Original Article

Association of apolipoprotein B XbaI gene polymorphism and lipid profile in northern Indian obese

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BACKGROUND: Over the last few decades, obesity, diabetes, and hypertension have become main health evils. The health problems of obesity are well-recognized. However, the fact that all obese individuals are not at the same risk of developing a disease is also recognized. The apolipoprotein B (APOB) plays a central role in lipid metabolism. So we compare the association of APOB XbaI gene polymorphism and lipid profile total in obese north Indian population.

MATERIALS AND METHODS: A total of 132 obese (body mass index [BMI] >25 kg/m^2) and 132 age matched non-obese (BMI $\leq$ 25 kg/m^2) subjects were studied after taking detailed clinical profile. Lipid profile in serum/plasma was done using commercial kits. Genetic analysis of APOB XbaI was done using Polymerase Chain Reaction-Restriction Fragment Leanth polymorphism (PCR-RFLP).

STATISTICAL ANALYSIS: Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS) (version 11.5) software (IBM Corporation). All continuous variables were expressed as mean ± SD and tested by analysis of variance test. Comparisons of categorical variables were assessed using χ^2 tests or Fisher's exact test. $P < 0.05$ was considered as significant.

RESULTS: Analysis showed that obese subjects had significantly higher value of the waist-to-hip ratio, blood pressure (systolic and diastolic), and lipid profile. In APOB XbaI gene polymorphism, we did not find significant differences in genotype or allele frequencies. Moreover, none of the studied metabolic parameters (lipid profile) showed any association with the gene polymorphism.

CONCLUSIONS: Study reveals no considerable association of APOB XbaI gene polymorphism with obesity and lipid profile in north Indians.

Key words: Body mass index, lipid profile, obesity, polymorphism

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Introduction

Obesity has a negative effect on health, being associated with cardiovascular disease, hypertension, and diabetes.[1] Obesity is also associated with adverse changes in plasma lipoprotein metabolism. Various lipid/lipoprotein abnormalities have been observed in obese individuals, including elevated cholesterol, triglycerides (TG), and lower high-density lipoprotein cholesterol (HDL-C) levels. Of these indicators, changes in TG and HDL-C levels are most consistent and pronounced.[2,3]

The apolipoprotein B (APOB) plays a central role in lipid metabolism as the main protein component of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). It also serves as the ligand for removal of LDL from the circulation by receptor-mediated endocytosis via the LDL receptor.[4] Variants of the APOB gene may, therefore, be involved in the pathogenesis of obesity.

The APOB gene is located on the short arm of chromosome[2] (2p23-24)[5] and several single nucleotide polymorphisms in the APOB gene have been described, including XbaI[6] and EcoRI.[7] The XbaI polymorphism arises due to a single base variation in exon 26 (at 2488th position ACC→ACT) of the APOB gene that does not lead to change in amino acid sequence.[5] This

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polymorphism has been found to be associated with inter individual variability of lipid levels, but the results are conflicting.[8] Epidemiological studies have shown an association between the APOB gene polymorphisms with generalized and regional obesity and an increase in various lipoprotein subfractions (total cholesterol [TC], low density lipoprotein cholesterol [LDL-C], and TG), and atherosclerosis[9,10] though most of these studies have been carried out in Caucasian subjects. On the other hand, Liu *et al.*[11] reported that X(+) allele of APOB gene is a risk factor for the development of gallstone in Chinese patients, in another study no association exists between the APOB XbaI polymorphism and cholelithiasis in Mexican population.[12] Saha *et al.*[10] observed a significant association of APOB XbaI gene polymorphism with obesity and serum lipid levels while contradictory results also available, Misra *et al.*[13] reported that APOB XbaI gene polymorphism did not associate with obesity. We have, therefore, attempted to correlate APOB XbaI gene polymorphism with obesity and lipid profile in obese and non-obese subjects of northern Indian population.

Materials and Methods

Subjects

A total of 634 subjects were enrolled initially from the out patients department of Chatrapati Shahuji Maharaj Medical University, Lucknow and volunteers from the general population of Lucknow (North India). Out of these, 88.20% were Hindus (Hindi speaking and residing in Lucknow for at least two generations) while 21.8% belonged to other religions. Individuals of South, East, and Central Indian origin were excluded. A process of disproportionate stratified and systematic sampling was used to select individuals between 19 and 60 years old, oversampling of the majority groups to ensure that prevalence estimates for the majority groups were reliable and to allow statistical comparison. Every individual has been classified as Hindu North Indian (Hindi speaking) depending on self-reported family origin from two generations. Moreover, the possibility of population admixture is slight because in this part of country, inter-religion marriage or consanguinity is rare.

