Title

Association between insulin receptor gene exon 17 rs1799817 variant and risk of nonalcoholic fatty liver disease

Authors

Touraj Mahmoudi, MSc ^a, Hossein Nobakht, MD ^b, Reza Dabiri, MD ^b, Iradj Maleki, MD ^c, Pooneh Dehghan, MD ^d, Fariborz Mansour-Ghanaei, MD, AGAF ^e, Faramarz Derakhshan, MD ^a, Mohammad Reza Zali, MD ^a

^a Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

^b Internal Medicine Department, Semnan University of Medical Sciences, Semnan, Iran

^c Gut and Liver Research Center, Mazandaran University of Medical Sciences, Sari, Iran

^d Taleghani Hospital Research Development Unit, Shahid Beheshti University of Medical Sciences, Tehran, Iran

^e Division of Gastroenterology and Hepatology, Gastrointestinal and Liver Diseases Research Center (GLDRC), Guilan University of Medical Sciences, Rasht, Iran

Corresponding author

Touraj Mahmoudi, MSc. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Velenjak, Shahid Chamran Highway, 1985711151, Tehran, Iran. Tel: +989124148851, Fax: +982122432517, E-mail: mahmouditouraj@gmail.com

Abstract

Background and objectives: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease characterized by accumulation of triglycerides in hepatocytes without alcohol consumption. Considering the key role of insulin resistance in NAFLD, we investigated whether insulin (*INS*) and insulin receptor (*INSR*) gene variants were associated with NAFLD risk.

Materials and methods: This study was conducted as a case-control study and 230 subjects, including 128 controls and 102 cases with biopsy-proven NAFLD, were enrolled and genotyped using PCR-RFLP method.

Results: We observed no significant difference in genotype or allele frequencies between the cases with NAFLD and controls for *INS* gene rs689 variant either before or after adjustment for confounding factors including age, BMI, sex, smoking status, systolic blood pressure, and diastolic blood pressure. However, the *INSR* rs1799817 “TT” genotype compared with the “CC” genotype and “CC+CT” genotypes appears to be a marker of decreased NAFLD susceptibility ($P=0.029$; OR=0.14, 95%CI=0.03-0.82 and $P=0.048$; OR=0.20, 95%CI= 0.04-0.98, respectively).

Conclusions: To our knowledge, our findings suggested for the first time that the *INSR* rs1799817 “TT” genotype compared with the “CC” genotype and “CC+CT” genotypes had 86% and 80% decreased risks for NAFLD, respectively. Nevertheless, further studies are needed to confirm these findings.

Keywords:

insulin resistance; *INS*; *INSR*; nonalcoholic fatty liver disease; gene variant

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a complex metabolic disease characterized by accumulation of triglycerides in hepatocytes without alcohol consumption and includes a wide spectrum of histologic abnormalities ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) that may progress to cirrhosis and even hepatocellular carcinoma. NAFLD is the most common form of chronic liver disease and a growing global health concern that its prevalence varies between 20% and 30% in the general population of Western countries (Bellentani, et al., 2010). To date, despite considerable efforts, the pathogenesis of NAFLD is not yet fully elucidated. However, previous studies show that NAFLD is associated with obesity (Fabbrini et al., 2010) and type 2 diabetes mellitus (Kotronen et al., 2008). Furthermore, insulin resistance (IR), a prominent feature of NAFLD independent of obesity, plays a major role in the pathogenesis of NAFLD (Marchesini et al., 2001; Eguchi et al., 2006). In actual fact, NAFLD is thought to be the liver manifestation of insulin resistance. Interestingly, the rates of elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels are much higher in NAFLD patients with IR than those without IR (Li et al., 2015) and IR is more severe in patients with NASH in comparison to patients with simple fatty liver (Gholam et al., 2007). Moreover, a positive association between serum levels of insulin and NAFLD has been reported (Siddiqui et al., 2015). The pleiotropic effects of insulin are mediated by its cell surface receptor, insulin receptor (INSR), through its ability to modulate the expression of target genes (White, 1999).

Accordingly, as mentioned earlier the clinical and metabolic features of NAFLD like insulin resistance and obesity support the hypothesis that the genes involved in insulin

signaling pathway including the *INS* (rs689) and *INSR* (rs1799817) gene variants might have a role in the pathogenesis of NAFLD. These two SNPs were selected based on their commonly use in previous genetic epidemiology studies, position in the gene (promoter or exon), functional importance, and somewhat degree of heterozygosity.

