive subjects

In light of above analysis, we further investigated whether; the 124-bp allele could trigger the risk for depression under influence of

Table 1

Demographic profile of males and females among cases and controls

	Cases				Controls			
	Females			Males		Females		Males
	N	Mean±SD	N	Mean±SD	N	Mean±SD	N	Mean±SD
Age (in yr)	84	38.77±13.80	126	37.63±14.64	113	43.36+18.02	120	43.68+19.51
BMI	84	23.27±5.20	120	22.24±4.90	96	23.43+6.00	108	21.24+4.85
WHR	53	0.90+0.07	113	0.92+0.06	96	0.88+0.08	106	0.94+0.13
Urea (mg/dl)	84	17.58±6.66	120	18.97±8.24	37	29.17+9.59	27	36.93+16.30
Creatinine (mg/dl)	84	0.70±0.57	120	0.79±0.45	37	0.65+0.14	24	0.69+0.12
SBP (mm/Hg)	84	117.9±15.00	126	123.7±11.28	103	122.8+14.09	114	125.7+18.08
DBP (mm/Hg)	84	78.6±10.41	126	81.8± 7.14	103	80.5+8.75	114	81.7+10.55

Table 2

Genotypic and allelic distribution at D2S2944 locus among cases and controls

		Genotype			Allele	
	N	X,X	X, 124-bp	124-bp, 124-bp	X	124-bp
cases	143	70 (0.49)	36 (0.25)	37 (0.26)	176 (0.62)	110 (0.38)
Controls	252	151 (0.60)	49 (0.19)	52 (0.21)	351 (0.70)	153 (0.30)

Chi-square = 5.038, p = 0.0248, Odds Ratio = 1.4338, 95 % CI = 1.0573-1.9445

Table 3

Genotypic and allelic distribution at D2S2944 locus am male and female cases and controls

		Genotype			Allele	
	N	X,X	X, 124-bp	124-bp, 124-bp	X	124-bp
Males (Cases)	93	51 (0.55)	18 (0.19)	24 (0.26)	120 (0.62)	66 (0.38)
Males (Controls)	124	80 (0.64)	23 (0.19)	21 (0.17)	183 (0.74)	65 (0.26)
Females (Cases)	50	19 (0.38)	18 (0.36)	13 (0.26)	56 (0.70)	44(0.30)
Females (Controls)	121	68 (0.56)	26 (0.22)	27 (0.22)	162 (0.65)	86 (0.35)

Odds Ratio = 1.5485, 95 % CI = 1.0249 to 2.3395 (Males for 124 bp allele)

Odds Ratio = 1.4801, 95 % CI = 0.9217- 2.3766 (Females for 124 bp allele)

environmental factors such as alcohol dependence. Indeed the homozygous 124 bp depressive males were found to be more prone to smoking (67%) as compared to the subjects without 124 bp allele (48%) (Fig 1). When analyzed for anthropometric markers the presence of a single or double copy of 124 bp allele doubled the incidence of being obese (Body Mass Index or BMI >30) in the cases (Fig 2a). About 14% depressive subjects with single or double copy of 124 bp allele were obese (>30 BMI) as compared to the 7% depressive subjects without presence of 124 bp allele (OR = 1.6233, 95 % CI = 1.0323 - 2.5526, X^2=3.954, p = 0.04). Out of the markers for obesity a strong association was observed with abdominal obesity as reflected by increased Waist-Hip Ratio or WHR (X^2 = 4.891, p = 0.02, OR= 3.0667, 95 % CI = 1.2299-7.6463) (Fig 2b). The study was able to depict the obesity depression associations with power of >80% with the given sample size and relative risk of 3.

DISCUSSION

The present study assessed the relationship between genotype and markers for obesity (BMI, WHR) in depressive subjects and controls. The 124 bp variety was the allele of major interest. Numerous previous reports provide evidence for association of the 124 bp allele with depression and associated co-morbidities.[12-14] Our data reiterates this strong association of D2S2944 124-bp allele with depression. Langbehn *et al*, [14] lend support to the apparent association between 124bp allele of the D2S2944 and a subset of depressive disorders but they were unable to confirm the earlier observations of a female specific risk.[12, 13] Our results indicated some