Out of these subjects, only 132 obese (body mass index [BMI] >25 kg/m^2) and 132 non-obese (BMI ≤ 25 kg/m^2) individual were selected befitting the strict inclusion criteria. All subjects were asked for detailed clinical history, and required measurements were done for height, weight, BMI, and waist-to-hip ratio (WHR). Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured in minimum clothing to the nearest 0.1 kg using a calibrated balance scale. BMI was calculated as the weight in kilogram divided by meter square of height. Only normotensive (systolic < 130 mmHg; diastolic < 85 mmHg) and non-diabetic (fasting blood sugar < 110 mg/dl) subjects were included. Subjects were considered as normal if they fall in normal ranges of various parameters. For example, leptin = 2-11 mg/ml, serum insulin = 0-30 μU/ml. Only non-smoker, non-diabetic, normotensive subjects who did not have a history of coronary artery disease, neoplasia, congenital and mental disorders, and endocrine disorders such as myxoedema and Cushing syndrome were included. The study was approved by an institutional ethics committee, and informed consent was obtained from each subject in accordance with principles of the declaration of Helsinki.

Sample collection

After an informed consent, overnight fasting blood samples (5 ml) were taken from all subjects. Two milliliter blood was taken in Ethylenediamine tetraacetic acid (EDTA) for analysis of DNA. The genomic DNA was extracted from peripheral blood leucocytes pellet using the standard salting out method (Miller 1988). Remaining 3 ml blood was used for serum/plasma isolation.

Anthropometry

Body weight was measured to the nearest 0.1 kg, and height was measured to the nearest 0.01 m.[14] The waist circumference was measured half way between the lower rib and iliac crest, the hip circumference was measured over the widest part in the gluteal region, and the WHR was calculated.[15]

Biochemical parameters

Lipid profile, total five biochemical parameters namely, TC, TG, HDL, LDL, and VLDL were estimated in obese and non-obese subjects. Fasting blood sugar was assayed by glucose oxidase-peroxidase) method.[16]

APOB XbaI polymorphism

Genomic DNA was isolated from peripheral blood according to standard procedures. The – 866A/G polymorphism in the promoter of human APOB gene was determined by digesting PCR products with restriction enzyme XbaI (Fermantas Inc., USA) as previously described.[17] The primers used were 5'-GGAGACTATTCAGAAGCTAA-3' as upstream primer and 5'-GAAGAGCCTGAAGACTGACT-3' as downstream primer.

Quality control

Quality control and assessment was done at every step of the study. The amount of isolated DNA was of good quality (absorbance 260 nm/280 nm, ratio > 1.75). One sample with known genotype and a reagent blank were included after every 20 samples in the PCR. A 50 base-pair marker was included during electrophoresis. Twenty percent of samples from patients and controls including samples of each genotype were re-genotyped by other laboratory personnel and no discrepancy was found.

Statistical analysis

All the statistical calculation for the continuous data of biochemical factors were performed using Statistical Package for the Social Sciences (SPSS) version 11.5 statistical software packages (IBM Corporation). For each variable, the values were expressed as mean ± SD. Data was evaluated by *t* test and one-way analysis of variance. Allele and genotypic frequencies for APOB XbaI was calculated with the gene counting method. Comparison of the categorical data i.e., different APOB genotypes among controls and obese subjects was done by Fischer's exact test and χ^2 test. Odd's ratios were calculated with a 95% confidence interval limit using 2 × 2 contingency table. $P < 0.05$ was considered significant. The observed genotype frequencies were compared with the expected frequencies to check for the Hardy–Weinberg equilibrium and $P < 0.05$ was used as the level of significance.