2. Materials and Methods

2.1. Participants

A case-control study of 128 controls (age range, 20-60 years) and 102 cases with biopsy-proven NAFLD (age range, 19-64 years) was performed between April 2010 and September 2012 in Tehran, Iran. The diagnosis of NAFLD was made according to the following criteria: (a) the presence of fatty liver on ultrasonography and elevated serum liver enzymes; (b) alcohol consumption < 20 g/day in men and < 10 g/day in women; and (c) excluding patients with other causes of liver disease including viral hepatitis, Wilson's disease, alpha-1 antitrypsin deficiency, use of drugs known to induce steatosis. On the other hand, none of the control subjects had a body mass index (BMI) ≥ 25 , liver steatosis examined by abdominal ultrasonography, elevated liver enzymes (ALT, AST, GGT), and viral hepatitis infection examined by blood test. None of them were also alcoholic or drank regularly and were on regular medications. The controls were recruited from the institute staff and medical students. An experienced pathologist, unaware of all patients clinical and biochemical data scored biopsies using the Brunt's criteria and steatosis and necroinflammation were graded from 0 to 3 and fibrosis was staged from 0 to 4 (Brunt et al., 1999). All 230 subjects were Iranian and genetically unrelated and informed consent was obtained from all of them before their entry into the study. Study protocol was approved by the Ethical Review Boards of the Institution and it was conducted according to the Helsinki Declaration. Information on demographic, anthropometric, and clinical characteristics of the cases and controls was recorded using self-administered questionnaire before diagnosis of NAFLD.

2.2. Genotype analysis

Genomic DNA was extracted from 5ml EDTA-anticoagulated whole blood using standard methods, and genotyping was done by PCR-RFLP method. Furthermore, genotyping process was performed by investigators who were blinded to the subjects' clinical data. Details of the studied gene variants and PCR and RFLP conditions are shown in Table 1. Both PCR products were digested overnight by corresponding restriction enzymes (Fermentas, Leon-Rot, Germany) and then electrophoresed on 2 to 3.5% agarose gels. Bands in gels stained with ethidium bromide for visualization under ultraviolet light. *INS* and *INSR* genotypes of each subject were identified according to the digestion pattern and the presence or absence of the Alw26I and Eco72I sites, respectively. The concordance of genotyping was confirmed by duplicate analysis of approximately 20% of the randomly selected samples.

2.3. Statistical methods

Differences in demographic, anthropometric or clinical factors were calculated using t-test or chi-square test when appropriate. χ^2 test was also used to calculate the differences in the allele frequencies between the groups. We used logistic regression to compare the distribution of the genotype frequencies between the different groups. To adjust confounding factors including age, BMI, sex, smoking status, systolic blood pressure (SBP), and diastolic blood pressure (DBP) logistic regression analysis was also used. The odds ratios (ORs) were the measure of associations and for all ORs, 95% confidence intervals (95% CI) were calculated. For statistical analyses, we used SPSS software, version 15.0 (SPSS Inc. Chicago, IL, USA). Significance was assumed for $P < 0.05$.

3. Results

3.1. Clinicopathological and biochemical analysis

Selected characteristics of the study population and their statistical significance are summarized in Table 2. In general, the cases with NAFLD were older and had higher BMI than the controls ($P<0.001$). Furthermore, the percentages of males and smokers were also higher in the cases compared with the controls ($P<0.001$). In addition, the cases with NAFLD had higher systolic blood pressure (SBP) and diastolic blood pressure (DBP), as well as higher serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) than the controls ($P<0.001$).

3.2. *INS* and *INSR* SNPs analysis

Table 3 shows the genotype and allele distributions for *INS* (rs689) and *INSR* (rs1799817) gene variants in the cases with NAFLD and controls. We observed no significant difference in genotype or allele frequencies between the cases with NAFLD and controls for *INS* gene rs689 variant either before or after adjustment for confounding factors including age, BMI, sex, smoking status, SBP, and DBP. However, the *INSR* rs1799817 “TT” genotype compared with the “CC” genotype occurred less frequently in the cases with NAFLD than the controls ($P=0.029$; OR=0.14, 95%CI=0.03-0.82). Furthermore, the *INSR* rs1799817 “TT” genotype compared with the “CC+CT” genotypes appears to be a marker of decreased NAFLD susceptibility ($P=0.048$; OR=0.20, 95%CI= 0.04-0.98).

