RESEARCH ARTICLE

Association between urine retinol-binding protein levels and nonalcoholic fatty liver disease: A cross-sectional study in Chinese population

Juanwen Zhang¹ | Zeyu Liu² | Xuyao Zhang³ | Li Zhang⁴ | Xi Jin⁵

¹Department of Laboratory Medicine, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

²Department of Endocrinology, Sir Run Run Shaw Hospital Affiliated to School of Medicine, Zhejiang University, Hangzhou, China

³Clinical Medicine, Hangzhou Normal University Qianjiang College, Hangzhou, China

⁴Kidney Disease Center, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

⁵Department of Gastroenterology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Correspondence

Xi Jin, Department of Gastroenterology, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China.
Email: jxfl007@zju.edu.cn

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Objective: The prevalence of nonalcoholic fatty liver disease (NAFLD) has been rapidly increased, becoming a public health problem worldwide. Our objective was to investigate the association between urine retinol-binding protein (RBP) and NAFLD in a Chinese population and develop a multivariate logistic regression model for NAFLD prediction.

Methods: A total of 317 NAFLD patients and 391 healthy controls were enrolled in this cross-sectional study based on inclusion and exclusion criteria, from whom fasting urine and blood were collected for further study. Urine RBP level and other parameters were measured and compared between NAFLD subjects and controls.

Results: Urine RBP levels (expressed by RBP/creatinine ratio) in NAFLD patients were significantly higher than controls (median 133.1 mg/g vs 110.7 mg/g; $P < .001$). Urine RBP/creatinine ratio was verified as an independent factor for NAFLD prediction after adjustment in multivariate logistic regression. The area under curve (AUC) of receiver operating characteristic (ROC) was 0.889 with the 95% confidence interval from 0.867 to 0.912. With a cutoff point of 0.215, the sensitivity and specificity of urine RBP/creatinine ratio in NAFLD prediction were 81.1% and 84.5%, respectively.

Conclusion: Our results demonstrated that urine RBP/creatinine ratio was an independent risk factor for NAFLD while the predictive model for NAFLD diagnosis is noninvasive with high sensitivity and specificity.

KEYWORDS

nonalcoholic fatty liver disease, retinol-binding protein

1 | INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is considered as lipid deposition in hepatocytes without excessive alcohol consumption. The progression spectrum of NAFLD ranges from nonalcoholic fatty liver (NAFL, also termed as simple steatosis) to nonalcoholic steatohepatitis (NASH) and cirrhosis, even hepatocellular carcinoma.¹ Although the “two-hit” hypothesis has been widely accepted as the major theoretical basis for NAFLD, the detailed mechanism is still vague.² Recently, the prevalence of NAFLD has been rapidly increased, reaching

approximately 10%-30% of the global population.³⁻⁵ Furthermore, NAFLD was associated with metabolic syndrome, including obesity and insulin resistance (IR).⁶ All these mentioned above have put NAFLD under the spotlight as a public health problem worldwide.

Retinol-binding protein (RBP), a liver-synthesized lipocalin with a molecular weight of about 21 kDa.⁷ As the retinol transport vehicle in the circulatory system, RBP delivers retinol from the liver to the desired target tissue.⁸ Retinol is bound deep inside the RBP cavity in plasma, then RBP-retinol complex interacts with transthyretin (TTR) in a molar ratio of 1:1, which prevents its loss through glomerular filtration.⁹ It has been identified that the cell-surface receptor of a target cell would recognize the retinol-RBP-TTR complex. After retinol uptake

Zhang and Liu contributed equally to this work.

