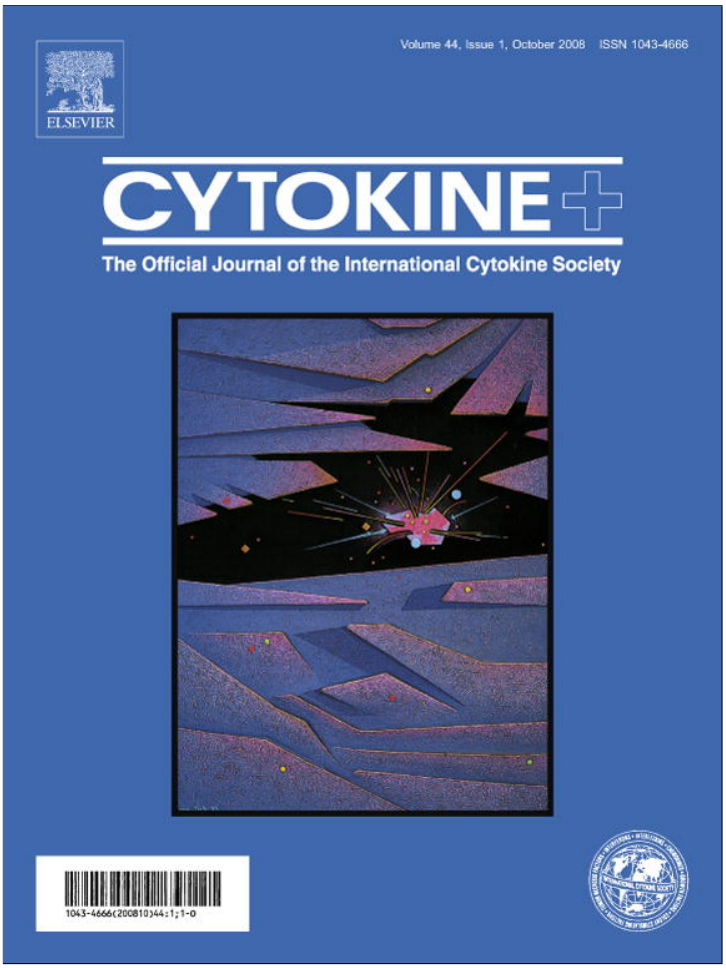

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Genetic variants in *STAT3* are associated with nonalcoholic fatty liver disease

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ABSTRACT

Aims: To investigate the role of gene variants and derived haplotypes of the *STAT3* transcription factor in nonalcoholic fatty liver disease (NAFLD) and their relation with the clinical disease severity. *Patients and methods:* 108 patients with NAFLD and different stages of clinical disease severity, and a group of 55 healthy individuals were included in a Hospital-based study. We selected 3 tagSNPs showing a minor allele frequency >10% (rs2293152 C/G, rs6503695 C/T, and rs9891119 A/C) encompassing 68.55 kb in chromosome 17, representing 24 polymorphic sites ($r^2 > 0.8$). *Results:* In univariate analysis, there were significant differences in the allele frequency of the rs6503695 and rs9891119 between the healthy individuals and NAFLD patients (empiric $P = 0.021$ and 0.020, respectively). The test results for the multimarker analysis showed that haplotypes TA and CC of tagSNPs rs6503695, rs9891119 were significantly associated with NAFLD (empiric $P = 0.035$ and 0.015, respectively). When we tested the hypothesis of a relation between the gene variants and the clinical and histological spectrum of NAFLD by multinomial analysis, a significant association was observed with rs9891119 ($P = 0.02$). *Conclusions:* Our study suggests a potential role of the *STAT3* polymorphisms and their haplotypes in susceptibility to NAFLD and disease severity.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common abnormality observed in patients with metabolic syndrome [1] and refers to a wide spectrum of liver diseases ranging from fatty liver alone to nonalcoholic steatohepatitis (NASH) with evidence of liver cell injury, a mixed inflammatory lobular infiltrate, and variable fibrosis [2].

