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Association of LEPR Gene Polymorphism in Type 2 Diabetic Patients in North India Population

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Abstract
Leptin and Leptin receptor gene have their direct role in controlling the food intake and thus in developing obesity. The prime objective of this study was to evaluate the association of three common SNPs (*rs1137100, rs1137101 and rs8179183*) in LEPR gene with T2DM.Blood samples from diabetic patient and normal peoples were collected and their biological parameters as Total cholesterol, trigycerides, LDL, HDL and Hba1c were analyzed. The sorted samples were then took for DNA isolation from the blood followed by amplifying Lep gene -human leptin gene (for product 242 bp) by the used of primers. Restriction analyses of the PCR products were performed with restriction enzyme. The statistical analysis thus conclude the odd ratio of all SNPs (SNP 1- rs1137100 (O.R.- 2.64); SNP 2- rs1137101 (O.R.- 3) and SNP 3- rs8179183 (O.R. – 1.5)) which signifies that there are correlation between these polymorphism and the disease of only nominal or borderline.

Keywords
LEPR gene, polymorphism, PCR-RFLP, SNPs

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INTRODUCTION:
Leptin, secreted by Adipose tissue, is a peptide hormone. Its role is to act as intermediary in satiety sensation. It also plays role in regulating lipid homeostasis, metabolism of energy and regulating insulin secretion in our body. Insulin secretion regulation is achieved by two varied processes. In the first mechanism, leptin simultaneously inhibits parasympathetic nervous system and excites sympathetic system, which is resultant of NPY gene (neuropeptide Y gene) expression. Thus, this process ultimately lowers secretion of insulin. It also regulates secretion of insulin by binding to the receptors on pancreatic beta cells (Kieffer *et al*., 1997; Emilsson *et al*., 1997).

Some of the major medical conditions caused due to obesity are Type 2 diabetes, cardiovascular disease, cancer, stroke, psychiatric illness and premature death (Nguyen and El-Serag, 2010). Cause of obesity is build-up in fat in body, thus causing a positive balance between catabolism of fat and total energy intake (Bray, 2006).

Leptin hormone plays integral part in weight gain and thus causing obesity. It is secreted by white adipose tissue, causing regulation of food intake, energy homeostasis and regulation of body temperature. The receptor for leptin, named LEPR (Leptin

receptor) located in hypothalamus does these functions (Considine, 2005; de Luis *et al*., 2009; Mantzoros *et al*., 2011). Malfunctioning of this receptor causes loss in regulation of appetite causing state of Leptin resistance leading to obesity related phenotypes. Some of the reasons of leptin resistance are defect in transportation of leptin across BB barrier and LEPR signaling pathways (Wauman and Tavernier, 2011).

Single nucleotide polymorphisms in Leptin (LEP) and Leptin receptor (LEPR) have been under study to explore their association with Type 2 Diabetes Mellitus, Obesity and in populations of different regions. Studies have suggested that *LEP* rs7799039 (-2548G>A) and rs2167270 (19A>G) SNPs are associated with either variation in increased leptinemia and/or obesity susceptibility in several populations (Yiannakouris *et al*., 2003; Jiang *et al*., 2004; Hinuy *et al*., 2010; Yu *et al*., 2012). Thus, these studies have suggested that *LEPR* polymorphisms may be linked to the onset and occurrence of obesity and Type 2 diabetes mellitus. Two *LEPR* polymorphisms, rs3806318 and rs1327118, are both located in near gene 5′ of *LEPR*. Various studies have showed that the two polymorphisms in *LEPR* may participate in the progression of cancer and inflammatory response (Chen *et al*., 2012; Teras *et al*., 2009; Zhang *et al*., 2007; Zhang *et al*., 2012).

The aim of this study is to investigate the association of LEPR gene polymorphism with Type II diabetes mellitus in population of North India. In this study, three SNPs were targeted rs1137100 (A>G), rs1137101 (A>G) and rs8179183 (G>C / G>T) were targeted and studied among patients from Kanpur.

MATERIAL AND METHODOLOGY

Subjects

We performed a case-control study which included of 50 samples of T2DM patients and 42 non-diabetic healthy subjects from Rama Medical College Hospital & Research Centre, Kanpur (U.P.). The samples consisted of 50 T2DM patient (38 females and 12 males) samples and 42 (31 females and 11 males) gender-matched healthy volunteers. All the subjects (≥35 years) who were subjected to routine physical examination were considered for our study. The subjects or their relatives were made to sign the consent form and also questionnaires were filled by them, it had a complete medical history and information about the subject.

Sample collection and DNA extraction

Peripheral Blood samples were collected freshly from subjects in early morning and stored in tubes containing EDTA. These blood samples were then used for DNA extraction using QIAamp DNA Blood Mini Kit (Cat No./ID: 51104). The extracted DNA was analyzed qualitatively by Electrophoresis and DNA concentrations were then normalized to 100 ng/µL for further genotyping analysis. The DNA samples with absorbance ratios (280/260) between 1.8 and 2.0 (UV-Vis Double Beam Spectrophotometer 2202, Systronics, India) were used in this study.