in peripheral tissue, unbound form of RBP is freely filtered by the renal glomeruli, which is virtually completely reabsorbed and broken down by megalin/cubilin-receptor-mediated endocytosis by proximal tubule epithelial cells. Schina et al found that NAFLD subjects had significant lower level of serum RBP than controls.¹⁰ However, the opposite results showed no statistical difference in serum RBP level between subjects with or without NAFLD.^{11,12} Despite of conflicting outcomes, the role of serum RBP in NAFLD was widely investigated.¹³⁻¹⁷ Currently, no studies explored the association between urine RBP and NAFLD. Therefore, we conducted a retrospective cross-sectional study with the aim to investigate the change of urine RBP level [expressed by RBP/creatinine ratio (RCR)] in ultrasonography proven NAFLD subjects with normal-range estimated glomerular filtration rate (eGFR) on a large Chinese population. We hypothesized that the analysis of urine RCR, along with anthropometric information (sex and age), makers of hepatocellular damage (alanine aminotransferase, ALT; aspartate aminotransferase, AST; gamma-glutamyltransferase, GGT; uric acid, UA) and metabolic factors (body mass index, BMI; systolic blood pressure, SBP; diastolic blood pressure, DBP; triglyceride, TG; high-density lipoproteins cholesterol, HDL-C) would give us a better understanding of the association between urine RBP and NAFLD as well as the NAFLD prediction capacity of urine RBP.

2 | METHODS

2.1 | Subjects

The participants enrolled in the study were from those who received regular health check-up in the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China between June 2016 and October 2016. The diagnosis of hepatic steatosis was made according to the guideline presented by Fatty Liver Disease Study Group of the Chinese Liver Disease Association.¹⁸ We excluded the following subjects: (i) those with alcohol consumption >140 g/wk for men and 70 g/wk for women; (ii) the history and/or laboratory evidences of viral hepatitis, autoimmune hepatitis, drug-induced liver disease or other forms of chronic liver disease and other causes of secondary fatty liver; (iii) those with a history of kidney dysfunction or eGFR <90 mL/min/1.73 m²; (iv) acute or chronic infections; and (v) the presence of pregnancy or lactation. The 317 NAFLD patients and 391 healthy controls were used in the analysis. Informed consent was obtained from all the subjects before participation. The guideline from the STROBE statement was followed in the stage of study design and manuscript preparation.¹⁹

2.2 | Laboratory assessment

Systolic blood pressure and DBP were measured by the same sphygmomanometer. Height and weight were measured after removing shoes and bulky clothing and other factors which may influence the accuracy of measurement. BMI is calculated as weight in kilogram divided by the square of height in meter. A Toshiba Nemio 20 sonography machine with a 3.5-MHz probe (Toshiba, Tokyo, Japan) was

used for liver ultrasound examinations when defining NAFLD patients. Given the subjects are from the population for routine health check, ultrasound is more practical with acceptable power of diagnosis.¹⁸ Liver ultrasound examinations were performed by a single experienced radiologist blinded to the clinical and laboratory data. After 12 hours of overnight fasting, blood and urine samples were obtained from each participant. All samples were analyzed within 1-2 hours after collection without freezing. The quantitative determination of RBP levels was assessed using latex particle-enhanced immune-turbidimetric assay by a commercial assay kit (Chongqing Zhongyuan Biotechnology Limited Company, Chongqing, China). In brief, RBP in urine binds to the specific anti-RBP antibody, which is coated on latex particles, and causes agglutination. The coefficients of variation were <6% and 8% for inter-assay and intra-assay, respectively. The normal level of urine RBP ranged from 0.00 to 0.70 mg/L. Blood samples were obtained from an antecubital vein and analyzed by clinical laboratory personnel. The parameters, including ALT, AST, GGT, UA, creatinine, TG, HDL-C and urine creatinine were measured using a Hitachi 7600 clinical analyzer (Hitachi, Tokyo, Japan).

2.3 | Statistical analysis

Statistical analysis was performed using SPSS, version 16 (SPSS, Chicago, IL, USA). Continuous variables were expressed as means $\pm$ standard deviations with normal distribution and analyzed by the Kolmogorov-Smirnov test, or presented as the median (first quartile-third quartile) with the skewed distribution. Quantitative variables were examined by the Student's *t* test for normal distribution or the Mann-Whitney *U* test for the skewed distribution. Multivariate logistic regression was utilized by choosing forward option. A receiver operating characteristic (ROC) curve was applied to test the sensitivity and the specificity as well as the cutoff point for the diagnostic predictive model. *P* values <.05 were considered as statistical significance.