4. Discussion

Given the important role of insulin resistance in the pathogenesis of NAFLD, our case-control study was design to clarify whether there was an association between *INS* and *INSR* gene variants and susceptibility to NAFLD risk in the Iranian population. The results of this study showed no significant differences in allele and genotype frequencies of *INS* gene rs689 variant between the cases with NAFLD and controls. However, our findings indicated for the first time that after adjustment for the confounding factors the *INSR* rs1799817 “TT” genotype compared with the “CC” genotype and “CC+CT” genotypes appears to be a marker of decreased NAFLD susceptibility.

4.1. *INSR* gene rs1799817 variant

Complex multifactorial diseases such as NAFLD result from the interaction between genetic and environmental factors. In these kinds of diseases it could be difficult to identify a majority of genes due to their relatively small individual effects and complex interactions. However, one approach of identifying novel susceptibility genes for the diseases is to study polymorphisms in candidate genes. Therefore, investigation into the association between DNA sequence variations and NAFLD has become a subject of interest in recent years. Previous epidemiologic studies have also suggested that the genes involved in insulin signaling pathway are potential candidate genes for NAFLD (Dongiovanni et al., 2010) because as mentioned earlier, the clinical and metabolic features of cases with NAFLD include the increased risk of insulin resistance, type 2 diabetes mellitus, and obesity (Fabbrini et al., 2010; Kotronen et al., 2008; Marchesini et al., 2001; Eguchi et al., 2006). However, no studies to date have evaluated the association between *INS* and *INSR* gene variants and NAFLD risk. In this study, we analyzed the

association between the rs1799817 variant in the exon 17 of *INSR* gene and NAFLD risk. The *INSR* rs1799817 “TT” genotype compared with the “CC” genotype and “CC+CT” genotypes had 86% and 80% decreased risks for NAFLD, respectively. In other words, the “TT” genotype has a protective effect on NAFLD and to the best of our knowledge this is the first study reporting that the *INSR* rs1799817 “TT” genotype is a marker of decreased NAFLD susceptibility. Since rs1799817, located in exon 17, is a "synonymous" polymorphism (His1085His) meaning that it does not alter the amino acid sequence of *INSR* protein, the exact molecular mechanism responsible for the biological effects of the variation is not known at the present time. However, there is emerging evidence for a possible role of silent polymorphisms in altering protein function (Kimchi-Sarfaty et al., 2007). The rs1799817 variant may also affect mRNA levels through regulation of mRNA splicing and/or stability. *INSR* gene contains 22 exons and the exon 17 of the gene encodes the partial region of the tyrosine kinase domain and hence, it is crucial for the function of *INSR* (Seino et al., 1990) and polymorphisms of exon 17 of *INSR* gene might play an important role in the pathogenesis of human disorders with insulin resistance such as NAFLD. Thus, a possible hypothesis is that as the “C” allele of the rs1799817 variant is less stable and translated less efficiently into *INSR* protein, reduced *INSR* abundance may impede *INSR* actions and may contribute to develop insulin resistance and NAFLD. Such a mechanism is speculative at the present but biologically plausible. In accordance with this hypothesis, however, previous studies have also shown significant associations between the *INSR* gene variants and both insulin resistance (Malodobra et al., 2011) and BMI (Gunter et al., 2007). Any defects in function of *INSR* protein might impair the biological response to insulin and lead to

insulin resistance and obesity which are involved in the etiology of NAFLD (Fabbrini et al., 2010; Marchesini et al., 2001; Eguchi et al., 2006). Interestingly, in a very recently study by Gangopadhyay et al. (2016), insulin resistance was significantly higher in the subjects with the rs1799817 ‘CC’ genotype. Accordingly, our finding that the INSR rs1799817 “TT” genotype appeared to be a marker of decreased NAFLD susceptibility is consistent with the notions above. Furthermore, it has been reported that the INSR rs1799817 “T” allele had significant protection against diabetes (Bodhini et al., 2012). Alternatively, a polymorphism like the rs1799817 do not need to be functionally relevant by itself, but may be in complete or near-complete linkage disequilibrium with a yet unknown functional variant of *INSR* gene that has functional relevance and explains the association observed in the present study. Therefore, combined with the previous findings, our finding raises the possibility that *INSR* gene may contribute to NAFLD development. Nevertheless, we cannot exclude chance as a possible explanation for the findings. For this reason, the results should be interpreted cautiously until further studies with increased numbers of NAFLD patients confirm these findings.