It was previously shown that most patients with NASH are also insulin resistant [3]. Recent data indicate that NAFLD should be considered the hepatic manifestation of the metabolic syndrome [4]. In fact, there is a nearly-universal association between both clinical entities as insulin resistance is a major contributor in the pathogenesis and disease progression of NAFLD [5].

Signal Transducer and Activator of Transcription 3 (*STAT3*)—initially described as an acute-phase protein and also as an ubiquitous transcription factor indispensable during early embryogenesis—contributes to various metabolic processes and, possibly, to the pathogenesis of certain diseases such as the metabolic syndrome.

For instance, it was reported that mice lacking *STAT3* specifically in the liver have insulin resistance and glucose intolerance when fed a high-fat diet, showing that hepatic *STAT3* signaling is essential for normal glucose homeostasis [6]. Interestingly, restoration of hepatic *STAT3* expression in these mice by using an adenovirus-mediated gene transfer, corrected the metabolic abnormalities and the alterations in the hepatic expression of gluconeogenic genes [6].

Moreover, it was shown that *STAT3* plays an important role in the induction of liver acute-phase genes in response to bacterial lipopolysaccharide [7], suggesting that *STAT3* is a key regulator of the anti-inflammatory signaling pathway.

Finally, previous studies have suggested that *STAT3* plays a critical role in the regulation of mammalian body weight and energy homeostasis [8].

Consequently, in view of the evidence mentioned above, we hypothesized that *STAT3* gene variants and their predicted haplotypes of linkage disequilibrium (LD) blocks may contribute to the susceptibility of NAFLD. Additionally, we tested the hypothesis of

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a relation between gene variants and clinical and biochemical disease severity. Here, then, we performed a candidate gene case-control association study.

2. Patients and methods

Between October 2005 and June 1, 2007 we performed a cross sectional study on NAFLD in a county Hospital of the city of Buenos Aires. The study involved 108 consecutive unrelated patients (30 males and 78 females) with features of NAFLD, including ultrasonographic examinations (US) suggestive of fatty infiltration [9] performed by the same operator.

Secondary causes of steatosis, including alcohol abuse ($\geqslant$30 g alcohol daily for men and $\geqslant$20 g for women), total parenteral nutrition, hepatitis B and hepatitis C virus infection, and the use of drugs known to precipitate steatosis were always excluded. By using standard clinical and laboratory evaluation as well as liver biopsy features when applicable, autoimmune liver disease, metabolic liver disease, Wilson's disease, and α-1-antitrypsin deficiency were likewise ruled out in all patients.

For the evaluation of the clinical and biochemical disease severity, NAFLD cases were classified as follows: fatty liver with persistently normal liver function test during 12 months of follow-up (FL-NLFT), fatty liver with persistently abnormal liver function test (FL-ALFT), and NASH proven through biopsy as described below. Patients were defined to have abnormal liver function test in the presence of at least one of the following biochemical criteria: (1) elevated serum alanine (ALT) and/or aspartate aminotransferase (AST), defined as >41 U/L, (2) gamma-glutamyl-transferase (GGT) >50 U/L, and (3) alkaline phosphatase (AP) >250 U/L.

Additionally, 55 healthy individuals (18 males and 37 females) with the same demographic background and who underwent an annual health examination during the same study period were included in the study as an additional control group. All healthy controls were subjected to US. None of them evidenced fatty change, biochemical abnormalities or features indicative of metabolic syndrome.

2.1. Physical, anthropometric and biochemical evaluation

Health examinations included anthropometric measurements, a questionnaire on health-related behaviors, and biochemical determinations.

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2) and was used as the index for relative weight. Additionally, waist and hip circumference were also assessed. Blood was drawn from fasting subjects who had lain in a supine resting position for at least 30 min. Serum insulin, total cholesterol, HDL and LDL-cholesterol, triglycerides, plasma glucose and liver function tests were measured by standard clinical laboratory techniques. Homeostasis Model Assessment (HOMA) was used to evaluate an insulin resistance index and was calculated as fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5. Elevated blood pressure was defined as systolic arterial blood pressure (SABP) $\geqslant$ 130 mm Hg and/or DABP $\geqslant$ 85 mm Hg or receipt of anti-hypertensive medications.