2.4 | Ethics statement

The study was approved by the ethics committee of the first affiliated hospital, school of medicine, Zhejiang University in China and conducted in accordance with the Helsinki Declaration.

3 | RESULTS

3.1 | Characteristics of subjects

A total of 708 subjects were chosen for evaluation, including 317 NAFLD patients and 391 healthy controls. Biographical information and laboratory characteristics of subjects enrolled in the study were illustrated in Table 1. There was no significant difference between NAFLD patients and healthy controls in age. However, compared with healthy controls, SBP, DBP, BMI, ALT, AST, GGT, UA, and TG were significantly increased, while HDL-C was significantly decreased in NAFLD patients. As expected, urine RBP/creatinine ratio in

TABLE 1 Anthropometric and biochemical features of the study subjects

Variable	NAFLD (n = 317)	Control (n = 391)	P value
Age (y)	54 (46-66)	54 (45-68)	.858
Gender (M/F)	231/86	228/163	<.001
SBP (mm Hg)	130 (118-140)	125 (113-135)	<.001
DBP (mm Hg)	80 (73-86)	76 (68-83)	<.001
BMI (kg/m ²)	25.48 (24.09-27.01)	21.78 (20.59-23.31)	<.001
ALT (U/L)	26 (19-41)	16 (12-21)	<.001
AST (U/L)	22 (19-29)	20 (17-23)	<.001
GGT (U/L)	28 (21-45)	17 (13-24)	<.001
UA (μmol/L)	369 (317.5-440.5)	316 (264-363)	<.001
TG (mmol/L)	1.79 (1.295-2.365)	1.02 (0.77-1.39)	<.001
HDL-C (mmol/L)	1.17 (1.01-1.34)	1.42 (1.21-1.7)	<.001
Creatinine (mg/L)	25.7 (70.5-89)	70 (59-78)	<.001
Urinary RCR (mg/g)	133.1 (17.7-221.6)	110.7 (9.6-179.7)	<.001

NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic pressure; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; UA, uric acid; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; ACR, albumin/creatinine ratio; RBP, retinol-binding protein; RCR, RBP/creatinine ratio; M, male; F, female. Data are presented as median (first quartile-third quartile). Analysis was conducted using Mann-Whitney *U* test.

NAFLD patients was significantly higher than that in controls (median 133.1 mg/g vs 110.7 mg/g; *P* < .001).

3.2 | Association between urine RBP/creatinine ratio and NAFLD

To get a better understanding of the relationship between urine RBP/creatinine ratio and NAFLD, we used multivariate logistic regression with forward selection to analyze the odds and *P* values. According to the results in Table 1, we put age, sex, SBP, DBP, BMI, ALT, AST, GGT, UA, TG, HDL-C, and urine RBP/creatinine ratio into the multivariate logistic regression analysis. The results before and after adjustments in multivariate logistic regression model were shown in Table 2. The *P* value for RBP/creatinine ratio in NAFLD prediction was <.001. After sequential addition of those above mentioned factors, the *P* values were always <.001. This result suggested that urine urinary RBP/creatinine ratio was an independent factor for NAFLD prediction.

