ORIGINAL RESEARCH

Association of Melanocortin 4 Receptor Gene Single Nucleotide Polymorphism [Rs17782313] In Young Obesity

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ABSTRACT

Background: Obesity is the result of complex relationship between food consumption patterns, lifestyle habits, socioeconomic, cultural, urban development, certain diseases or pharmacological treatment and genetics. Single-nucleotide polymorphisms(SNP) present in particular genes are associated with eating behavior in obese individuals. SNP of melanocortin -4 receptor (MC4R) gene was found to contribute satiety responsiveness and eating in the absence of hunger leading to childhood and adolescent obesity. Aim: To analyze the presence SNP of MC4R gene(rs17782313 CC genotype) in young obese and to find its association with BMI ≥ 30 kg/m² and waist hip ratio. **Materials and Methods:** This case control study was done at KAP Vishwanatham Government Medical college,Trichy, during the period of JUNE 2020 – MAY 2021. Total 35 young obese adults BMI ≥ 30 kg/m² in the age group of 15-25 years and age and sex matched 35 controls were included in the study. Data from all the individuals for blood pressure, anthropometric and biochemical characteristics were obtained, and single nucleotide polymorphism of MC4R gene (rs17782313 CC genotype) was assessed by PCR RFLP. **Results:** Genotype and allele distribution in controls and cases finds CC genotype,C allele distribution was higher in obese cases with high statistical significance ($p < 0.0001$). The logistic regression analysis for C allele at rs 17782313 had odds ratio of 4.03 with 95 % confidence interval and p value of 0.001. As body mass index increases, the risk of development of obesity increases. This is evidenced in our study as statistical significance is in positive correlation between body mass index and obesity. **Conclusion:** Findings in present study, suggest the statistical significant association of single nucleotide polymorphism of MC4R gene with presence of C allele for T allele at rs 17782313 and obesity.

Keyword:melanocortin receptor, allele, body mass index, childhood obesity.

INTRODUCTION

Worldwide prevalence of obesity in children, adolescent and adult population has increased more than threefold from 4 –18% globally[1] The prevalence of overweight was 10 % and obesity is about 5 % in South Indian adolescent population[2]. Among various common causes of obesity like increased calorie intake and decreased physical activity, genetics also contribute for around 40% with more than 50 genes which are strongly associated and contributes to higher rates of obesity.[3] `Recently, single nucleotide polymorphism of melanocortin -4 receptor (MC4R) gene found to contribute to satiety responsiveness, eating in the absence of hunger which leads to childhood and adolescent obesity. MC4R activation is both necessary and sufficient for the control of dietary fat intake by melanocortin signals.[4] MC4R gene resides on chromosome 18 at position q21. The melanocortin-4 receptor gene *(*MC4R*)* encodes a 332-amino-acid, which belongs to a family of seven trans-membrane G-protein-coupled receptors. This protein is well-known major regulator of food intake and energy expenditure. Polymorphisms within the MC4R coding region have been described to be related with obesity in humans.[5] In the melanocortin pathway, the fed state is signaled by abundance of circulating leptin and insulin, which binds to receptors expressed at the surface of pro-opiomelanocortin neurons (POMC) to promote processing of the POMC to mature hormone alpha –melanocyte stimulating hormone (α- MSH).The α- MSH released by POMC neurons that signals to decrease food intake by binding to melanocortin -4 receptors (MC4R) expressed by MC4R neurons to the paraventricular nucleus.[6]

1. Obesity in both adults and children are associated with numerous comorbidities, including hypertension, type II diabetes mellitus, dyslipidemia, obstructive sleep apnea and sleep-disordered breathing, certain cancers, and major cardiovascular diseases [7]. So early identification of the disease is needed to prevent disease progression and reduce the risk of cardiovascular morbidity and mortality. The reduced expression of MC4R receptor results in defective satiation and increase rate of food intake. The defective satiety signaling leads to melanocortin obesity syndrome [8].
2. There are studies which demonstrate the association of MC4R variants and related traits in Indian children and adults, with higher impact during childhood. It was proposed that Type 2 diabetes and insulin resistance was associated on adult Indians residing in different geographical regions [9]. This study is a hospital based case control study aimed to find the association between single nucleotide polymorphism of MCR4 gene rs 17782313 SNP (CC genotype) and young obese individuals with BMI ≥ 30 kg/m².

