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Leptin Gene Polymorphism in Under, Normal, Over Weight and Obese Depressed Patients

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ABSTRACT

Background: Initially identified as anti-obesity gene, leptin gene is believed to play a role in mood control. The current study was planned to monitor leptin gene polymorphism in different BMI groups of male and female depressed patients. **Methods:** Diagnosed depressed patients were divided into groups according to their BMI and severity of depression. Their leptin gene was sequenced to learn the relationship of leptin gene polymorphism with BMI and severity in depression. **Results:** Leptin gene polymorphisms rs36219260, rs17151914 and rs17151922 were found to be related with BMI and depression. **Conclusion:** Further studies are required to learn the relationship of these SNPs with the hormonal levels and the frequency of these SNPs in undepressed population.

Keywords: Leptin gene, polymorphism, obese, underweight, depression

INTRODUCTION

Leptin hormone targets different organs including CNS and hence regulates different mechanisms of the body; majorly body weight, eating habits and reproduction.[1] It is major regulator of metabolism.[2] As leptin is secreted from white adipose tissues, more the percentage of white adipose tissues in body, higher are the leptin levels leading to leptin resistance.[3] Gene for leptin protein is positioned at 7q31.3 chromosome location and encrypts 3.5-kb cDNA.[4] Leptin and its receptor genes influence heritability of BMI (body mass index) and differences that exist among individuals.[5] Mutated product of the gene is major cause of recessively inherited obesity of mice.[6] A study in obese girls demonstrated that polymorphism in leptin gene at -2549bp locus is associated to leptin secreted and variable adiposity in various individuals.[7] It is not obvious that all leptin polymorphic loci are within leptin gene, however polymorphism is also observed in promoter regions.[7] A polymorphism at locus C-188A was found in promoter region of leptin gene that was firstly recognized in obesity patients.[8] These polymorphisms may have various effects in obesity patients as described above and may also contribute to various other diseases. Various single nucleotide polymorphisms in leptin gene are discovered till now that are associated to obesity and several other diseases. In 1996, Comings and his team suggested that not only obesity, but behavioural disorders may also be associated with variations in leptin gene.[9] A study on leptin gene polymorphism shows presence of a SNP in 5' flanking region of exon number 2 in women with either no or minimal stress.[10]

Initially identified as an anti-obesity hormone, leptin has been shown to have important role in behaviour and responses to stress. Preclinical studies show that injected leptin produces antidepressant like effect.[11] While, there is inadequate and controversial information about the role of leptin signalling in human depression. Deuschle et al, 1996 reported that there is no difference in circulating leptin levels in depressed patients and undepressed controls.[12] On the other hand, other data show that circulating leptin levels are higher in depressed patients.[13,14] On the contrary, lower levels of circulating leptin have also been reported in depression.[15-17]

Several studies have proposed that leptin gene variations are associated with the pathophysiology obesity in humans.[18, 19] c-2548 G>A, a common SNP in the promoter region of leptin gene, is linked to the alterations in circulating leptin and BMI in obesity.[7,8] In view of leptin having an important role in weight gain and mood control, the current study is therefore, planned to monitor leptin gene polymorphism in different BMI groups of male and female depressed patients.

MATERIALS AND METHODS

Patients visiting OPD of Karachi Psychiatric hospital were selected for study after informed written consent. 95 depressed patients [underweight (n=10), normal weight (n=40), overweight (n=27) and obese (n=18)] consented for the study during January - March 2016. Informed written consent was obtained from all individual participants included in the study. Blood samples were collected in ACD vaccutainers for the DNA sequencing studies. DNA extraction was

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done via salting out method. [20] The DNA extract was purified and used to isolate leptin gene by PCR. The optical densities (OD) of the samples were taken by spectrophotometer at 260nm and 280nm. OD in a range of 1.7-2.0 considered as best ratio, Ratio>2.0 salt contamination; Ratio<1.7 protein contamination. The 50ng/µl dilutions of stock DNA were further used in the study. The purified DNA product (leptin gene product) was submitted to Institute of Biomedical and Genetic Engineering, Islamabad for sequencing. The sequencing was done by using Sanger Sequencer. A part of the promoter region, exon 2 and exon 3 were sequenced to study the polymorphisms in coding part of the gene. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethical committee (Independent Ethical Committee, ICCBS, University of Karachi + ICCBS-001-BC-2014/Protocol/1.0) and with the Helsinki declaration of 1975, as revised in 2000 (5).