All the investigations performed in this study were conducted in accordance with the guidelines of The Declaration of Helsinki. Written consent from individuals was obtained in accordance with the procedures approved by the Ethical Committee of our institution.

2.2. Liver biopsies and histopathological evaluation

A percutaneous liver biopsy (LB) was performed in 68 patients that showed US fatty changes plus persistently abnormal liver function tests (in at least three different determinations in a follow-up 12 month period). LB was performed with ultrasound guidance and modified 1.4 mm diameter Menghini needles (Hepafix, Braun, Germany) on an outpatient basis. Liver biopsy specimens were routinely fixed in 40 g/L formaldehyde (pH 7.4) embedded in paraffin and stained with hematoxylin and eosin, Masson trichrome and silver impregnation for reticular fibers. The same liver pathologist, who was blinded to patient details, read all biopsies. The diagnosis of NASH was confirmed by liver histology, and grading necroinflammatory activity or staging fibrosis was scored according to the system developed by Brunt et al. [10]. NASH was defined as steatosis plus any stage of fibrosis or as steatosis plus lobular inflammation plus ballooning degeneration [2].

2.3. Genotype and haplotype analysis

The genetic analyses were done on genomic DNA extracted from white blood cells by a standard method as previously described [11].

To assess the contribution of *STAT3* gene variants to NAFLD, we selected tag SNPs by using an aggressive tagging approach to construct single-marker and multi-marker tests (test based on combinations of tags) to capture alleles of interest [12] and the phase II genotyping data from the HapMap project for Caucasians from the CEU dataset with a minor allele frequency (MAF) $\geqslant$0.10 and a minimum r^2 of 0.8.

Genotyping was performed by a high-throughput genotyping method involving PCR amplification of genomic DNA with two tailed allele-specific primers that introduce priming sites for universal energy-transfer-labeled primers as previously described [13].

To ensure genotyping quality, we included DNA samples as internal controls, hidden samples of known genotype, and negative controls (water). Genotypes with a signal below a negative control were not scored. The analysis error was estimated by replicating eight times a blinded sample (always belonging to the same individual) across the templates of the project. On 216 genotypes for the "blinded sample", we had only 1 not-matched genotype (0.46% error); then, the observed error rate is estimated to be less than 0.5%. Overall genotype completion rate was 85.18% and 87.27% for rs2293152 and 96.29% and 96.36% for rs6503695 in cases and controls, respectively, and 100% in both cases and controls for rs9891119.

To explore a possible stratification in the population we used a collection of 13 SNPs at different loci (located in chromosome 4, 15, 17, 13, 1, and 3) and then analyzed the data with the Structure program Version 2 [14]. We found no evidence of stratification in our sample, because cases and controls showed similar *Q* values and were assigned with a similar distance to clusters by the program Structure with no further improvement in the fitting model by adding up to four clusters (the ln of likelihood was maximum for $K = 1$).

A a-priori power estimation for the utilized sample was performed for single-point allelic effects, odds ratio of 1.5, at a nominal significance level of 0.05 for HapMap-predicted MAF of 0.457 (rs2293152) to 0.26 (rs6503695 and rs9891119) of a potential susceptibility marker and a 30% prevalence of the disease. This analysis gave us an estimated power of 70% under the additive model for both markers, and 84% for the multiplicative model for rs2293152 and 79% for rs6503695 and rs9891119.

The PLINK software was used for assessing the association between SNPs and the affection status and quantitative traits as well as for testing Hardy–Weinberg equilibrium and LD measures [15]. SNP haplotype analysis was performed by Haploview software [16]. This tool was also used to obtain haplotype frequencies. Control for multiple testing was done by permutation testing (100,000

permutations) of individual traits to obtain an empirical *P*-value. Differences in genotype frequencies between cases and controls were analyzed as described using PLINK software. Multi-marker haplotype test was performed by Haploview.