3.3 | Optimizing the predictive model for NAFLD

To form a more practical model for early predicting NAFLD, we prefer to include the parameters that are painless and noninvasive. Therefore, we eliminated invasive sera parameters, including ALT, AST, GGT, UA, TG, and HDL-C. In detail, we utilized the multivariate logistic regression analysis with the remained parameters, involving age, sex, SBP, DBP, BMI, and urine RBP/creatinine ratio. Only BMI (OR 2.055; 95%CI 1.827-2.311, *P* < .001) and urine RBP/creatinine ratio (OR 30.718; 95%CI 14.759-47.691, *P* < .001) entered into the new model. The new model is presented as logit (N index) = 1.942*urine RBP/creatinine ratio + 0.714*BMI - 18.370. Furthermore, we applied a ROC curve to test the sensitivity and

specificity of this new predictive model (Figure 1). The area under curve (AUC) was 0.889 with the 95% confidence interval from 0.867 to 0.912. According to Youden's index, the best cutoff point was 0.215, the sensitivity and the specificity of which were 81.1% and 84.5%, respectively.

4 | DISCUSSION

In the current study from a large Chinese population, we provided evidence that NAFLD subjects even with normal-range eGFR had a higher urinary RBP/creatinine ratio. The results also indicated that urine RBP/creatinine ratio was an independent risk factor for NAFLD. Finally, we established a predictive model for NAFLD by utilizing BMI and urine RBP/creatinine ratio with a high sensitivity and specificity of 81.1% and 84.5%, respectively.

To our knowledge, our study is the first to demonstrate a significant correlation between urinary RBP/creatinine ratio and NAFLD. The underlying mechanism by which RBP/creatinine ratio interacts with NAFLD remained unclear, but several intertwined pathways were proposed. Firstly, previous studies suggested that serum RBP was correlated to NAFLD.^{10,13,14} NAFLD is reported to be associated with genetic, environmental, and metabolic factors.²⁰⁻²⁵ Currently, insulin resistance is regarded to play central role in NAFLD while elevated serum RBP levels were found in insulin-resistance mice and humans, indicating its potential role in NAFLD.^{2,26,27} Serum RBP can promote hepatic steatosis by inducing hepatic mitochondrial dysfunction.²⁸ Besides, Yang et al suggested that there might be retinol-independent mechanisms for RBP through influencing insulin resistance.²⁷ The other possible mechanism linking the increased urine RBP levels with NAFLD is the inflammatory response

Model	OR (95% CI)	P value
Unadjusted	32.021 (15.010-49.112)	<.001
Adjusted for age and gender	31.763 (15.189-49.661)	<.001
Adjusted for age, gender, SBP, DBP and BMI	30.311 (14.609-47.831)	<.001
Adjusted for age, gender, SBP, DBP, BMI, ALT, AST, GGT, UA	30.172 (13.219-47.066)	<.001
Adjusted for age, gender, SBP, DBP, BMI, ALT, AST, GGT, UA, TG and HDL-C	29.498 (12.710-45.121)	<.001

TABLE 2 Independent predictors for NAFLD according to urine RBP in unadjusted and adjusted models

NAFLD, nonalcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic pressure; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; UA, uric acid; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; RBP, retinol-binding protein; OR, odds ratios; CI, confidence intervals.

Odds ratios were determined using logistic regression analyses.

FIGURE 1 Receiver operating characteristic curves analysis of NAFLD predictive model. ROC, receiver operating characteristic

during the “second hit” phase of NAFLD pathogenesis. A heterogeneous class of pro-inflammatory adipokines and hepatokines such as tumor necrosis factor (TNF)- α and interleukin-6 (IL-6) can modulate reversed renal lipid accumulation, glomerular sclerosis, and tubulointerstitial inflammation.²⁹⁻³¹

Given that NAFLD has become a public health problem all over the world, its diagnosis should be made early, safely, time-efficiently, and cost-effectively. The accuracy and safety are under fierce discussion despite of several expression patterns reported for the diagnosis of NAFLD.^{32,33} Compared with serum RBP, the use of urine RBP benefits more.³⁴ Firstly, the diagnosis of NAFLD in its early stage is of clinical importance, since NAFLD patients may sequentially progress from simple steatosis into NASH, cirrhosis, and even hepatocellular carcinoma.^{35,36} Especially, most