DNA sequence of leptin gene was retrieved from Ensembl (www.ensembl.org) and primers were designed by using online primer3 software (http://simgene.com/primer3) and synthesized from Invitrogen (Carlsbad CA, USA). The primers used were GCTGGTCTCGAAATCCTGA (Forward1) and CATACTGTGGGTCTCCATCCT (Reverse1), AGCCAGAGCAGAAAGCAAAG (Forward 2) and AGAAAGAGTGGAGCCCTGTG (Reverse 2), CTGGGTGCAGGATACAAG (Forward 3) and CCAGAGTTCCTTCCCTTAAC (Reverse 3), GCTTCAGGCTACTCCACAGAG (Forward 4) and CTATGGGATTGGAAACTGCAC (Reverse 4), GGAAGGTTTGGTGTGTGGAG (Forward 5) and CGGAATCTCGCTCTGTCATC (Reverse 5).

The PCR was carried out in thermal cycler of Bio-Rad Laboratories Inc. California, USA, Model T-100. For PCR 20µl (De-ionized water 11.6ul, PCR buffer 10X 2ul, MgCl2 25mM 1.2 ul, dNTPs 25uM 1ul, forward primer 10mM 1ul, Reverse primer 10mM 1ul, Taq DNA polymerase 5U/ µl 0.2ul, DNA dilutions of 50ng/µl 2ul) sample of the total reaction volume was used. Confirmation of the PCR product was performed using agarose gel electrophoresis using 2.5% agarose (containing ethidium bromide) and visualized under UV.

Statistical Analysis

The sequenced electropherograms were analyzed by using BioEdit and Biostar software to find polymorphisms. The results are represented in frequencies of polymorphisms in various groups.

RESULTS

Three different polymorphisms rs36219260, rs17151914 and rs17151922 were found in the leptin gene [Table 1]. The table shows the frequency of rs36219260, rs17151914 and rs17151922 in depressed patients which was found to be 8.42%, 5.26% and 4.21%, respectively.

[Table 2] shows the detailed genotyping and allelic frequencies of SNP rs17151914 in leptin gene. 94.2% (n=82) were homozygous for CC, 2.2 % (n=2) were heterozygous for CT and 3.4% (n=3) were homozygous for TT. The frequency of the major allele and minor was 95.3% and 4.5%, respectively.

[Table 3] shows the detailed genotyping and allelic frequencies of SNP rs17151922 in leptin gene. 95.4% (n=83) were homozygous for GG and 4.6% (n=4) were homozygous for TT. There was no heterozygous minor allele. The frequency of the major allele and minor was 95.4% and 4.6%, respectively.

[Table 4] shows the frequencies of rs36219260, rs17151914 and rs17151922 at Global, South Asian and Pakistani/Japanese population already reported via 1000Genome project compared to the current study in depressed patients (Ensemble.org). There is no previous population genetic data of rs36219260 whereas in current study it is found to be 9.19%. The global, SAS and PJL frequency of minor allele for rs17151914 already reported is 4%, 4% and 2% respectively. The minor allele frequency of rs17151914 was 4.5% in depressed patients. The global, SAS and PJL frequency of minor allele for rs17151922 already reported is10%, 4% and 2%. The minor allele frequency of rs17151922 was 4.6% in depressed patients.

Table 1: Frequency of SNPs found in depressed patients.

SNP ID	Change	Position	Frequency	
rs36219260	Deletion CAAAACAAAA/-	7:128240410-128240419	8.42%	upstream gene variant
rs17151914	C/T	7:128254354	5.26%	intron variant
rs17151922	G/T	7:128255163	4.21%	3 prime UTR variant

Table 2: Detail of genotyping of SNP rs17151914 in leptin gene.

SNP ID		Genotype	Patients	Frequency	Allele Frequency	
rs17151914	Homo for major	CC	82	94.2%	C	95.3%
	Hetro for minor	CT	2	2.2%	T	4.5%
	Homo for minor	TT	3	3.4%		

Table 3: Detail of genotyping of SNP rs17151922 in leptin gene.

SNP ID		Genotype	Patients	Frequency	Allele Frequency	
rs17151922	Homo for major	GG	83	95.4%	C	95.4%
	Hetro for minor	GT	00	00%	T	4.6%
	Homo for minor	TT	4	4.6%		

Table 4: Frequency at Global, South Asian (SAS) and Pakistani/Japanese (PJL) population already reported via 1000Genome project compared to the current study in depressed patients.