2.4. Statistical analysis

Phenotypic quantitative data were expressed as means ± SE. For univariate analysis and to avoid any assumption about variable distribution and homoscedasticity, differences between groups were assessed by the non-parametric Mann–Whitney Test. For testing the association between markers and disease severity, we used a regression analysis for a multinomial distribution (Logit as the Link function) with disease severity as the dependent (response) variable coding controls, FL-NLFT, FL-ANFT and NASH subjects as 0, 1, 2, and 3, respectively; HOMA and BMI as continuous predictor variables and genotypes as a grouping variable. We used the CSS/Statistica program package, StatSoft V 6.0 (Tulsa, USA) to perform these analyses.

3. Results

Clinical features, anthropometric variables and laboratory findings at diagnosis available in patients and healthy individuals are shown in Table 1. NAFLD patients were older and showed most of the risk factors of the metabolic syndrome: elevated BMI, waist-hip ratio, fasting insulin, and HOMA index.

In the patients' group, 40 out of 108 were classified as having FL-NLFT, 23 as FL-ANFT, and 45 as NASH proven through biopsy. Patients in the FL-NLFT showed persistently normal ALT, AST, AP, and GGT.

3.1. STAT3 gene variants

The *STAT3* gene contains 24 exons and spans over 75.17 kb in chromosome 17q21 at location 37.718.69–37.794.039. To diminish the burden of genotyping the complete number of variants of the *STAT3* (The HapMap B35 full set database includes 28 polymorphic sites with MAF > 0.05) we selected 3 tagSNPs showing a MAF > 10% (rs2293152 C/G, rs6503695 C/T, and rs9891119 A/C) encompassing 68.55 kb of the gene. The 3 tagSNPs represent 24 polymorphic sites with an $r^2 > 0.8$ considering the HapMap project data. Table 2 illustrates the tagSNPs description. Test results from the *Tagger* algorithm showed the above mentioned single-marker tags and 3 multi-marker tags (haplotypes composed of 2 or 3 markers that capture additional variants) capturing all SNPs of MAF $\geqslant$ 5% (Table 3). The haplotype TA of the multi-marker composed of tagSNPs rs6503695–rs9891119 captures two additional single-markers with an $r^2 > 0.8$. Additionally, the haplotype CC of the multi-marker composed of the same tagSNPs captures 12 additional variants, five of them in high LD showing an $r^2 > 0.8$. Finally, the haplotype TC captures two additional variants with high LD. However, this haplotype was found at a very low frequency.

Table 1
Clinical and biochemical characteristics of the studied individuals

Variables	Healthy individuals	NAFLD patients	Nominal *P* value
Number of subjects	55	108	
Age, years	46.2 ± 1.3	56.0 ± 1.12	0.00001
BMI (kg/m^2)	25.65 ± 0.68	36.09 ± 3.33	0.00001
Waist-hip ratio	0.84 ± 0.01	0.91 ± 0.01	0.00001
SABP (mm Hg)	120.77 ± 1.8	124.71 ± 1.58	NS
DABP 8(mm Hg)	75.81 ± 1.36	78.93 ± 1.04	NS
Fasting plasma glucose (mmol/L)	4.75 ± 0.08	5.88 ± 0.23	0.00004
Fasting plasma insulin (pmol/L)	45.83 ± 3.36	94.86 ± 7.35	0.00001
HOMA index	1.43 ± 0.12	3.55 ± 0.32	0.00001
Total cholesterol (mmol/L)	6.05 ± 0.14	5.59 ± 0.14	NS
HDL cholesterol (mmol/L)	1.10 ± 0.08	1.21 ± 0.05	NS
LDL-cholesterol (mmol/L)	2.98 ± 0.23	3.16 ± 0.15	NS
Uric acid (μmol/L)	220.07 ± 6.82	243.86 ± 2.63	NS
Triglycerides (mmol/L)	1.74 ± 0.02	2.02 ± 0.01	NS
ALT (U/L)	19.0 ± 2.2	45.0 ± 3.42	0.003
AST (U/L)	19.83 ± 1.77	37.5 ± 2.44	0.001
γGT (U/L)	19.66 ± 2.52	57.12 ± 5.81	0.043
AP (U/L)	232.5 ± 15.9	248.2 ± 12.1	NS

NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; SABP and DABP, systolic and diastolic arterial blood pressure; HOMA; homeostatic model assessment; ALT and AST, serum alanine and aspartate aminotransferase; γGT, gamma-glutamyl-transferase; AP, alkaline phosphatase. Results are expressed as means ± SE. Nominal *P* value stands for statistical significance using Mann–Whitney test. NS, non significant.
All measurements are in SI units.

Table 2
Characteristics of the Tag Single Nucleotide Polymorphisms of the *STAT3* gene genotyped in the study

NCBI SNP reference[a]	Location in the *STAT3* gene	Hetero-zygosity	dsSNP allele	Minor allele	MAF
rs2293152	Intron 13	0.49	C/G	C	0.38
rs6503695	Intron 2	0.47	C/T	C	0.37
rs9891119	Intron 1 (non-coding region near the gene promoter)	0.42	A/C	C	0.37

MAF, minor allele frequency (within controls in the study). *STAT3*, Signal Transducers and Activator of Transcription 3.
[a] Single Nucleotide Polymorphisms on NCBI Reference Assembly.

The distribution of the genotypes was in Hardy–Weinberg equilibrium (data not shown).

In univariate analysis, after multiple comparison correction by permutation tests, there were significant differences in the allele frequency of the rs6503695 and rs9891119 between the control group and NAFLD patients (empiric *P* value = 0.021 and 0.020, respectively).

Association studies of single-tagSNPs of the *STAT3* with NAFLD using extended Mantel–Haenszel test for trend and genotype counts according to disease status are shown in Table 4.

Test results for the multi-marker analysis showed that the haplotype TA of tagSNPs rs6503695 and rs9891119 (capturing rs744166 and rs12949918 with an $r^2 \geqslant 0.8$) and haplotype CC of tagSNPs rs6503695 and rs9891119 (a tag for rs8069645, rs6503696, rs6503697, rs4103200, and rs9912773 with an $r^2 \geqslant 0.8$) were significantly associated with NAFLD (Table 5), and the association remained after multiple testing correction by permutation test.

When we tested the hypothesis of a relation between the gene variants and the clinical and histological spectrum of NAFLD (disease severity by using the variable coding of disease' grade ranging from healthy subjects to nonalcoholic steatohepatitis patients as follows: controls, FL-NLFT, FL-ANFT, and NASH subjects as 0, 1, 2, and 3), a significant association was observed with the rs9891119 A allele (nominal $P = 0.02$, Spearman Rank Test). Using a multinomial with Logit function test, this association (χ^2: 15.02, $p = 0.02$) persisted after adjusting for HOMA (χ^2: 22.36, $p = 0.000055$) and BMI (χ^2: 53.88, $p = 1 \times 10^{-11}$) as independent continuous predictor variables. Then, we observed significantly higher scores of disease severity in individuals carrying the AA genotype (1.73 ± 0.16) in comparison with AC genotype (1.10 ± 0.21) and CC genotype (1.14 ± 0.36), $p < 0.04$. A more robust difference was observed in the dominant model of inheritance: AA

Table 3
Test results from the *Tagger* algorithm that we used to select single and multi-marker tests to capture all SNPs of MAF ⩾ 5% and $r^2 \leqslant 0.8$

