A study on association of leptin receptor gene polymorphism with obstructive sleep apnea syndrome in overweight and obese north indian subjects

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Abstract

Obesity is an important risk factor of Obstructive Sleep Apnea Syndrome (OSAS). Previous studies suggested Leptin Receptor (LEPR) gene Polymorphisms is associated with obesity and OSAS. Study was conducted to assess association of LEPR gene polymorphism K109R, Q223R and K656N with OSAS in North Indian subjects. Genotyping and estimation of serum Leptin levels were done in 190 subjects. Polysomnography, anthropometrical measures and biochemical investigations were done in all the subjects who qualified for inclusion in the study. We observed significant association of Q223R gene polymorphism with blood pressure (BP) (P<0.05) and nocturnal max pulse rate (P<0.05). K656N gene polymorphism was associated with AHI (P<0.05), average desaturation levels (P<0.05) and HDL-C (P<0.05). No association was observed in genotype distribution of these subjects according to obesity and disease severity. These findings suggest that LEPR Q223R and K656N gene Polymorphism may influence BP, Max Pulse rate, AHI, Average desaturation levels and HDL levels in these Subjects.

Introduction

Obstructive sleep apnea syndrome (OSAS) is commonly associated with loud snoring, periodic reduction and or cessation of breathing, witnessed gasping or choking and awakenings due to narrowing of the upper airways during sleep. These conditions lead to nocturnal hypoxemia, arousals, excessive day time sleepiness (EDS) and other related symptoms (1, 2). OSAS has been found to be associated with increased cardiovascular and cerebro-vascular morbidity and mortality (3). Some other important health consequences of OSAS are cognitive dysfunction, impaired work performance, anxiety, difficulties in personal relations, and an increased risk of fatal and non fatal automobile accidents. These circumstances lead to loss of human life and huge economical burden to the society worldwide (4). The adult prevalence of OSAS ranges within 2-4% in total population. In India, its prevalence varies from 3.9 to 14% in males and 1.2 to 7.5% in females (5). Significant progress has been made in identifying the genetic basis of sleep disorder such
as restless leg syndrome (RLS) and narcolepsy, whereas the genetic basis of obstructive sleep apnea Syndrome (OSAS) remains to be determined. Therefore, the identification of genetic variants associated with increased risk for OSAS could potentially translate into earlier recognition and treatment with reduced morbidity, and may also serve to identify potential targets for novel therapies.

Obesity is the main risk factor for occurrence of OSAS sharing multiple pathophysiological mechanisms such as insulin resistance, hyperleptinemia, & hypoventilation (6). Obese individuals have nearly four times higher risk of having OSAS as compared to non obese individuals independent of age and gender (7). Leptin plays a critical role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure. Defects in leptin production cause severe hereditary obesity in rodents and humans. Leptin acts through the leptin receptor (LEPR), a single-transmembrane-domain receptor of the cytokine receptor family, which is found in many tissues in several alternatively spliced forms (8). Leptin levels have significantly increased in OSAS subjects and positively correlate with the severity of OSAS (9).

Several studies have been reported showing importance of LEPR gene polymorphisms as risk factors of obesity and hyperleptinemia. LEPR K109R (13, 15, 18, 24), Q223R (10, 13, 15, 16, 19, 20, 25) and K656N (14, 21, 23, 26) gene polymorphisms were observed associated with obesity, blood pressure, insulin resistance, dyslipidemia, fat mass and leptin levels. Leptin may affect regulation of the respiratory centre. Consequently these gene polymorphisms may predispose an individual for risk of occurrence and increase in severity of the OSAS. Hence aim of the present study was to study the significance of LEPR K109R, Q223R, and K656N gene polymorphisms on severity of OSAS which is measured by Apnea Hypopnea Index (AHI).