PCR amplifications and genetic typing assay

PCR amplification was done for the LEPR gene polymorphism rs1137100 (A>G), rs1137101 (A>G) and rs8179183 (G>C / G>T). Primers were designed for these gene Polymorphisms using Primer3web (version 4.1.0). And their sequences were identified (Primer sequence Forward Primer: 5' TGACATTTCACACACGTCGGTA 3’; Reverse Primer 5' CCCCCAAAGCCTGAAAAAGC3'). PCR amplification was performed in a total volume of 25µl, containing 5 µl of 10 × Buffer, 2 µl template DNA, 1 µl upstream primer, 1 µl downstream primer, 0.5 µl Taq DNA polymerase, 2 µl dNTP, and 13.5 µl deionized sterile water. PCR procedures were carried out as follows: initial denaturation at 94℃ for 5 min; followed by 35 cycles of denaturing at 95℃ for 30s, annealing at 65℃ for 30s and extension for 30s at 72℃; and a final extension at 72℃ for 10 min. PCR products were analyzed using 1.2% agarose gel electrophoresis. LEPR gene SNPs were analyzed using restriction fragment length polymorphism (RFLP). The RFLP was carried out with the enzyme Hhal that cuts the LEPR mutant amplicons. The restriction digestion product was loaded on the 1% agarose gel stained with ethidium bromide.

Statistical analysis

OEGE - Online Encyclopedia for Genetic Epidemiology studies statistical software was used for statistical analysis. The cases and controls for the population were tested through Hardy-Weinberg equilibrium (HWE) test using the online software. Genotype and allele frequencies of the polymorphisms rs1137100 (A>G), rs1137101 (A>G) and rs8179183 (G>C / G>T) were manually counted based on gel analysis Chi square test was used to define difference between genotype and allele frequency of case and control groups. The association of LEPR gene polymorphisms with diabetes was determined by odds ratios (ORs) and 95% confidence intervals (CIs).

RESULT AND DISCUSSION

The Genotype and allele frequency distributions of LEPR gene polymorphisms polymorphisms rs1137100, rs1137101 and rs8179183 are evaluated in Table 1. Genotype frequencies of the three polymorphisms were all in accordance with Hardy-Weinberg Equilibrium (HWE) expectation, indicating fine suitability of the participants in control-case group as general population.

Table 1: The distribution of genotypes and alleles of LEPR gene polymorphisms rs1137100, rs1137101 and rs8179183 in the case and control groups

S.No.	SNP								
1	**rs1137100**	**Alteration**	A>G						
		Case Genotype/Allele frequency		**P**	**Control Genotype/Allele frequency**			**P**	**O.R (at 95% CI)**
AA		3.13			6.25				
AG		4.9			4				
GG		2.08	0.08	0.05	1.56		0.11	0.05	**2.64**
A		0.55			0.65				
G		0.45			0.35				
2	**rs1137101**	**Alteration**				A>G			
AA		1.33			∞				
AG		6			0				
GG		1.33	0.01	0.05	1.25		0.11	0.05	**3**
A		0.5			0.75				
G		0.5			0.25				
3	**rs8179183**	**Alteration**	G>C						
GG		1.33			9				
GC		6			3.46				
CC		1.33	0.03	0.05	3		0.18	0.05	**1.5**
G		0.5			0.6				
C		0.5			0.4				

The odd ratio of all SNPs (SNP 1- rs1137100 (O.R.- 2.64); SNP 2- rs1137101 (O.R.- 3) and SNP 3- rs8179183 (O.R. – 1.5)) signifies that there is correlation between these polymorphisms and the disease. Though the associations observed are nominal or borderline which could be due to small sample size. As the Odd ratio is directly associated to the sample size, therefore the sampling process does hold a significant role in deriving any prominent role of these polymorphisms in patients of T2DM.

T2DM is studied to be related closely to factors like obesity, hypertension and malfunctioning of lipid metabolism. LEPR gene, also known as T2DM gene has its significant role in regulating energy metabolism and body lipid homeostasis. The risk of association of T2DM with polymorphism has been explained based on different mechanism. One mechanism explained by Yan-Li *et al.* (2017) is alteration in glutamine residue which causes lowered down expression of LEPR on plasma membrane, thus resulting in improper signal transduction through receptor. Secondly, alteration in vagus nerve sensibility causing lowering of insulin secretion leading to IR. It thus causes, disturbance in fat and glucose metabolism (Ying *et al.,* 2009).

Liu et al and Su et al. both performed meta-analysis on the association between *LEPR* Gln223Arg gene polymorphism and T2DM in 2015 and 2016, respectively (Liu *et al*., 2015; Su *et al*., 2016). They both concluded that *LEPR*Gln223Arg gene polymorphism had no effect on the susceptibility with T2DM. Both of these papers, however, do not take into account differences in ethnicity. Liu's meta-analysis grouped all Asians, including Koreans, Indians, Malaysia, and Chinese into a single subgroup while Su's meta-analysis analyzed data that combined Asians and Europeans. These meta-analyses also included studies with the controls' genotype number that deviated from HWE, such as individual studies by Zhang, Zhao, and Liu (Zhang *et al*., 2011; Zhao *et al*., 2008; Liu, 2004). These factors combined may help account for the differences in results and also lend further credibility to the results of this study. The result of our study suggests significant association between SNP1, SNP2 and SNP3 and the risk of type 2 diabetes mellitus but a

more detailed study and approach to considering other risk factors and parameters will provide better insight of the correlation.

CONCLUSION

The study deals with the leptin gene polymorphism and impact, the gene has on the patients of Type 2 diabetes mellitus. It has been reported that genetic variations in the LEPR gene, single nucleotide polymorphisms, have increased the risk of type 2 diabetes mellitus (T2DM). The study was designed for a very small population of 50 subjects from hospital of North Indian region. All the subjects were genotyped for the leptin gene, SNP by polymorphism chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods, using biochemical methods to detect fasting glucose and other biochemical factors in the blood sample. The result of our study suggests significant association between snp1, snp2 and snp3 and the risk of type 2 diabetes mellitus. In other words, leptin gene shows a major diabetes gene pool capacity in north indian population, the alleles were not protective. The odd ratio of all SNPs signifies that there is huge correlation between genotype and disease. A further detailed study could be laid down on north Indian population with huge population size.

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