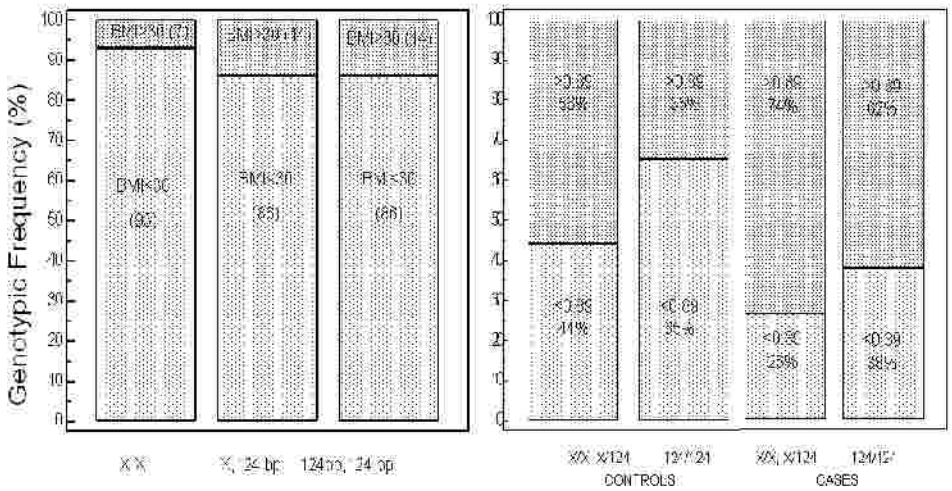

Fig 2a, b: Association of 124 bp allele with markers related to obesity and metabolic disorders in depressive subjects; BMI among cases: X^2= 3.954, p = 0.04, OR = 1.6233, 95 % CI = 1.0323 to 2.5526; WHR among cases and controls (>0.89 and presence of 124 bp genotype) X^2 = 4.891, p = 0.02, OR= 3.0667, 95 % CI = 1.2299-7.6463

interesting potential gender effects. Our findings are in line with the findings of Beem *et al*,[15] who found an association of this allele with anxiety in male patients only. Our dataset also reveals an association of 124 bp allele with depression in males, however, no significant association was observed in females (Fig 2a, 2b, Table 3).

The association between 124 bp allele and depression becomes even stronger after the adjustment for BMI and WHR, which indicates a probable interaction between D2S2944 marker and development of obesity with or without subsequent sequel of depression. It has already been shown that in lean men WHR tends to be associated with a high level of stress [19]. Present study also shows that the homozygous 124 bp allele cases with WHR >0.89 are at 3 times more risk for depression than the homozygous 124 bp allele controls with >0.89 WHR. These results are of much importance in the light of fact that depression is associated with obesity but there is lack of information about the common link between the two. Iwasaki et al [20] carried out whole genome scan and found linkage with type 2 diabetes and BMI near D2S2944 marker (LOD = 1.45) in an analysis of 164 Japanese families. Li *et al*,[10] provided evidence for linkage between obesity and D2S2944 on the 2q34 region for total cholesterol (TC) and LDL. This further strengthens our observation that this marker might be the common link between depression and obesity.

The COGA alcoholism genetics study reported region of maximum linkage to combined alcohol-depression phenotype [21] to lie at 248cM on chromosome 2. However, our data for D2S2944 showed no association with use of alcohol in depressed patients. Strine et al. [22] showed that, adults with current depression or a lifetime diagnosis of depression or anxiety more likely smoke, get obese, or be physically inactive. Phillibert *et al* [13] have suggested that sequence variations which increases the susceptibility to depression and possibly substance use disorder is in close proximity to D2S2944. The presence

of higher number of smokers within the 124 bp genotype group does indicate that there might be possible association between nicotine dependence and D2S2944 marker. In India social taboo restricts smoking in females; therefore this observation is limited to males only.

There are many significant strengths and limitations in the present study. Strengths include the inclusion of BMI, WHR and clinical markers for both cases and controls and sufficient power of study to detect the underlying associations. The weakness is the lack of other markers for obesity such as skinfold and body fat measurements. In spite of this limitation we were able to uncover a hitherto unreported link between depression and obesity. A detailed analysis of this loci is warranted and could unravel specific genetic link/mediations