**Materials and Methods**

**Subjects**

One hundred and ninety subjects were enrolled after taking written/informed consent from the pool of 320 randomly screened subjects from OPD of King George’s Medical University, Lucknow, India. All the subjects fitted with inclusion criteria (body mass index >25 kg/m² and age 18-65 years). Study was conducted between November 2008 to May 2012 after approval from IEC. Subjects with history of Liver disease, COPD, uncontrolled asthma, cancer, End Stage Renal Disease, heart failure and any other endocrine disorder (except type 2 diabetes mellitus) like Cushing syndrome and thyroid abnormalities were excluded from the study.

**Polysomnography**

All subjects underwent full night polysomnography sleep study (S-7000, Cogent technologies, EMBLA System Inc). The parameters studied were Electroencephalograms (EEG), (C3-A2, C4-A1, O2-A1, O3-A2), ECG and O₂ Saturation measurement by finger Pulse oximeter. Apnea Hypopnea Index (AHI) was calculated with the help of Somnologica Studio software. The apnea episodes were defined as complete cessation of airflow for ≥10 s, and hypopnea was defined as a ≥50% reduction in oronasal airflow accompanied by a reduction of at least 4% oxygen saturation calculated by pulse oximetry. Apnea events were classified as obstructive, mixed, or central, according to the presence or absence of breathing efforts with thoraco-abdominal paradox. AHI was determined by the frequency of these events per hour during sleep time based on the results of the overnight polysomnography. Recorded Polysomnographic data was cross checked manually for scoring of sleep stages, apneas and Hypopnea events regarding each subject.

**Biochemical and genetic analysis**

5 ml Fasting venous blood samples were taken in EDTA (2 ml), plain (2 ml) and in fluoride vial (1 ml) just after completion of the overnight polysomnography study (within 30 minutes). Serum lipid profile (Total Cholesterol, Triglyceride and HDL-C) and Fasting Plasma glucose estimation was done by enzymatic method (Merck) using Microlab Semi-autoanalyzer (Merck, Germany). LDL and VLDL calculated by Friedewald formula. Serum samples were stored in deep freezer at −20°C for serum Leptin assay which
was done by ELISA (AviBion). DNA isolation was done using DNA isolation kit (Medox Biotech, India) according to manufacturers instructions. PCR was performed using 100 ng genomic DNA, 10pmol each primer, 200 mmol/L deoxynucleotides, and 1U Taq polymerase with 1.5 mmol/L MgCl₂, for a final volume of 50 μL. PCR conditions were as follows: 30 cycles at 94°C for 45 s, 55°C for 45 s, and 72°C for 90s, with initial denaturation at 94°C for 2 min, and a final extension at 72°C for 7 min. PCR products were digested for overnight at 37°C with 5 U HaeIII (New England Biolabs), 5U MspI (New England Biolabs), and 5U BstUI (New England Biolabs) restriction enzymes for the K109R, Q223R, and K656N polymorphisms respectively. The resulting fragments were separated on 3% agarose gels.

Statistical analysis

Statistical analysis was performed using SPSS 20(SPSS Inc, USA). After assessing for approximate normal distribution, all continuous variables were summarized as mean±SD and categorical variables were expressed as n (%). Comparison between groups was done with one way ANOVAs test for continuous variables and χ² test for categorical variables. Post hoc test (Bonferroni) test was applied to analyzing intergroup association.

Results

Comparative analysis of sleep structure, obtained through overnight polysomnography, was done in all subjects. Significant difference was observed only in N1 stage duration (% of TST) of the sleep architecture between OSAS and NON-OSAS (Table I). In our setting significant difference was observed in (%)

distribution of degree of severity of disease (p=0.04) in different BMI group (Table II). Mean Serum Leptin levels (ng/ml) in four severity grade groups were not found significantly different (Table III). No significant differences were observed in distribution of these subjects according to disease severity in targeted LEPR K109R, Q223R and K656N gene polymorphism (Table IV). In this study, obesity variables body mass index (BMI), neck circumference (NC), waist
circumference (WC), hip circumference (HC) and waist to hip ratio (WHR) were not found to be associated with any of studied LEPR polymorphisms (Table V).