MATERIALS AND METHODS

This case control study was conducted in outpatients department of Medicine and Endocrinology departments at KAP Vishwanatham Government Medical College, Trichy during the period of JUNE 2020 – MAY 2021. The study was conducted in compliance with Helsinki declaration 1964 and its amendments, after approval from institutional ethical committee [I.E.C.NO:14/2020]. Informed and written consent were obtained from study and control group.

Inclusion criteria: study included 70 Subjects with aged between 15-30 yrs. Out of the 70, 35 subjects were obese cases with BMI ≥ 30 and 35 subjects were controls with BMI >18.5-25. Healthy individuals were selected from master health check– up OPD.

Exclusion criteria: Patients with Type 2 Diabetes mellitus, Metabolic syndrome, congenital abnormalities (such as Down syndrome, Prader willi syndrome, Laurence Moon Beidl syndrome, Generalised Lipodystrophy) Hypothyroidism, Cushing's syndrome, Polycystic ovaries and Patients on long term steroid therapy were excluded.

SAMPLE SIZE

Data collection: Each individuals included in this study were subjected to detailed history taking clinical examination, Anthropometric measurements of Height, Weight, Body Mass index, Waist hip ratio, Systolic and Diastolic blood pressure were measured.

Under aseptic precautions, following an overnight fasting, 5 ml of blood was collected from study group and controls by venepuncture. 2ml of blood was collected in anti coagulant tube (EDTA tube) and 3 ml of blood were collected in plain tube and centrifuged at 1000 X g for 15 minutes. Serum were separated for the estimation of glucose, urea, creatinine, total cholesterol, triglycerides, All the biochemical parameters were estimated in fully automated analyser (Biosystem A25) by enzymatic procedure using commercially available kit.

DNA was extracted from 2 ml whole blood sample obtained from both case and control group by using kit method (Spin column protocol).After DNA extraction, it is amplified by polymerase chain reaction.

POLYMERASE CHAIN REACTION

DNA extracted for MC4R gene was amplified by Polymerase chain reaction with Forward Primer:5′ -AAGTTCTACCTACCATGTTCTTGG -3′ and Reverse Primer:5′ -TTCCCCCTGAAGCTTTTCTTGTCATTTTGAT-3′of the mentioned sequence.

PCR was performed using the kit HiGenoMB manufactured by Himedia laboratories, India.The procedure of PCR includes preparation of master mix contains the following components Taq Polymerase – 2units,DNA concentration–200ng,10µM concentration of forward and reverse primers,Catalyst– 1.5Mm magnesiumchloride, dNTP - 200Mm, Reaction Buffer-50mM – KCl,10Mm - Tris Hcl(ph – 8.3). PCR conditions were Initial denaturation - 95˚C for 5minutes, 35 cycles Cyclic denaturation at 95˚C for 30seconds, Cyclic annealing - 55˚C for 30seconds, Cyclic extension - 72˚C 30seconds and Final extension - 72˚C for 7 minutes.