Results

Two hundred sixty four subjects comprising obese (60 male and 72 female) and non-obese (79 male and 53 female) adults were evaluated. The mean BMI of the obese and non-obese subjects was 29.40 ± 4.13 and 21.73 ± 2.11, respectively. In obese subjects, the WHR ($P < 0.001$), Systolic ($P < 0.001$) and diastolic ($P < 0.002$) blood pressures, though in normal range, were significantly higher in obese subjects as compared to non-obese subjects. Fasting blood glucose was significantly higher in obese subjects as compared to non-obese subjects. TC, TG, LDL, and VLDL was also higher ($P \leq 0.001$) in obese subjects as compared to non-obese subjects. On the contrary, HDL was significantly higher ($P < 0.001$) in non-obese as compared to obese subjects [Table 1].

Association of APOB XbaI gene polymorphism with obesity

This polymorphism was in Hardy–Weinberg equilibrium in non-obese population. The allele frequencies were X–, 75.4% versus 71.2%; and X+, 24.6% versus 28.8% in obese and non-obese subjects. The frequencies of X+X+ genotype did not differ significantly in obese and

Table 1: Basic characteristic of study subjects

Variable	Obese (*n*=132)	Non-obese (*n*=132)	*P* value
Demographic profile of subjects			
WHR	0.93±0.05	0.84±0.06	<0.001
SBP (mm Hg)	122.97±11.82	115.17±8.53	0.001
DBP (mm Hg)	80.83±9.58	76.26±6.48	0.002
Metabolic profile of subjects			
TC (mg/dl)	262.61±37.64	170.29±27.44	<0.001
HDL-C (mg/dl)	30.39±1.74	42.96±5.66	<0.001
VLDL-C (mg/dl)	31.01±11.23	20.95±3.18	<0.001
TG (mg/dl)	196.45±22.47	104.65±15.79	<0.001
LDL-C (mg/dl)	175.11±54.98	105.53±25.86	<0.001

Total number of obese (132) and non-obese subjects (132). All values are expressed in mean±SD. WHR: Waist-to-hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol; TG: Triglyceride

Table 2: Association of APOB XbaI gene polymorphism with obesity

Genotype	Obese *n**(%)	Non-obese *n**(%)	*P* value	OR (95% CI)
X-X–	79 (59.8)	70 (53.0)	-	1.0 (reference)
X-X+	41 (31.1)	48 (36.4)	0.300	0.757 (0.447-1.281)
X+X+	12 (9.1)	14 (10.6)	0.519	0.759 (0.329-1.751)
Allele				
X–	199 (75.4)	188 (71.2)	-	1.0 (reference)
X+	65 (24.6)	76 (28.8)	0.280	0.808 (0.549-1.189)

Total number of obese (132) and non-obese subjects (132) (for genotype). Total number of chromosomes in obese (264) and non-obese subjects (264) (for alleles). APOB: Apolipoprotein B; CI: Confidence interval; OR: Odds ratio; *n**: Number

non-obese. Similarly, frequency of X+ allele was not significantly different in the two groups [Table 2].

Association of APOB XbaI gene polymorphism with obesity related parameters

In obese and in non-obese subjects, none of the obesity related parameters like lipid levels specially TC, TG, LDL, HDL, and VLDL were associated with genotypes of APOB [Table 3]. The APOB X+ allele also did not show any statistically significant difference with clinical profile of obese or non-obese subjects [Table 4].

Lipid levels like TC, TG, LDL, HDL, and VLDL were found significantly different between obese and non-obese subjects. When we analyzed the association between genotypes of APOB XbaI gene polymorphism with lipid profile in obese and non-obese subjects, none of the variables associated with APOB XbaI polymorphism [Table 4].

Discussion

The present study examined the genotypic and allelic frequencies and the effect of APOB XbaI polymorphism on obesity and lipid profile in the northern Indian obese and non-obese population. The APOB XbaI polymorphism was selected for the present study by virtue of its documented association with obesity and dyslipidemia in North Indian population.[10] Various studies have shown an association between the APOB gene polymorphisms with lipoprotein subfractions (TC, LDL-C, and TG).[18,19] Asian Indians are prone to develop dyslipidemia and accelerated atherosclerosis.[20] Genetic investigations of the Asian Indian populations established in other part of the world show a correlation of APOB gene polymorphisms with hyperlipidemia.[10]

Present study shows that there was no significant difference in the genotype and allele frequencies of APOB XbaI polymorphism between obese and non-obese subjects. Saha *et al.*,[19] also reported no significant difference in allelic frequencies of XbaI gene polymorphism of the APOB gene.