We observed an association of LEPR Q223R gene polymorphism with systolic and diastolic blood pressure and nocturnal max pulse rate in these subjects (Table V). In our setting, LEPR K656N gene polymorphism was found to be associated with AHI, average desaturation levels and HDL levels (Table V). Fasting Plasma Glucose levels were not found associated with any of three LEPR polymorphism. Serum Fasting Leptin levels were also not found associated with three studied LEPR polymorphism (Table V). Bonferroni test was applied intergroup analysis and we didn’t find any significant association.

**Discussion**

In our study, Sleep architecture (% of N1, N2, N3 and REM phases) was found similar with previous study (33) showing reduced slow wave Sleep and significant increase in N1 stage in OSAS cases. Our results with Q223R gene polymorphism are in accordance with previous studies (13, 15, 16, 19, 25, and 34). In a previous study, subjects with Q allele (Q/Q and Q/R) had higher heart sympathetic activity, body fat percentage, and leptin levels and subjects with higher prevalence of Q223 allele shown higher insulin levels (25). Similarly a Chinese study (13) found associations between the frequencies of AA genotype and A allele of Q223R and hypertension and raised diastolic blood pressure compared with GG carriers. In few other studies (15, 16, 19, 34)

<table>
<thead>
<tr>
<th>Variables</th>
<th>K109R</th>
<th>P value</th>
<th>Q223 R</th>
<th>P value</th>
<th>K656N</th>
<th>P value</th>
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<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NC</td>
<td>16.2 ±1.4</td>
<td>15.9 ±1.6</td>
<td>15.0 ±0.5</td>
<td>0.276</td>
<td>16.1 ±1.7</td>
<td>16 ±1</td>
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<td>WC</td>
<td>110.8 ±12.1</td>
<td>109.1 ±11.4</td>
<td>97.7 ±9.2</td>
<td>0.076</td>
<td>109.9 ±12</td>
<td>109.3 ±11.2</td>
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<td>HC</td>
<td>104.9 ±9.3</td>
<td>103.7 ±6.6</td>
<td>98.2 ±9.7</td>
<td>0.146</td>
<td>104 ±8.7</td>
<td>103.5 ±8.1</td>
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<td>WHR</td>
<td>1.05 ±0.06</td>
<td>1.05 ±0.07</td>
<td>1.02 ±0.06</td>
<td>0.203</td>
<td>1.05 ±0.07</td>
<td>1.06 ±0.06</td>
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<td>AHI</td>
<td>39.4 ±29.8</td>
<td>34.8 ±32</td>
<td>26.4 ±22.9</td>
<td>0.434</td>
<td>36.3 ±32.2</td>
<td>37.5 ±29.8</td>
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<td>ESS</td>
<td>11.8 ±5.4</td>
<td>11.5 ±5.7</td>
<td>7.7 ±6.8</td>
<td>0.308</td>
<td>10.9 ±5.2</td>
<td>12.0 ±5.7</td>
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<td>Obstructive</td>
<td>28.5 ±26.2</td>
<td>26.3 ±30.4</td>
<td>20.2 ±20.4</td>
<td>0.669</td>
<td>25.4 ±29.6</td>
<td>28.7 ±27.1</td>
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<td>Hypopnea</td>
<td>8.4 ±8.2</td>
<td>7.6 ±10.8</td>
<td>4.6 ±3.9</td>
<td>0.671</td>
<td>7.5 ±8.7</td>
<td>8.4 ±10.2</td>
</tr>
<tr>
<td>Desat Fall &lt;5%</td>
<td>39.8 ±29.6</td>
<td>34.1 ±30.5</td>
<td>27.9 ±19.2</td>
<td>0.380</td>
<td>33.2 ±27.3</td>
<td>39.6 ±32.1</td>
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<td>Avg Oxy Sat</td>