SNP ID	GLOBAL	SAS	PJL	CURRENT STUDY
rs36219260	NA	NA	NA	8.42%
rs17151914	C: 96% T: 4%	C: 96% T: 4%	C: 98% T: 2%	C: 95.3% T: 4.5%
rs17151922	G: 90% T: 10%	G: 96% T: 4%	G: 98% T:2%	G: 95.4% T: 4.6%

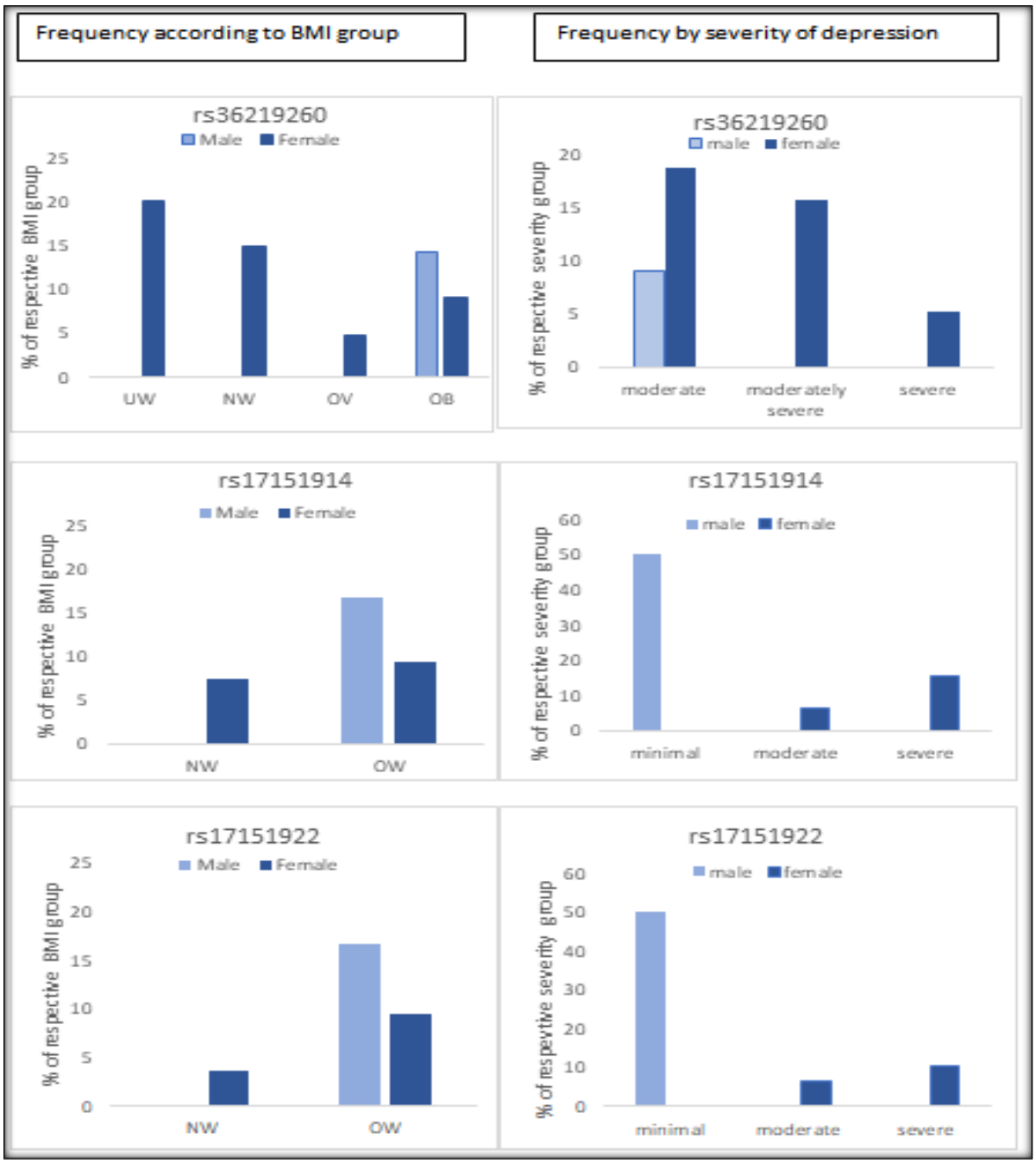

Figure 1: Frequency of rs36219260, rs17151914 and rs17151922 according to BMI groups and severity of depression. Where UW, NW, OW and Ob are underweight, normal weight, overweight and obese, respectively.