SNP ID	MB Position	Allele capture	Freq	Tagging test	Genotype	r^2
rs1053023	37719142	T	0.8417	rs6503695, rs9891119	C,C	0.438
rs1053005	37719436	T	0.8417	rs6503695, rs9891119	C,C	0.438
rs3744483	37719964	T	0.8417	rs6503695, rs9891119	C,C	0.438
rs8074524	37723124	C	0.8417	rs6503695, rs9891119	C,C	0.438
rs3809758	37725506	C	0.8417	rs6503695, rs9891119	C,C	0.438
rs8078731	37733907	A	0.8583	rs6503695, rs9891119	C,C	0.37
rs2293152	37735055	C	0.6	rs2293152	C	1
rs2306580	37745206	C	0.8917	rs6503695, rs9891119	T,C	0.92
rs8069645	37748428	A	0.7583	rs6503695, rs9891119	C,C	1
rs7217655	37749550	C	0.65	rs9891119	A	0.964
rs3816769	37751799	T	0.65	rs9891119	A	0.964
rs6503695	37753059	T	0.675	rs6503695	T	1
rs6503696	37753330	C2	0.7583	rs6503695, rs9891119	C,C	1
rs6503697	37755105	A	0.7583	rs6503695, rs9891119	C,C	1
rs4103200	37760591	G	0.7583	rs6503695, rs9891119	C,C	1
rs9891119	37761506	A	0.6417	rs9891119	A	1
rs9912773	37764060	C	0.775	rs6503695, rs9891119	C,C	0.911
rs17593222	37766516	C	0.8917	rs6503695, rs9891119	T,C	0.92
rs744166	37767727	A	0.5583	rs6503695, rs9891119	T,A	0.869
rs3785898	37768646	C	0.75	rs6503695, rs9891119	C,C	0.788
rs957970	37773416	A	0.6417	rs9891119	A	0.86
rs12949918	37779799	T	0.5667	rs6503695, rs9891119	T,A	0.838
rs1026916	37783361	G	0.6417	rs9891119	A	0.86
rs7211777	37787601	A	0.6417	rs9891119	A	0.86

SNP ID, Single Nucleotide Polymorphisms on NCBI Reference Assembly; MB position, Mapped chromosome position (International HapMap Project).

(1.73 ± 0.16) vs. AC+CC (1.12 ± 0.18), $p < 0.02$. In addition, rs9891119 was significantly associated with AST values (a surrogate of disease severity), as quantitative trait ($\beta = 10.14$, $p = 0.025$, linear regression analysis) in the additive model.

Finally, when we analyzed the allele frequencies of tagSNPs in patients with NASH, no association was observed between either the necroinflammatory grade or the overall fibrosis score and the tagSNPs (data not shown).

4. Discussion

We examined the genetic influence of gene variants and their haplotypes of the LD block of the *STAT3* factor on NAFLD and found that, in the analysis of individual markers, rs6503695 and rs9891119 were significantly associated with the disease being the rs6503695-T and rs9891119-A allele carriers 2.3 and 2.5-fold, respectively, more likely to have NAFLD in comparison with non-carriers. Moreover, the test of haplotypes based on multi-marker predictors showed that frequencies of haplotypes TA and CC of tag SNPs rs6503695 and rs9891119 in NAFLD individuals significantly differed from those in healthy subjects showing that the haplotype TA confers an almost 2-fold increase in the risk of suffering from NAFLD and haplotype CC confers a 2.5-fold protection against NAFLD.

Additionally, we evaluated the role of the gene variants in the clinical disease severity and observed a significant association between the clinical or histological spectrum of NAFLD and the rs9891119 tagSNP located near the gene promoter independently of the effect of the BMI or insulin resistance.

To our knowledge, the potential contribution of *STAT3* to the NAFLD susceptibility and severity has not been described in humans and our study is the first to provide evidence of association to the disease. This significant association is hardly explained by a possible stratification of the sample as we found no evidence of substructure either in cases or controls using independent multi-markers and the program Structure [14].