NAFLD patients are asymptomatic at its early stages, resulting in the neglect of NAFLD onset and progress. Secondly, the invasive nature of liver biopsy hampers its utilization in NAFLD diagnosis for increased risk of bleeding and infection. Therefore, it is not the best and convenient way for routine NAFLD detection. In terms of safety, our model precludes parameters derived from invasive way. We even eliminated serum parameters for its nature of vein puncture that also has risk of cross-infection. Actually, we previously tested a model adding ALT, AST, GGT, UA, TG, and HDL-C to the final model mentioned above. Among these added variables, only ALT and TG entered into the model. Moreover, BMI and urine RBP/creatinine ratio were ahead of ALT and TG in entering into the model with forward selection and the change of AUC was only 0.022, indicating that our model still works well without sera parameters. Finally, time-efficient and cost-effective are also required for the diagnosis of NAFLD. With the advance of high throughput techniques, the method for NAFLD diagnosis with no invasiveness has been rapidly developed, such as ultrasound and other imaging methods. Nevertheless, an experienced specialist is needed when performing ultrasound or imaging. Besides, the process is also time-consuming as only one patient at a time can be examined. In fact, sometimes the waiting list is so long that NAFLD patients may miss the “early” time as mentioned previously. Besides, the fee for ultrasound or imaging is high. Under this point of view, these methods may be not fully qualified for the rule of time-efficient and cost-effective. In our model, BMI can be calculated immediately by calculator according to the formula. For urine RBP/creatinine ratio, a laboratory specialist can analyze tens to hundreds samples in one round on different machines. Accordingly, we successfully established a new noninvasive diagnostic model consisted of BMI and urine RBP/creatinine ratio for NAFLD that was characterized by acceptable sensitivity and specificity. There is, without doubt, a significant association between central obesity and the prevalence and severity of NAFLD over the past few years. The relationship between urine RBP/creatinine ratio and NAFLD may be partially modulated by changes in urine RBP excretion under many conditions. It should be mentioned that RBP/creatinine ratio in urine is

sensitive, but not specific for NAFLD diagnosis and prediction. It must be confirmed in further prospective cohort studies to achieve better diagnostic accuracy.

Several limitations exist in our study that should be acknowledged. Firstly, RBP/creatinine ratio could not be reviewed in patients with different stages of NAFLD. Because the diagnosis of NAFLD was based on ultrasonographic examination in our study, which is not sensitive enough to grade and stage the NASH or NASH with advanced fibrosis. Secondly, we did not test serum RBP and the remaining kidney injury biomarkers and would be compensated in future larger cohort study. Thirdly, only Chinese population was analyzed in this study. According to genetic or environmental susceptibility, different population should be included when conducting a prospective study to verify the relationship between RBP and NAFLD. Further mechanistic study is needed to explore their precise interrelationship.

5 | CONCLUSIONS

It is the first study reporting the relationship between urine RBP/creatinine ratio and NAFLD. The results demonstrated that urine RBP/creatinine ratio was an independent risk factor for NAFLD diagnosis. Furthermore, a predictive model is established with high sensitivity and specificity. It is a safe, time-efficient and cost-effective tool for NAFLD prediction. Future researches on the mechanism of NAFLD are urgently needed for better NAFLD diagnosis, treatment, and prognosis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. This study is supported by the National Natural Science Foundation of China (81370008 and 81000169), and the Natural Science Foundation of Zhejiang province (R2110159, LY16H030003 and LY17H030007). The funding sources had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHORS' CONTRIBUTIONS

J.Z and Z.L performed the majority of data collection and partial analysis; X.Z and L.Z carried out major data analysis and statistic analysis; X.J designed the study and wrote the manuscript. All authors reviewed the manuscript.