From the amplified PCR product 5µl taken and submarine gel electrophoresis done in 1.8% agarose gel. Appearance of 137 bp band confirmed the presence of PCR amplified products. To the amplified product, restriction enzyme is added and submarine gel electrophoresis done to visualize the bands in Gel documentation system.MC4R gene polymorphism at rs 17782313 is detected by digestion of 107 and 30 bp amplified product with Bc1I restriction enzyme (10 U/ µL) and submarine electrophoresis was done with 1.8% agarose gel. Restriction digestion principle includes TT genotype/T allele has the restriction site and cleaved bands are seen at 107 bp and 30 bp (not visually prominent).CT genotype (Heterozygous individuals) had three bands(137bp, 107bp, 30bp), out of which two are visible at 137bp and 107bp (30bp is not visually prominent). CC genotype/C allele was devoid of BcII restriction site and band is seen at 137 bp. Based on restriction band pattern genotyping, in cases and controls were calculated.[10]

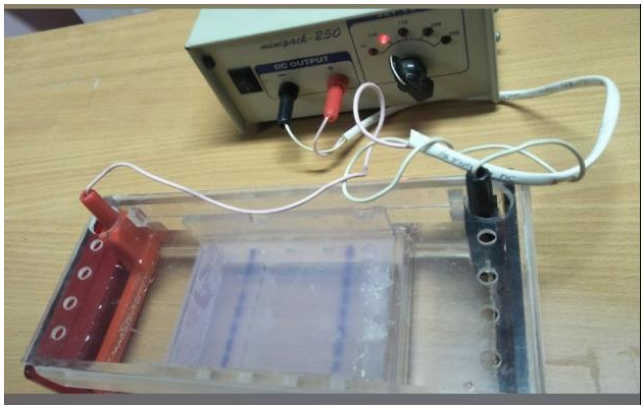
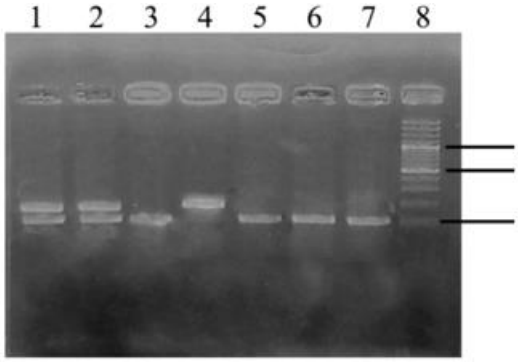


Figure 1: Submarine gel electrophoresis



1 - CT Genotype
2 - CT Genotype
3 - TT Genotype
4 - CC Genotype
5 - TT Genotype
6 - TT Genotype
7 - TT Genotype
8 - 100 Bp Ladder

Figure 2: Genotyping of Cases

STATISTICAL ANALYSIS

Study data were entered in Microsoft excel and analysed using statistical software version 20.0. Students 't' test was used to compare anthropometric, biochemical parameters of controls and cases. Chi Square test was used to analyze frequency of genotype distribution in controls and cases. Hardy-Weinberg law applied to find whether the study population is in equilibrium. Logistic regression analysis, odds ratio, 95% Confidence Interval (CI) were calculated to find out the association of C allele and obesity. P-value of <0.05 was set as Statistical significance level.

RESULTS

In present study, the case group had significantly higher BMI,Waist Hip Ratio and Systolic Blood Pressure than control subjects and statistically significant [Table/fig-1]. They showed significantly higher total cholesterol and triglyceride levels (Table 1).

[Table/fig 1]:Anthropometric and Serum Biochemistry profile characterization of the study cohort

Variable	CONTROLS(n=35)	CASES(n=35)	P Value
Age(years)	22.9 ±5.5	23.42±4.0	0.6
Male	19	19	0.02
Female	16	16	0.01
Body Mass Index(Kg/m2)	21.8±1.5	31.1±0.9	<0.001
Waist Hip Ratio	0.4±0.1	1.6±0.5	<0.001
Systolic Blood Pressure(mm/HG)	109.9±10.4	120.8±9.8	<0.001

Diastolic Blood Pressure(mm/Hg)	72.4±7.9	73.2±6.2	0.6
Blood Glucose(mg/dL)	109.3±13.1	117.4±11.2	0.007
Urea(mg/dL)	31.5±5.5	33.9±8.0	<0.1
Creatinine(mg/dL)	0.9±0.1	0.5±0.2	<0.001
Total Cholesterol(mg/dL)	160.4±20.0	190.7±17.5	<0.001
HDL(mg/dL)	48.3±7.9	44.5±9.9	0.08
Triglycerides(mg/dL)	131.4±9.3	173.4±30.7	<0.001

p value < 0.05-significant.