Although association does not necessarily mean a causal-relation, in this case a body of evidence supports that this may be the case, particularly considering the biological plausibility of this relation. In fact, these results are consistent with several indepen-

Table 4
Genotype counts according to disease status and association study of single-tagSNP in *STAT3* with NAFLD

Single tagSNP	Genotype	Disease status		NAFLD association test		
		Control group	NAFLD	OR (95% CI)	Cumulative OR (95% CI)	*P* value
	GG	18	27			
rs2293152	CG	24	35	0.97 (0.44–2.13)	2.05 (1.07–3.93)	0.037
	CC	6	30	3.33 (1.18–9.44)		
	CC	4	6			
rs6503695	CT	25	34	2.04 (0.53–7.84)	2.32 (1.20–4.48)	0.011
	TT	24	64	4.00 (1.05–15.18)		
	CC	8	6			
rs9891119	AC	21	28	1.77 (0.55–5.79)	2.54 (1.31–4.91)	0.005
	AA	26	74	3.79 (1.22–11.83)		

Odds ratios (OR) and 95% confidence intervals (95% CI) in relation to the first genotype. Cumulative OR using proportional odds model (Liu–Agresti method) [26] is also indicated. *P* value stands for two-sided alternative (cases ≠ controls) significance from the extended Mantel–Haenszel (MH) test for trend.

Table 5
Association study of multi-marker tag SNP tests (haplotypes) in *STAT3* with NAFLD

Multi-marker tagSNP test	Associated haplotype	Frequency	OR (95% CI)	Nominal *P* value	Empirical *P* value
rs6503695, rs9891119	TA	0.738	1.96 (1.18–3.26)	0.0097	0.035
rs6503695, rs9891119	CC	0.220	0.39 (0.23–0.66)	0.0039	0.015
rs6503695, rs9891119	TC	0.019	0.50 (0.10–2.52)	0.42	0.89

Odds ratios (OR) and 95% confidence intervals (95% CI). Empiric *P* value stands for statistical significance adjusted for multiple testing by permutation test.

dent observations in animal models. For instance, previous studies in *STAT3* mutant mice showed that these animals develop insulin resistance associated with an increased glucose production [6,17]. Moreover, the disruption of neural *STAT3* causes obesity, diabetes, infertility, and thermal dysregulation [18].

On the other hand, it was shown that IL-6 through *STAT3* signaling in the liver contributes to the brain insulin action leading to the suppression of hepatic glucose production [17,19].

As a final point, *STAT3* is able to activate several pathways related with liver regeneration and acute inflammatory reaction after hepatocyte necrosis [20]. This particular role of the *STAT3* protein is important considering that the immunologic response occurring in fatty liver may be a factor that impairs the liver regenerative capacity.

It is well known that NAFLD—like other complex diseases—is polygenic and multifactorial, and the disorder develops from the interplay between genes and the environment. In view of the critical role that insulin resistance and obesity play in the pathogenesis of NAFLD, it is reasonable to speculate that *STAT3* variants may be involved in the molecular pathogenesis of NAFLD.

Although on the basis of clinical data and follow-up studies NAFLD was initially regarded as a benign disorder, it is currently known that, in some but not all affected individuals, the disease may progress to more severe clinical forms. In fact, even after exposure to the same risk factors (either dietary or lifestyle-related aspects), it is not clear why some persons develop simple steatosis, whereas others progress to severe cirrhosis. However, NASH is observed only in a fraction of patients with NAFLD clearly suggesting a genetic predisposition. We observed that when we divided the NAFLD patients into categories of cases according to the clinical, biochemical or histological spectrum, a significant association was seen between the disease progression and the rs9891119 tagSNP.

To perform liver biopsies on asymptomatic patients without evidence of abnormality during a long follow-up period in none of the LFT (ALT, AST, AP, and GGT) is, at least, questionable, particularly because no intervention besides lifestyle measures should be recommend. In our study we pre-classified patients for clinical disease severity evaluation according to a panel of 4 LFT monitored during a 12-month follow-up period as it was shown that liver biopsy is invasive, costly, and prone to severe complications that have been reported to occur in 0.57% [21].