[Figure 1] shows the frequency of rs36219260, rs17151914 and rs17151922 according to BMI groups and severity of depression. The highest percentage of rs36219260 was found in underweight females (20%) followed by 14.8% in normal weight, 9.1% in obese and 4.7% in overweight. The rs36219260 was only found in 14.2% in obese males. Results show that rs36219260 is more related to underweight depression in females and obese depression in males.

Interestingly, rs17151914 and rs17151922 were found to be co-expressed. In females these SNPs were

more frequent in overweight (9.5%) followed by normal weight (7.4% of rs17151914; 3.7% of rs17151922) depressed females. In males, both these SNPs were only found in overweight patients with a frequency of 16.67%. Results suggest that rs17151914 and rs17151922 are related to overweight depression in both males and females.

Results on levels of severity in depressed shows that in the females rs36219260 was found to be more frequent in moderate depression (18.75%) followed by moderately severe (15.78%) and severe (5.2%) depression. While, in the males, rs36219620 was only found in (9.1%) in moderate depression. Suggesting that rs36219260 is related to moderate depression in both males and females.

rs17151914 and rs17151922 were most frequent in obese females (rs17151914 15.7%; rs17151922 10.5%) followed by moderate depression (rs17151914 6.25%; rs17151922 6.25%). In the males, however, these SNPS were found only in the patients with minimal depression (50% in both). The results show that rs17151914 and rs17151922 are related to obese depression in females and minimal depression in males.

DISCUSSION

Many SNPs have been reported in the leptin gene, while a few have been studied in relation with obesity. In leptin gene, rs7799039 and rs2167270 have been frequently studied for associations with obesity.[21] 5´- and 3´-UTRs region variants have also been linked with obesity.[22,23] An association of leptin gene rs10954174 and rs6966536 were found to be associated with BMI.[24] While, in the depressed patients, rs4731429 and rs3828942 in leptin gene has previously been shown to be related with BMI.[25] However, we report that rs36219260 of leptin gene is associated with obesity while rs17151914 and rs17151922 are associated with overweight in depressed males [Figure 1]. On the other hand, in the females rs36219260 is associated with underweight and rs17151914 and rs17151922 are associated with overweight in depressed females [Figure 1]. rs36219260 and rs17151914 have not been reported to be associated with obesity or BMI previously. The relationship of rs17151922 with BMI in 13 years old adolescent females was studied but the results did not show any relationship.[24] In another study rs17151922 was found to have a positive correlation to breast cancer.[26]

An important finding of our study is rs36219260, rs17151914 and rs17151922 are related to depression in our part of the world [Table 1]. These SNPs were not previously reported in depression although data shows the presence of rs17151914 (2%) and rs17151922 (2%) in general population of Pakistan while, rs17151914 is present in 4% of overall world population and rs17151922 is present in 10% of world population. The data of rs36219260 in population of Pakistan and the world is not available. The current study reports that rs36219260 is present in 8.42% of depressed people, rs17151914 and rs17151922 are present in 5.26% and 4.21% of depressed population, respectively.

The finding that rs36219260 was associated with moderate depression in both males and females suggest its role in pathophysiology of depression. rs17151914 and rs17151922 on the other hand, show a gender dimorphism in our study [Figure 1] To the best of our knowledge, the relationship of SNPs with severity of depression has not been previously reported. Although, pharmacogenetics of leptin gene in depression have been reported previously. An association of rs10487506 of leptin gene was however associated with response to tricyclic antidepressants.[27] A more recent study it was reported that rs4731426, rs12706832, rs2278815, rs10487505, rs4731429, rs10487506, rs2167270, rs11763517, rs11761556, rs3828942 and rs4731423 and had significant association with treatment responses in depression.[25]

CONCLUSION

Leptin gene polymorphisms rs36219260 is related to obese moderate depression in males and underweight moderate depression in females. rs17151914 and rs17151922 was found to be related to overweight minimal depression in males and overweight severe depression in females. Future studies are vital to learn the relationship of these SNPs with the hormonal levels and the frequency of these SNPs in undepressed population.

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