The Hardy-Weinberg equation here is TT+CT+CC=1. The frequency of TT genotype is 30% in controls and 75 in cases. The frequency of CT genotype is 15 % in controls and 17% in cases. The frequency of genotype CC distribution is 25.7 % in cases. Chi square value is 20.603, p value is < 0.0001 which is higher and statistically significant than TT and CT genotype (Table 2).

Table 2: The Hardy-Weinberg equation

Genotype	TT	CT	CC
Allele frequency in one generation	0.026	0.023	0.021
Allele frequency in next generation	0.013	0.022	0.010

The frequency of genotype CC distribution is 25.7 % in cases. Chi square value is 20.603, p value is < 0.0001 which is higher and statistically significant than TT and CT genotype (Table 3).

Table 3: Distribution of Genotype frequencies

MC4R gene rs 17782313 GENOTYPE	Controls (N%)	Cases (N%)	Chi Square	P Value
TT	21(30%)	5(7.1%)	20.603	< 0.0001
CT	11 (15.7%)	12 (17.1%)		
CC	3 (4.2%)	18 (25.7%)		

In frequency of allele distribution among patients with obesity and normal controls, C allele was observed in 30 cases as compared with 14 normal controls. T allele was observed in 17 cases as compared to 32 normal controls. Chi square value is 10.4005.p value is < 0.001 and is statistically significant (Table 4).

Table 4: Allele frequency distribution of MC4R gene polymorphism between cases and controls.

Allele	Controls	Cases	Total	Chi Square	P Value
c	30	14	44	10.400	< 0.0001
T	17	32	49		

Subjects with C allele are observed to have 4 times increased risk of having Obesity than those subjects having T allele. C allele has a statistical significance with p value - 0.001(Table 5).

Table 5: Logistic regression of C allele for finding out BMI associated Obesity (with n = 70) as dependent variable

Variable (Independent)		Odds Ratio OR	95%CI Confidence Interval	p - Value
Specific Allele	T	1	1.6981-9.5813	0.001
	C	4.3		

Comparison of body mass index between subjects with T allele and C allele gives Mean BMI with T allele is 25.3 ±4.9, Mean BMI with C allele is 28.1 ±4.3. p value of < 0.0001 which is statistically significant.

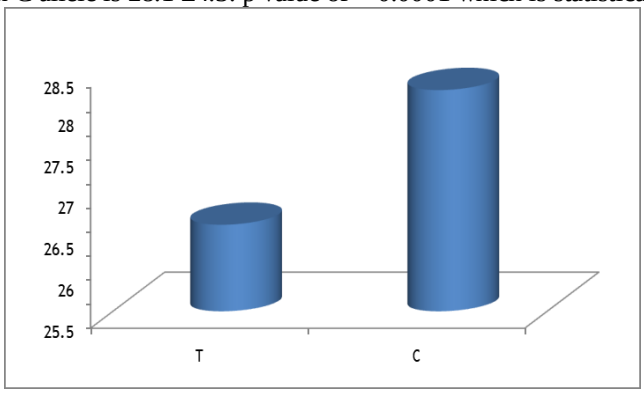


Figure 1: Comparison of body mass index between subjects with T allele and C allele