This issue could be a drawback in our study when making conclusions about histological disease severity. However, it is noteworthy that we did not pre-classified patients taking into account an isolated value of ALT and AST. By the contrary, we stratified and selected for biopsy those patients who showed a combination of abnormal LFT, which were previously shown to be strongly associated with disease severity and its complications [22–24].

In summary, genetic factors contribute to virtually every human disease by conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental factors that modify disease course and expression.

Our study suggests a potential role of *STAT3* variants and their haplotypes in an increased susceptibility to NAFLD and disease progression in our population.

Understanding how the reported variants can affect the *STAT3* function may require additional studies. However, it is worth mentioning that even robust genetic findings cannot always be readily explained at a functional level; however, they are seeds for future research [25].

We hope our study can serve as a primer because further research is needed to confirm and extend the current findings in larger populations to reveal the intimate mechanism by which the *STAT3* variants may lead fatty liver disease.

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References

[1] Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology 2002;35:367–72.
[2] Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37:1202–19.
[3] Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, et al. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373–9.
[4] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–23.
[5] Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: a potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology 2001;34:738–44.
[6] Inoue H, Ogawa W, Ozaki M, Haga S, Matsumoto M, Furukawa K, et al. Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo. Nat Med 2004;10:168–74.
[7] El Kasmi KC, Holst J, Coffre M, Mielke L, de PA, Lhocine N, et al. General nature of the STAT3-activated anti-inflammatory response. J Immunol 2006;177:7880–8.
[8] Cui Y, Huang L, Elefteriou F, Yang G, Shelton JM, Giles JE, et al. Essential role of STAT3 in body weight and glucose homeostasis. Mol Cell Biol 2004;24:258–69.
[9] Mendler MH, Bouillet P, Le Sidaner A, Lavoine E, Labrousse F, Sautereau D, et al. Dual-energy CT in the diagnosis and quantification of fatty liver: limited clinical value in comparison to ultrasound scan and single-energy CT, with special reference to iron overload. J Hepatol 1998;28:785–94.
[10] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–74.
[11] Kawasaki ES. Sample preparation from blood, cells, and other fluids. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols. A guide to methods and applications. San diego: Academic Press, Inc.; 1990. p. 146–52.
[12] de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet 2005;37:1217–23.
[13] Myakishev MV, Khripin Y, Hu S, Hamer DH. High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. Genome Res 2001;11:163–9.
[14] Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000;155:945–59.
[15] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
[16] Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
[17] Yang SQ, Lin HZ, Mandal AK, Huang J, Diehl AM. Disrupted signaling and inhibited regeneration in obese mice with fatty livers: implications for nonalcoholic fatty liver disease pathophysiology. Hepatology 2001;34:694–706.
[18] Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, et al. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. Proc Natl Acad Sci USA 2004;101:4661–6.
[19] Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, et al. Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. Cell Metab 2006;3:267–75.
[20] Moh A, Iwamoto Y, Chai GX, Zhang SS, Kano A, Yang DD, et al. Role of STAT3 in liver regeneration: survival, DNA synthesis, inflammatory reaction and liver mass recovery. Lab Invest 2007;87:1018–28.
[21] Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). Hepatology 2000;32:477–81.
[22] Lee DS, Evans JC, Robins SJ, Wilson PW, Albano I, Fox CS, et al. Gamma glutamyl transferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart Study. Arterioscler Thromb Vasc Biol 2007;27:127–33.

[23] Nannipieri M, Gonzales C, Baldi S, Posadas R, Williams K, Haffner SM, et al. Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City diabetes study. Diabetes Care 2005;28:1757–62.
[24] Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care 1998;21:732–7.
[25] Craddock N, Owen MJ, O'donovan MC. The catechol-*O*-methyl transferase (COMT) gene as a candidate for psychiatric phenotypes: evidence and lessons. Mol Psychiatry 2006;11:446–58.
[26] Liu IM, Agresti A. Mantel–Haenszel-type inference for cumulative odds ratios with a stratified ordinal response. Biometrics 1996;52:1223–34.