The association of the XbaI polymorphism with serum lipid levels has been found in several studies.[21-23] On the other hand, we did not find any association of APOB XbaI gene polymorphism with serum lipid levels in obese and non-obese subjects. In the present study, a comparison of clinical variables was also done in relation to the genotypes of the APOB XbaI gene in obese and non-obese subjects but no association was found with serum lipid levels. Our results were in agreement with a previous study,[24] while Saha *et al.*,[19] reported no association between the APOB XbaI gene and serum lipids levels.

On the other hand it appears that APOB XbaI polymorphism exhibits population specific variation, which

Table 3: Association of APOB XbaI gene polymorphism with obesity related parameters (lipid levels)

Variables	Obese subjects			*P* value	Non-obese subjects			*P* value
	X-X- (79)	X+X- (41)	X+X+ (12)		X-X- (70)	X+X- (48)	X+X+ (14)	
Metabolic profile of subjects								
TC (mg/dl)	262.50±6.57	262.28±8.91	264.45±4.41	0.982	169.99±9.73	167.75±7.11	171.47±4.38	0.891
HDL-C (mg/dl)	31.03±2.45	30.39±1.67	30.29±1.66	0.394	43.24±5.93	42.83±5.47	42.03±5.12	0.752
VLDL-C (mg/dl)	28.63±4.43	30.60±1.24	31.60±1.42	0.673	21.00±3.35	19.63±3.14	21.25±2.89	0.234
TG (mg/dl)	185.17±9.53	196.00±2.63	198.41±2.49	0.161	104.65±6.59	98.14±5.67	106.56±4.38	0.213
LDL-C (mg/dl)	175.12±5.72	174.15±6.57	178.29±6.32	0.972	104.35±6.69	105.94±6.51	107.13±4.87	0.842

Total number of obese (132) and non-obese subjects (132). All values are expressed in mean±SD. APOB: Apolipoprotein B; TC: Total cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol; TG: Triglyceride

Table 4: Association of APOB XbaI polymorphisms with obesity related parameters (lipid levels) (X+carrier and non-carrier)

Variable	Obese APOB		*P* value	Non-obese APOB		*P* value
	X+carrier (65)	X+non-carrier (199)		X+carrier (76)	X+non-carrier (188)	
Metabolic profile of subjects						
TC (mg/dl)	272.87±6.75	272.54±4.82	0.517	218.99±4.96	207.98±7.17	0.077
HDL (mg/dl)	31.91±3.71	32.03±3.77	0.951	39.94±3.95	40.03±2.12	0.085
VLDL (mg/dl)	29.75±2.32	29.57±1.94	0.373	24.57±2.28	22.93±3.38	0.233
TG (mg/dl)	148.41±3.82	147.56±4.85	0.381	122.54±3.35	114.49±6.86	0.250
LDL (mg/dl)	211.27±7.45	210.99±4.74	0.481	154.51±4.72	145.16±9.97	0.069

Total number of obese (132) and non-obese subjects (132). All values are expressed in mean±SD. APOB: Apolipoprotein B; TC: Total cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; VLDL-C: Very low density lipoprotein cholesterol

may be due to gene and environment interactions. APOB XbaI polymorphism does not lead to changes in the amino acid sequence and cannot be implicated at structure level. It is possible that some other polymorphism in its vicinity might be present, which is in linkage disequilibrium with APOB XbaI polymorphism and accountable for the observed association with obesity and lipid levels in other studies. The effect of APOB XbaI polymorphism on the lipid levels is due to linkage disequilibrium with ins/del polymorphism, which causes an amino acid change in the signal peptide of the APOB gene. Saha *et al*.,[10] showed a strong disequilibrium between ins/del polymorphism and the XbaI polymorphism of APOB gene. Moreover, gene–environment interaction may also responsible for the inconsistency of data due to differences in the diet and lifestyle of populations of various part of the world.

In summary, there was no evidence of association of APOB polymorphisms (XbaI) with obesity and serum lipid levels. There may be several reasons for the differences observed in the various studies. The populations studied by in different studies were genetically different.[9,10,19] In view of the strong associations reported in some studies, studies are needed with a larger sample size to study association of XbaI polymorphism with obesity.

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