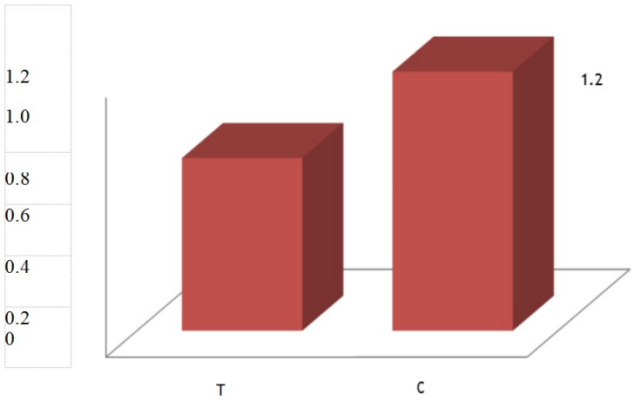


Figure 2: Comparison of waist hip ratio between subjects with T allele and C allele

Comparison of waist hip ratio between subjects with T allele and C allele shows Mean waist hip ratio with T allele is 0.8±0.6. Mean waist hip ratio with C allele is 1.2± 0.7**.**

DISCUSSION

The main finding of the present study demonstrates the association of SNP of rs 17782313 of MC4R gene with BMI ≥30 kg/m².There are population based study reports in association of SNP of rs 17782313 of MC4R gene with obesity in a North Indian population,This study results are noteworthy because it was a hospital based cross sectional study done in south India in the young obese subjects of age 15 to 25 years.The strength of the study is it excludes the secondary causes of obesity and compared with the age matched control individuals.. Genetic and environmental factors also play significant roles in development of obesity.[11] Among the genetic factors MC4R gene plays an important role in regulation of food intake and energy expenditure.[12] Being highly polymorphic, SNPs of MC4R gene rs 2331841, rs 6567160, rs 17782313 associated with obesity are recently evidenced.[13] Among these rs 17782313 is the common SNP leading to obesity. Hardy-weinberg law here predict the genetic variation in a population which will remain constant from one generation to next, in the absence of disturbing factors. If the population is not evolving, same number of allele frequency of TT, CT, CC is present in next generation. If proportions of TT, CT, CC changes, then the population is considered as evolving. The present study obeys Hardy-Weinberg Law and the study population is in disequilibrium. However large scale population study is needed to confirm the allele frequency changes.

In this present study Allele and Genotype frequency association analysis between the cases and control shows C-allele carriers in the rs17782313 site have an increased susceptibility to obesity. Therefore, findings are in agreement that MC4R rs17782313 plays a role in predisposition to obesity BMI ≥30 in view of the fact that it is expressed in the brain and part of the melanocortin pathway controlling food intake and energy expenditure.[14] Expression of the pro-opiomelanocortin gene in the hypothalamic arcuate nucleus causes reduction of food intake and weight loss.[15]When obesity progresses there is a definite increase in waist hip ratio. Our study compares Mean waist hip ratio among T allele and C allele determines presence of C allele increases with increase in waist hip ratio. The Pro-opiomelanocortin gene expression in the arcuate nucleus is decreased in leptin-deficiency.[16]Administration of leptin causes a significant stimulation of pro-opiomelanocortin gene expression in the arcuate nucleus, and also increases the frequency of action potentials in arcuate nucleus of pro-opiomelanocortin neurons. This supports the concept that the melanocortin signaling system basically lies downstream of leptin signaling in the arcuate nucleus.[17]

CONCLUSION

In our study we found there is statistically significant association of single nucleotide polymorphism of MC4R gene with presence of C allele for T allele at rs 17782313 and obesity. Mutated MC4R is associated with higher risk of childhood onset obesity.[18]Individuals with homozygous mutations are more likely to suffer from obesity while hetergenous mutant genotype needs further studies to clarify. Heterogenous coding mutations in MC4R gene are implicated in 1 to 6 % of early onset or severe adult obesity.[19]Genotyping for the presence of C allele at rs 17782313 of MC4R gene can be used as screening tool for early detection of determination of obesity and prevent further progression of non communicable diseases.