4.2. *INS* gene rs689 variant

This study also represented the first investigation into the association of the *INS* gene rs689 variant and NAFLD risk. This polymorphism is located in the promoter region and alterations in promoter sequence may influence protein expression. Furthermore, previous epidemiologic studies have indicated higher serum level of insulin in the cases with NAFLD than controls (Siddiqui et al., 2015) and significant associations have been found between the *INS* gene rs689 variant and Type 1 diabetes (Ayabe et al., 2016) and Type 2 diabetes (Sokhi, et al., 2016). Finally, because it is possible no clear functional

role in the regulation of *INS* gene expression can be attributed to the rs689 variant (Todd et al., 2007; Plagnol et al., 2011), it has been proposed that the variation of the DNA methylation patterns within the promoter of *INS* gene could affect its transcription (Kuroda et al., 2009; Yang et al., 2010). According to this opinion, certain genetic polymorphisms indicating significant associations with complex diseases may not have any functional effects on per se, but may be in association with epigenetic variations that have functional effects on promoter activity and gene expression. Despite the biological plausibility, however, our findings did not support the hypothesis that the *INS* gene rs689 variant might play a role in the pathogenesis of NAFLD in the Iranian population. In complex diseases, such as NAFLD, the effect of a majority of genes may be difficult to identify due to their modest individual effects and complex interactions (Lusis et al., 2008). This means that to conclude that *INS* gene is not involved in the pathogenesis of NAFLD, other *INS* gene variants should also be investigated in other populations and larger studies.

4.3. Study limitations

A number of limitations of the present study should be noted. First, the sample size of our study was small and the genotype differences may be attributable strictly to chance. Small sample size also precludes doing detailed analyses. Second, is that by testing only one variant in each gene, the coverage of the genes was incomplete. Third, is that we did not have data on serum levels of insulin and insulin resistance which could modify the effects observed here. Given the fact that we made a comparison between the cases with NAFLD and controls with BMI<25, the lower prevalence of the “T” allele in the cases could suggest this allele either may reduce the risk of developing NAFLD per se or

decrease the risk of insulin resistance – as it has been reported in a study by Gangopadhyay et al. (2016). In contrast, however, in another study by Mukherjee et al. (2009), “T” allele was a risk factor for insulin resistance. There is no simple explanation for this discrepancy. But, these inconsistent results may be due to false positive results, differences in genetic makeup, or statistical methods. Nevertheless, in spite of these limitations, we used liver biopsy as the gold standard for diagnosing NAFLD and NASH, and our study protocol was well designed and certainly provides interesting information and the possibility of true finding should not be excluded.

4.4. Conclusions

In conclusion, to our knowledge, this study shows for the first time that the *INSR* rs1799817 “TT” genotype compared with the “CC” genotype and “CC+CT” genotypes appears to be a protective factor for NAFLD susceptibility. This observation is relevant from a scientific standpoint; however, further large-scale studies will be needed to firmly establish the relationships between *INSR* gene and NAFLD risk. Furthermore, our findings do not support a role for effect of the *INS* gene rs689 variant on NAFLD in the Iranian population.

Acknowledgments

The authors thank all patients and healthy blood donors for providing blood samples.

This project was supported by grants from Iran National Science Foundation (INSF) and Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences.

References

- Ayabe T., Fukami M., Ogata T., et al., 2016. Variants associated with autoimmune type 1 diabetes in Japanese children: implications for age-specific effects of cis-regulatory haplotypes at 17q12-q21. *Diabet. Med.* (In Press)
- Bellentani S., Scaglioni F., Marino M., Bedogni G., 2010. Epidemiology of non-alcoholic fatty liver disease. *Dig. Dis.* 28, 155-161.
- Bodhini D., Sandhiya M., Ghosh S., et al., 2012. Association of His1085His INSR gene polymorphism with type 2 diabetes in South Indians. *Diabetes Technol. Ther.* 14, 696-700.
- Brunt E.M., Janney C.G., Di Bisceglie A.M., et al., 1999. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am. J. Gastroenterol.* 94, 2467-2474.
- Dongiovanni P., Valenti L., Rametta R., et al., 2010. Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. *Gut* 59, 267-273.
- Eguchi Y., Eguchi T., Mizuta T., et al., 2006. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. *J. Gastroenterol.* 41, 462-469.
- Fabbrini E., Sullivan S., Klein S., 2010. Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. *Hepatology* 51, 679-689.
- Gangopadhyay S., Agrawal N., Batra A., Kabi B.C., Gupta A., 2016. Single nucleotide polymorphism on exon 17 of insulin receptor gene influences insulin resistance in PCOS: a pilot study on North Indian women. *Biochem. Genet.* 54, 158-168.