<td>91.7 ±5.6</td>
<td>92.5 ±4.9</td>
<td>93.3 ±4.4</td>
<td>0.566</td>
<td>92.5 ±5.2</td>
<td>92.1 ±5</td>
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<td>Low Oxy Sat</td>
<td>74.4 ±12.8</td>
<td>76.7 ±11.9</td>
<td>80.2 ±9.8</td>
<td>0.393</td>
<td>78.1 ±11.6</td>
<td>74.9 ±12.3</td>
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<td>Avg Desat</td>
<td>8.2 ±3.7</td>
<td>7.7 ±3.7</td>
<td>6 ±0.7</td>
<td>0.512</td>
<td>7.2 ±3.1</td>
<td>8.3 ±3.8</td>
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<td>LEPTIN</td>
<td>17.8 ±19.3</td>
<td>19 ±19.7</td>
<td>5.8 ±6.2</td>
<td>0.428</td>
<td>22.4 ±23.5</td>
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<td>TC</td>
<td>190 ±49.8</td>
<td>192.6 ±48.3</td>
<td>205.6 ±73.8</td>
<td>0.879</td>
<td>195.3 ±51.5</td>
<td>192.1 ±50.4</td>
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<td>HDL</td>
<td>42.5 ±9.7</td>
<td>43.2 ±8.7</td>
<td>46.7 ±2.9</td>
<td>0.723</td>
<td>43 ±8.4</td>
<td>42.4 ±1</td>
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<td>LDL</td>
<td>110.9 ±52</td>
<td>110.9 ±51.4</td>
<td>132.3 ±72.8</td>
<td>0.818</td>
<td>116.1 ±55.3</td>
<td>110.8 ±52.4</td>
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<td>VLDL</td>
<td>30.5 ±15.4</td>
<td>30.6 ±16.6</td>
<td>26.5 ±5.5</td>
<td>0.752</td>
<td>31.8 ±18.2</td>
<td>30.1 ±13.9</td>
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<td>TG</td>
<td>159.4 ±72</td>
<td>162 ±76.4</td>
<td>132.7 ±27.5</td>
<td>0.760</td>
<td>161.2 ±87.5</td>
<td>160.2 ±61.1</td>
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<td>FPG</td>
<td>103.6 ±27.4</td>
<td>105.5 ±27.8</td>
<td>100.5 ±24.3</td>
<td>0.945</td>
<td>103.7 ±23.8</td>
<td>102.2 ±26.7</td>
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<tr>
<td>Systolic BP</td>
<td>137 ±17.4</td>
<td>134.7 ±13.5</td>
<td>133 ±4.7</td>
<td>0.622</td>
<td>137 ±16</td>
<td>135.1 ±16.3</td>
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<td>Diastolic BP</td>
<td>88.2 ±10.5</td>
<td>87.1 ±7.3</td>
<td>85.2 ±4.1</td>
<td>0.600</td>
<td>87.6 ±7.3</td>
<td>87.6 ±10</td>
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<tr>
<td>Max Pulse</td>
<td>103.8 ±10.6</td>
<td>109.9 ±33.8</td>
<td>99.2 ±12.2</td>
<td>0.249</td>
<td>108.6 ±23.4</td>
<td>104.2 ±20.4</td>
</tr>
</tbody>
</table>

One way univariant analysis was performed using one way ANOVA test for group analysis and post hoc test (Bonferroni) test to analyze intergroup association. NC: Neck Circumference in inches, WC: Waist Circumference in centimeters, HC: Hip Circumference, WHR: Waist Hip Ratio, ESS: Epworth Sleepiness Score Desat: % Desaturation, Avg Oxy Sat: % Average Oxygen Saturation, Low Oxy Sat: % Lowest Oxygen Saturation, Desat Fall <5%: Desaturation events of Fall <5%, Avg Desat: % Average Desaturation, BP: Blood Pressure, bpm: beats per minute. All of following in mg/dl; TC: Total Cholesterol, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very Low Density Lipoprotein, TG: Triglyceride, FPG: Fasting Plasma Glucose.
BMI, leptin levels and systolic blood pressure means were observed lowest in the R223 homozygotes group and higher body weight in carriers of the 223Q alleles of LEPR.