LIMITATIONS OF THE STUDY

Mutated Gene analysis offer a diagnosis and cannot always predict the severity of the disease. Testing for mutations associated diseases such as in obesity can predict the risk but not definitive.Accuracy is high but can vary depending on the disorder[20]. Quantitative measurement of α- melanocyte stimulating hormone, leptin hormone in blood would have given better understanding about pathophysiology of obesity due to single nucleotide polymorphism of MC4R gene. Large scale population study is needed to confirm the

presence of allele frequency changes in MC4R Gene polymorphism rs17782313.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare that are relevant to the content of this article.

REFERENCES

1. LobsteinT,Baur L Uauy R,IOTF Taskforce,NCD Risk factor collaboration, Lancet, World obesity federation-Sep 2018.
2. Prasanna Kamath B.T.,GirishM,Bengalokar, Deepthi,R., Muninarayan C., Ravishankar S, Prevalence of overweight and obesity among adolescent school going children in urban, South India Vol 04,Oct 2012
3. Jeffry SicatMD, Obesity and Genetics : Nature and Nurture July 23,2018
4. Philippe Samama, Leonid Rumennik, Joseph F Grippo-The melanocortin receptor MCR4 controls fat consumption.2003 May15:113(1-3):85-8.
5. AgataLeonska- DuniecPawelIldus I. Ahmeto*et al.*-Genes and individual responsiveness to exercise-induced fat loss,2018 March 1:17(1):134-144
6. Giulia Baldini and Kevin D Phelan -The melanocortin pathway and control of appetite – progresss and therapeutic implications –Journal of Endocrinology. 2019 April1; 241(1);R1-R33.
7. Carl J Lavie, Richard V Milani, Hector O Ventura - Obesity and cardiovascular disease: risk factor, paradox, and impact of weight loss. 2003 Aug 20; 42(4); 677-9
8. DolladaSrisai, Mathew P Gillum, Brandon L Panaro, Xian-Man Zhang,Endocrinolgy,2011,152(3),890-902
9. Otto Tscchritter,Axel haupt, Hubert Preissl, Caroline Ketterer, Anits M hennige- Journal of Obesity 2011.283153.
10. HiGenoMB Himedia 2XPCR TaqMixture kit
11. M Switonski*et al.*J Appl Genet Family of Melanocortin receptor genes in mammals, mutations, polymorphisms and phenotypic effects, Nov 2013.5494);461-72.
12. DolladaSrisai,MathewPGillum,Brandon L Panaro, Xian-Man Zhang,Endocrinolgy,2011,152(3),890-902
13. Vettori, G. Pompucci, B. paolini, I. Del ciondolo, S. Bressan4, M. dundar, S. Kenanoğlu, V. Unfer,*et al.* ;geneob project Genetic background, nutrition and obesity: a review European Review for Medical and Pharmacological Sciences 2019; 23: 1751-176
14. Giuseppe D'Agostino and Sabrina Diano-alpha melanocyte stimulating hormone production and degradation Journal of molecular medicine Berlin, Germany. 2010 Dec;88(12):1195-1201.
15. Jeffry SicatMD, Obesity and Genetics : Nature and Nurture July 23,2018.
16. David L. Nelson, Micheal M Cox, Lehninger-Principles of Biochemistry, Eigth edition pg 913
17. Hiroyuki Shimizu, Kinji Inoue and Masatomo Mori - The leptin-dependent and -independent melanocortin signaling system: regulation of feeding and energy expenditure, 2007 April;193(1):1-9.
18. Manpreet Doulla,Adam D Mclntyre,Rober A hegele,Ptricia H Gallego Pediatric Child health,2014 Dec; 19(10): 515-518
19. Cecile Lubrano-Berthelier,MarthaCavazos,BeatriceDubern-Molecular Genetics of Human Obesity- associated MC4R Mutations- Annals of New York Academy of sciences-994 (1) 49-57.
20. Najat Mahdieh,BaharehRabbani,Iran J Pediatric –An Overview of Mutation detection methods in genetic disorders -2013August ;23(4);375-388.