- Gholam P.M., Flancbaum L., Machan J.T., et al., 2007. Nonalcoholic fatty liver disease in severely obese subjects. *Am. J. Gastroenterol.* 102, 399-408.
- Gunter M.J., Hayes R.B., Chatterjee N., et al., 2007. Insulin resistance-related genes and advanced left-sided colorectal adenoma. *Cancer Epidemiol. Biomarkers Prev.* 16, 703-708.
- Kimchi-Sarfaty C., Oh J.M., Kim I.W., et al., 2007. A silent polymorphism in the MDR1 gene changes substrate specificity. *Science* 315, 525-528.
- Kotronen A., Juurinen L., Hakkarainen A., et al., 2008. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care* 31, 165-169.
- Kuroda A., Rauch T.A., Todorov I., et al., 2009. Insulin gene expression is regulated by DNA methylation. *PLoS One* 4, e6953.
- Li M., Zhang S., Wu Y., et al. 2015. Prevalence of insulin resistance in subjects with nonalcoholic fatty liver disease and its predictors in a Chinese population. *Dig. Dis. Sci.* 60, 2170-2176.
- Lusis A.J., Attie A.D., Reue K., 2008. Metabolic syndrome: from epidemiology to systems biology. *Nat. Rev. Genet.* 9, 819-830.
- Malodobra M., Pilecka A., Gworys B., Adamiec R., 2011. Single nucleotide polymorphisms within functional regions of genes implicated in insulin action and association with the insulin resistant phenotype. *Mol. Cell Biochem.* 349, 187-193.
- Marchesini G., Brizi M., Bianchi G., et al., 2001. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50, 1844-1850.

- Mukherjee S., Shaikh N., Khavale S., et al., 2009. Genetic variation in exon 17 of INSR is associated with insulin resistance and hyperandrogenemia among lean Indian women with polycystic ovary syndrome. *Eur. J. Endocrinol.* 160, 855-862.
- Plagnol V., Howson J.M., Smyth D.J., et al., 2011. Genome-wide association analysis of autoantibody positivity in type 1 diabetes cases. *PLoS Genet.* 7, e1002216.
- Seino S., Seino M., Bell G.I., 1990. Human insulin receptor gene: partial sequence and amplification of exons by polymerase chain reaction. *Diabetes* 39, 129-133.
- Siddiqui M.S., Fuchs M., Idowu M., et al. 2015. Severity of nonalcoholic fatty liver disease and progression to cirrhosis associate with atherogenic lipoprotein profile. *Clin. Gastroenterol. Hepatol.* 13, 1000-1008.
- Sokhi J., Sikka R., Raina P., et al., 2016. Association of genetic variants in INS (rs689), INSR (rs1799816) and PP1G.G (rs1799999) with type 2 diabetes (T2D): a case-control study in three ethnic groups from North-West India. *Mol. Genet. Genomics* 291, 205-216.
- Todd J.A., Walker N.M., Cooper J.D., et al., 2007. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat. Genet.* 39, 857-864.
- White M.F., 1997. The insulin signaling system and the IRS proteins. *Diabetologia* 40, S2-17.
- Yang B.T., Dayeh T.A., Kirkpatrick C.L., et al., 2010. Insulin promoter DNA methylation correlates negatively with insulin gene expression and positively with HbA(1c) levels in human pancreatic islets. *Diabetologia* 54, 360-367.