Results of above mentioned previous studies and of present study suggest that leptin is associated with blood pressure regulation through the leptin receptor by activating the central sympathetic nervous system. When BMI and leptin are elevated, increased blood pressure is found only with the most prevalent LEPR genotype at codon 223, whereas variants of this receptor seem to protect from hypertension.

In previous studies LEPR Gene Polymorphism K109R, Q223R and K656N were found positively associated to physiological environment/correlates and risk factors of OSAS like obesity (10, 16, 19, 21, 22) high body fat composition (10, 17) increased plasma leptin level (17, 19, 21-23) abnormal insulin and glucose metabolism (11, 20, 23) low glucose oxidation rates (26) sweet preference (24) bone mineral content, physical activity (12) and other pathologies of High blood pressure (13) non alcoholic fatty liver disease (14) early atherosclerosis (15) and dyslipidemia (17) in different and sex groups in different populations. On the other hand we find contradictory pattern of these variables from several other studies and meta-analysis (27-30) conducted to assess such associations. In a study evaluating frequency distribution of LEPR Q223R polymorphism in OSAS cases and controls in Polish subjects, Popko et al (31) found significant correlation between LEPR Q223R polymorphism and OSAS. They also observed that presence of Arg allele was associated with obesity and higher lipid levels in OSAS subjects. Previously similar investigation as of ours was made by Hanaoka et al (32) in Japanese population and they found similar results of marginally significant association in a dominant model between wild-type alleles of LEPR Q223R and K656N SNPs in the LEPR gene with mild OSAS (AHI ≥10 to <20).

OSAS is a multi-factorial disease. Various factors other than gene structure may set off and or facilitate disease progression in due course of time. Obesity, Craniofacial morphology, soft tissue structures of upper airways, inflammation, ventilatory control pathways and multiple pathways, through biological pleiotropy may have an effect on OSAS occurrence and or progression in an individual. Physical activity profile, dietary composition and caloric intake may also affect its occurrence and advancement in a person. OSAS is not expected to be a simple condition linked with one or two genes or proteins; instead, this syndrome is probably an expression of several interrelated abnormal pathways and various molecular aberrations. OSAS is a risk factor for many diseases and numerous other diseases (such as T2DM and Hypertension) increase the risk of OSAS. Confounding variables like age, sex, and obesity represent a significant challenge in design of valid and reproducible OSAS study. Obesity is the most important amongst them in adult cases. There is no single common phenotype of OSAS. It occurs in consequence of one or more of contributing phenotypic factors acting single-handedly or in connections with each other. We have to make use of this network or cascade to identify the genetic basis of the disease. Intermediate phenotypes such as craniofacial morphology, upper airway control, ventilator control, and sleepiness should be studied independently. The advantages of using intermediary phenotypes over clinical variables will reduce heterogeneity and increased strength against confounders.

Although it is very difficult to obtain control individuals with matched physiological metrics, these results represent effect and association of LEPR gene polymorphism at position K109R, Q223Rand K656N on obesity, disease phenotype, leptin levels, fasting serum lipid levels, fasting plasma glucose levels and on Systolic and diastolic blood pressure in Indian Population and may be applied for assessment of high risk population for OSAS and associated clinical conditions in obese overweight Indian population. These results may also facilitate uncovering new markers of OSAS expression as well as its progression and consequences, such as hypertension and increased metabolic abnormalities in Indian population. Understanding of such genetic risk factors is crucial in drawing predictive models that integrate both genetic and phenotypic markers, thereby enabling early diagnosis and treatment.
Eventually, these measures will result in reduction of morbidity and public health concern associated with OSAS. It will also allow for advanced monitoring and lessening in personal risk of OSAS by lifestyle changes. As this study was conducted in a tertiary health care center so sampling biased could not be entirely ruled out. These results may be further validated in multi-centric design with appropriate matching of subjects according to age, sex and BMI.

**References**