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REFERENCES

1. Faith MS, Matz PE, Jorge MA. Obesity-depression associations in the population. *J Psychosom Res* 2002; 53:935-42.
2. Roberts RE, Deleger S, Strawbridge WJ, Kaplan GA. Prospective association between obesity and depression: evidence from the Alameda county study. *Int J Obes* 2003, 27:514-521.
3. Goodman E, Whitaker RC. A prospective study of the role of depression in the development and persistence of adolescent obesity. *Pediatrics* 2002; 110:497-504.
4. Richardson LP, Davis R, Poulton R, McCauley E, Moffitt TE, Caspi A. A longitudinal evaluation of adolescent depression and adult obesity.*Arch Pediatr Adolesc Med* 2003; 157:739-745.
5. Hasler G, Pine DS, Kleinbaum DG, Gamma A, Luckenbaugh D, Ajdacic V, et.al. Depressive symptoms during childhood and adult obesity. *Mol Psychiatry* 2005; 10:842-850.
6. Markowitz S FMA, Arent S M. Understanding the Relation between Obesity and Depression: Causal Mechanisms and Implications for Treatment. *Clinical Psychology Science and Practice* 2008; 15 :1-20.
7. Fuemmeler BF, Agurs-Collins T, McClernon FJ, Kollins SH, Garrett ME, Ashley-Koch AE. Interactions between genotype and depressive symptoms on obesity. *Behav Genet* 2009; 39 :296-305.
8. Stunkard AJ, Faith MS, Allison KC. Depression and obesity. *Biol Psychiatry* 2003; 54 : 330-7.
9. Damcott CM, Sack P, Shuldiner AR. The genetics of obesity. *Endocrinol Metab Clin North Am* 2003; 32 :761-86.
10. Li WD, Yuan G, Price RA. Family Based Linkage Disequilibrium Mapping On Human Chromosome Region 2q34 For Total Cholesterol In An Obesity Cohort. Genetics of metabolic Risk for Cardiovascular Disease.*Circulation* 2007; 116:II-508
11. Zubenko GS, Hughes III HB, Stiffler JS, Zubenko WN, Kaplan BB. D2S2944 identifies a likely susceptibility locus for recurrent, early-onset, major depression in women. *Mol Psychiatry* 2002; 7 : 460-467.
12. Zubenko GS, Hughes HB, Stiffler JS, Zubenko WN, Kaplan BB. Genome survey for susceptibility loci for recurrent, early-onset major depression: results at 10cM resolution. *Am J Med Genet* 2002; 114 : 413-422.
13. Philibert R, Caspers K, Langbehn D, Troughton EP, Yucuis R, Sandhu HK et al. The association of the D2S2944 124 bp allele with recurrent early onset major depressive disorder in women. *Am J Med Genet B Neuropsychiatr Genet* 2003; 121B :39-43.
14. Langbehn DR, Philibert R, Caspers KM, Yucuis R, Cadoret RJ. Association of a D2S2944 allele with depression specifically among those with substance abuse or antisocial personality. *Drug Alcohol Depend* 2006; 83 :33-41.
15. Beem AL, Geus EJ, Hottenga JJ, Sullivan PF, Willemsen G, Slagboom PE, et. al. Combined linkage and association analyses of the 124-bp allele of marker D2S2944 with anxiety, depression, neuroticism and major depression. *Behav Genet* 2006; 36 : 127-136.
16. Herva A, Laitinen J, Miettunen J, Veijola J, Karvonen JT, Läksy K, et.al. Obesity and depression: results from the longitudinal Northern Finland 1966 Birth Cohort Study. *Int J Obes* 2006; 30 :520-527.

17. Linden M. ICD-10 primary health care. WHO support for family practice psychotherapy. *MMW Fortschr Med.* 2002; 144 :26-28.
18. Hammond JB, Spanswick G, Mawn JA. Extraction of DNA from preserved animal specimens for use in randomly amplified polymorphic DNA analysis. *Anal Biochem* 1996; 240 :298-300.
19. Räikkönen K, Hautanen A, Keltikangas-Järvinen L. Association of stress and depression with regional fat distribution in healthy middle-aged men. *J Behav Med* 1994; 17 :605-616.
20. Iwasaki N, Cox NJ, Wang YQ, Schwarz PE, Bell GI, Honda M, et.al. Mapping genes influencing type 2 diabetes risk and BMI in Japanese subjects. *Diabetes* 2003; 52 :209-213.
21. Nurnberger JI Jr, Foroud T, Flury L, Su J, Meyer ET, Hu K, et.al. Evidence for a locus on chromosome 1 that influences vulnerability to alcoholism and affective disorder. *Am J Psychiatry* 2001; 158 :718-24.
22. Strine TW, Mokdad AH, Dube SR, Balluz LS, Gonzalez O, Berry JT, et. al. The association of depression and anxiety with obesity and unhealthy behaviors among community-dwelling US adults. *Gen Hosp Psychiatry* 2008; 30 :127-137.

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