

# Association of single nucleotide polymorphisms in *TNFA*, *PNPLA3*, *ADIPOQ* and *APOC3* genes with obesity and non-alcoholic fatty liver disease in north Indian adolescents

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## Background

- India has the world's second highest number of children with obesity.
- More than 60% of obese children and adolescents have non-alcoholic fatty liver disease (NAFLD).
- Single nucleotide polymorphisms in several genes may confer a higher risk of obesity and NAFLD.

## Objective

- To examine the association of the following eight single nucleotide polymorphisms (SNPs) with obesity and NAFLD in north Indian adolescents.
  - SNPs -238G>A, -1031 T>C and -863 C>A of Tumor Necrosis Factor-  $\alpha$  (*TNFA*) gene
  - SNP rs738409 C>G of patatin-like phospholipase domain containing 3 (*PNPLA3*) gene
  - SNPs +276 G>T and +45 T>G of Adiponectin (*ADIPOQ*) gene
  - SNPs 455 T>C and 482 C>T of Apolipoprotein C3 (*APOC3*) gene

## Subjects and Methods

In this case control study, 214 overweight/ obese adolescents aged 10 to 16 years and 86 healthy lean adults were enrolled.

Ultrasonography done by a single experienced pediatric radiologist to diagnose NAFLD and grade its severity.

BMI, waist circumference, fasting glucose, ALT, and lipids were measured in adolescents as well as controls.

Serum insulin, adiponectin, apolipoprotein C3 and tumor necrosis factor- $\alpha$  (TNF-  $\alpha$ ) only in the adolescents

PCR and RFLP were done to evaluate the genotype for -238 G>A, -1031 T>C and -863 C>A of *TNFA*; rs738409 C>G of *PNPLA3*; +276 G>T and +45 T>G of *ADIPOQ*; and 455 T>C and 482 C>T of *APOC3* genes

The frequency of hetero- and homozygous variant genotypes of the SNPs were compared with the lean adult controls for the the overweight/ obese adolescents as a whole, and for the subgroups without NAFLD, with mild NAFLD and with moderate or severe NAFLD.

## Results

- The mean age of the overweight/ obese adolescents was  $11.9 \pm 1.6$  years, and their mean BMI was  $27.3 \pm 4.3$  Kg/m<sup>2</sup>.
- NAFLD was present in 62.5% of the subjects, with the disease being mild, moderate and severe in 40.4, 18.8, and 3.3%, respectively.

Table 1. SNPs that showed significant association with obesity

Gene	SNP	OR (95% C.I.)
<i>TNF- <math>\alpha</math></i> ( <i>TNFA</i> )	*Variant genotypes of -1031 T>C	2.47 (1.46 – 4.18)
	*Variant genotypes of -863 C>A	2.52 (1.45 – 4.35)
Adiponectin ( <i>ADIPOQ</i> )	Wild genotype of +276 G>T	2.58 (1.40-4.75)
Apolipoprotein C3 ( <i>APOC3</i> )	*Variant genotypes of 455 T>C	2.00 (1.11 – 3.61)
Presence of all the above 4 SNPs		14.4 (2.5 to 99.6)
Presence of any 3 of the above four SNPs		8.1 (2.1 - 32.7)
Presence of any 2 of the above four SNPs		4.9 (1.3-18.2)

\*Homozygous or heterozygous variants

Table 2. SNPs that showed significant association with moderate or severe NAFLD

Gene	SNP	OR (95% C.I.)
<i>PNPLA3</i>	Variant genotypes of rs738409 C>G	2.39 (1.09 – 5.28)
Apolipoprotein C3 ( <i>APOC3</i> )	Variant genotypes of 455 T>C	5.04 (1.62 – 15.67)
Presence of both the above SNPs		6.6 (1.7- 25.7)

Table 3. Comparison of serum biochemical parameters in the adolescents with wild vs. homozygous variant genotypes

Genotype	Biochemical parameter [Median (range)]	P-value	
<i>TNFA</i> -863 C>A	Insulin ( $\mu$ U/ml)	0.060	
	CC (n = 67)		13.9 (1.8 – 43.0)
	AA (n = 14)		17.0 (8.0 – 46.9)
<i>TNFA</i> -1031 T>C	Insulin ( $\mu$ U/ml)	0.022	
	TT (n = 54)		14.7 (1.8 – 43)
	CC (n = 8)		28.6 (8.0 – 46.9)
<i>APOC3</i> 455 T>C	Triglyceride (mg/dl)	0.041	
	TT (n = 40)		95.3 (59.2 – 314.8)
	CC (n = 50)		115.4 (60.8 – 312.4)
<i>APOC3</i> 455 T>C	Apolipoprotein C3 (mg/dl)	0.060	
	TT (n = 40)		7.2 (0.8 – 26.0)
	CC (n = 50)		8.6 (0.7 - 30.9)

## Conclusions

- Four polymorphisms, -1031 T>C and -863 C>A of *TNFA* gene, and 455 T>C of *APOC3* gene, and wild type of the polymorphism +276 G>T of *ADIPOQ* gene had a significant association with overweight/ obesity in Asian Indian adolescents.
- Two polymorphisms, rs738409 C>G of *PNPLA3* gene and 455 T>C of *APOC3* gene were associated with moderate or severe NAFLD.
- Higher fasting insulin was present in adolescents with homozygous variant types of SNPs -1031 T>C and -863 C>A of *TNFA* gene.
- Higher triglyceride and apolipoprotein C3 levels were seen in those with homozygous variant genotype of 455 T>C of *APOC3* gene.
- The study provides useful insight into the contributory role of genetic polymorphisms in the pathogenesis of predominantly lifestyle related conditions of obesity and NAFLD.

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