ORCID

Xi Jin <http://orcid.org/0000-0002-0296-2516>

REFERENCES

- Kunde SS, Lazenby AJ, Clements RH, Abrams GA. Spectrum of NAFLD and diagnostic implications of the proposed new normal range for serum ALT in obese women. *Hepatology*. 2005;42:650-656. PubMed PMID: WOS:000231554800020.
- Day CP, James OFW. Steatohepatitis: a tale of two "hits"? *Gastroenterology*. 1998;114:842-845. PubMed PMID: WOS:000072770100030.
- Jin X, Yang YD, Chen K, et al. HDMCP uncouples yeast mitochondrial respiration and alleviates steatosis in L02 and hepG2 cells by decreasing ATP and H(2)O(2) levels: a novel mechanism for NAFLD. *J Hepatol*. 2009;50:1019-1028. PubMed PMID: WOS:000266384600023.
- Carr RM, Oranu A, Khungar V. Nonalcoholic fatty liver disease: pathophysiology and management. *Gastroenterol Clin North Am*. 2016;45:639-652. Epub 2016/11/14. PubMed PMID: 27837778.
- Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol*. 2013;10:686-690. PubMed PMID: WOS:000326632100010.
- Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes*. 2001;50:1844-1850. PubMed PMID: 11473047.
- Blaner WS. Retinol-binding protein - the serum transport protein for Vitamin-A. *Endocr Rev*. 1989;10:308-316. PubMed PMID: WOS:A1989AM98200005.
- Gliniak CM, Brown JM, Noy N. The retinol-binding protein receptor STRA6 regulates diurnal insulin responses. *J Biol Chem*. 2017;292:15080-15093.
- Girard AW, Grant F, Watkinson M, et al. Promotion of orange-fleshed sweet potato increased Vitamin A intakes and reduced the odds of low retinol-binding protein among postpartum Kenyan women. *J Nutr*. 2017;147:955-963.
- Schina M, Koskinas J, Tiniakos D, et al. Circulating and liver tissue levels of retinol-binding protein-4 in non-alcoholic fatty liver disease. *Hepatol Res*. 2009;39:972-978. Epub 2009/07/25. PubMed PMID: 19624771.
- Milner KL, van der Poorten D, Xu A, et al. Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. *Hepatology*. 2009;49:1926-1934. Epub 2009/05/29. PubMed PMID: 19475694.
- Cengiz C, Ardicoglu Y, Bulut S, Boyacioglu S. Serum retinol-binding protein 4 in patients with nonalcoholic fatty liver disease: does it have a significant impact on pathogenesis? *Eur J Gastroenterol Hepatol*. 2010;22:813-819. PubMed PMID: WOS:000278569900007.
- Terra X, Auguet T, Broch M, et al. Retinol binding protein-4 circulating levels were higher in nonalcoholic fatty liver disease vs. histologically normal liver from morbidly obese women. *Obesity (Silver Spring)*. 2013;21:170-177. PubMed PMID: WOS:000322086900054.
- Seo JA, Kim NH, Park SY, et al. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. *Clin Endocrinol*. 2008;68:555-560. PubMed PMID: WOS:000253977800009.
- Yan HM, Chang XX, Xia MF, et al. Serum retinol binding protein 4 is negatively related to beta cell function in Chinese women with non-alcoholic fatty liver disease: a cross-sectional study. *Lipids Health Dis*. 2013;12:157. PubMed PMID: WOS:000326435900001.
- Alkhoury N, Lopez R, Berk M, Feldstein AE. Serum retinol-binding protein 4 levels in patients with nonalcoholic fatty liver disease. *J Clin Gastroenterol*. 2009;43:985-989. PubMed PMID: WOS:000271307200018.
- Chen X, Shen T, Li Q, et al. Retinol binding protein-4 levels and non-alcoholic fatty liver disease: a community-based cross-sectional study. *Sci Rep*. 2017;7:45100. Epub 2017/03/24. PubMed Central PMCID: PMC5362806.
- Zeng MD, Fan JG, Lu LG, et al. Guidelines for the diagnosis and treatment of nonalcoholic fatty liver diseases. *J Dig Dis*. 2008;9:108-112. PubMed PMID: WOS:000256983200009.
- von Elm E, Altman DG, Egger M, et al. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE)