Table 1. Information for the studied markers in *INS* (insulin) and *INSR* (insulin receptor) genes

Gene (SNP ID)	Location (Base change)	Forward Primer Reverse Primer	PCR program (35 cycles)	PCR fragment size (bp)	Restriction enzyme, Incubation temperature	Alleles: RFLP fragments size (bp)
<i>INS</i> (rs689)	Promoter (T/A)	5'-TCCAGGACAGGCTGCATCAG-3' 5'-AGCAATGGGCGGTTGGCTCA-3'	93 °C 45s, 57 °C 30s, 72 °C 45s	441	Alw26I, 37 °C	Allele A: 441 Allele T: 230+211
<i>INSR</i> (rs1799817)	Exon 17 (T/C)	5'-CCAAGGATGCTGTGTAGATAAG-3' 5'-TCAGGAAAGCCAGCCCATGTC-3'	93 °C 45s, 60 °C 30s, 72 °C 45s	317	Eco72I, 37 °C	Allele T: 317 Allele C: 274+43

Table 2. General characteristics of the study groups ^a

Variables	Controls (n=128)	Cases (n=102)	P-value
Age (years)	31.8(8.1)	40.7(10.1)	<0.001
BMI(kg/m ²)	22.9(2.8)	28.7(4.9)	<0.001
Gender			
Men	71(55.5)	81(79.4)	
Women	57(44.5)	21(20.6)	<0.001
Smoking status			
Never smoker	119(93.0)	73(71.6)	
Former smoker	6(4.7)	18(17.6)	
Current smoker	3(2.3)	11(10.8)	<0.001
SBP (mmHg)	113.7(10.3)	122.8(16.8)	<0.001
DBP (mmHg)	70.4(8.2)	75.1(10.4)	<0.001
AST (IU/L)	19.6(6.6)	37.6(16.7)	<0.001
ALT (IU/L)	19.4(9.8)	66.4(38.3)	<0.001
GGT (IU/L)	18.3(7.9)	53.7(29.3)	<0.001
Steatosis			
Grade 0		-	
Grade 1		29(28.4)	
Grade 2		54(53.0)	
Grade 3		19(18.6)	
Necroinflammation			

Grade 0	34(33.3)
Grade 1	38(37.3)
Grade 2	28(27.5)
Grade 3	2(1.9)
Fibrosis	
Stage 0	67(65.7)
Stage 1	32(31.4)
Stage 2	3(2.9)
Stage 3	-
Stage 4	-

^a Variables presented as mean (SD) or number (%) ; BMI: Body mass index, SBP: Systolic blood pressure, DBP: diastolic blood pressure, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase.

Table 3. Association between genotypes and alleles of insulin (*INS*) and insulin receptor (*INSR*) gene variants and risk of nonalcoholic fatty liver disease ^a

Gene (Variant)	Controls (n=128)	Cases (n=102)	OR (95% CI) P-value ^b
<i>INS</i> (rs689) ^c			
Genotype-wise comparison			
AA	84(67.7)	61(61.6)	1.0(reference)
AT	35(28.3)	36(36.4)	2.28(0.90-5.76)0.081
TT	5(4.0)	2(2.0)	0.83(0.10-6.79)0.859
AT and TT	40(32.3)	38(38.4)	2.00(0.83-4.85)0.121
TT versus others	5(4.0)	2(2.0)	0.61(0.08-4.87)0.643
Allele-wise comparison			
A	203(81.9)	158(79.8)	1.0(reference)
T	45(18.1)	40(20.2)	1.14(0.71-1.85)0.583
<i>INSR</i> (rs1799817) ^d			
Genotype-wise comparison			
CC	80(67.2)	69(70.4)	1.0(reference)
CT	24(20.2)	22(22.5)	0.63(0.24-1.65)0.342
TT	15(12.6)	7(7.1)	0.14(0.03-0.82)0.029
CT and TT	95(79.8)	76(77.5)	0.54(0.21-1.38)0.195
TT versus others	15(12.6)	7(7.1)	0.20(0.04-0.98)0.048
Allele-wise comparison			
C	184(77.3)	160(81.6)	1.0(reference)

T	54(22.7)	36(18.4)	0.86(0.58-1.25) 0.419
---	----------	----------	-----------------------

^a Variables presented as number (%)

^b Adjusted for age, body mass index (BMI), sex, smoking status, systolic blood pressure (SBP), and diastolic blood pressure (DBP) in genotype-wise comparisons

^c Distribution of *INS* gene variant in 124 controls and 99 cases

^d Distribution of *INSR* gene variant in 119 controls and 98 cases