- statement: guidelines for reporting observational studies. *Lancet*. 2007;370:1453-1457. PubMed PMID: 18064739.
20. Ballestri S, Lonardo A, Romagnoli D, et al. Ultrasonographic fatty liver indicator, a novel score which rules out NASH and is correlated with metabolic parameters in NAFLD. *Liver Int*. 2012;32:1242-1252. PubMed PMID: WOS:000307722000008.
 21. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the dionysos nutrition and liver study. *Hepatology*. 2005;42:44-52. PubMed PMID: WOS:000230189200008.
 22. Petta S, Miele L, Bugianesi E, et al. Glucokinase regulatory protein gene polymorphism affects liver fibrosis in non-alcoholic fatty liver disease. *PLoS One*. 2014;9:e87523. PubMed PMID: WOS:000330626900078.
 23. Angulo P. Medical progress - nonalcoholic fatty liver disease. *N Engl J Med*. 2002;346:1221-1231. PubMed PMID: WOS:000175033400008.
 24. Miele L, Dall'Armi V, Cefalo C, et al. A case-control study on the effect of metabolic gene polymorphisms, nutrition, and their interaction on the risk of non-alcoholic fatty liver disease. *Genes Nutr*. 2014;9:383. PubMed PMID: WOS:000333704000003.
 25. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet*. 2008;40:1461-1465. PubMed PMID: WOS:000261215900023.
 26. Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med*. 2006;354:2552-2563. PubMed PMID: WOS:000238233400005.
 27. Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*. 2005;436:356-362. PubMed PMID: WOS:000230623400034.
 28. Liu Y, Mu D, Chen H, et al. Retinol-binding protein 4 induces hepatic mitochondrial dysfunction and promotes hepatic steatosis. *J Clin Endocrinol Metab*. 2016;101:4338-4348. PubMed PMID: 27504850.
 29. Bernard AM, Vyskocil AA, Mahieu P, Lauwerys RR. Assessment of urinary retinol-binding protein as an index of proximal tubular injury. *Clin Chem*. 1987;33:775-779. PubMed PMID: WOS:A1987H746400006.
 30. Musso G, Cassader M, Cohnen S, et al. Emerging liver-kidney interactions in nonalcoholic fatty liver disease. *Trends Mol Med*. 2015;21:645-662.
 31. Gonzalez-Calero L, Martin-Lorenzo M, Ramos-Barron A, et al. Urinary kininogen-1 and retinol binding protein-4 respond to acute kidney injury: predictors of patient prognosis? *Sci Rep*. 2016;6:19667. PubMed PMID: 26792617; PubMed Central PMCID: PMC4726181.
 32. Jin X, Ye YF, Chen SH, Yu CH, Liu J, Li YM. MicroRNA expression pattern in different stages of nonalcoholic fatty liver disease. *Dig Liver Dis*. 2009;41:289-297. PubMed PMID: 18922750.
 33. Jin X, Chen YP, Kong M, Zheng L, Yang YD, Li YM. Transition from hepatic steatosis to steatohepatitis: unique microRNA patterns and potential downstream functions and pathways. *J Gastroenterol Hepatol*. 2012;27:331-340. PubMed PMID: 21793903.
 34. Domingos MA, Moreira SR, Gomez L, et al. Urinary retinol-binding protein: relationship to renal function and cardiovascular risk factors in chronic kidney disease. *PLoS One*. 2016;11:e0162782. PubMed PMID: 27655369; PubMed Central PMCID: PMC45031461.
 35. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther*. 2011;34:274-285. PubMed PMID: WOS:000292394800002.
 36. Glen J, Floros L, Day C, Pryke R, Guideline Development Group. Non-alcoholic fatty liver disease (NAFLD): summary of NICE guidance. *BMJ*. 2016;354:i4428. PubMed PMID: WOS:000383101100002.

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