



# Systematic Review and Meta-analysis of Circulating Fetuin-A Levels in Nonalcoholic Fatty Liver Disease

Shousheng Liu<sup>#1,2</sup>, Jianhan Xiao<sup>#3</sup>, Zhenzhen Zhao<sup>1,2</sup>, Mengke Wang<sup>3</sup>,  
Yifen Wang<sup>3</sup> and Yongning Xin<sup>\*2,3</sup>

<sup>1</sup>Clinical Research Center, Qingdao Municipal Hospital, Qingdao University, Qingdao, Shandong, China; <sup>2</sup>Digestive Disease Key Laboratory of Qingdao, Qingdao, Shandong, China; <sup>3</sup>Department of Infectious Disease, Qingdao Municipal Hospital, Qingdao University, Qingdao, Shandong, China

## Abstract

**Background and Aims:** Accumulated studies have reported the key role of circulating fetuin-A in the development and progression of nonalcoholic fatty liver disease (NAFLD) but the results have not been consistent. In this study, we performed a systematic review and meta-analysis to explore the relationship between circulating fetuin-A level and the development and classification of NAFLD. **Methods:** The PubMed, EMBASE, and Cochrane Library databases were searched to obtain the potentially relevant studies up to May 2020. Standardized mean differences (SMD) and 95% confidence intervals of circulating fetuin-A levels were extracted and summarized. Sensitivity, subgroup analysis and meta-regression analysis were performed to investigate the potential heterogeneity. Association of circulating fetuin-A level with classification of NAFLD was also reviewed. **Results:** A total of 17 studies were included, composed of 1,755 NAFLD patients and 2,010 healthy controls. Meta-analysis results showed that NAFLD patients had higher circulating fetuin-A level (SMD=0.43, 95% confidence interval [CI]: 0.22–0.63,  $p<0.001$ ) than controls. Subgroup analysis indicated that circulating fetuin-A level was markedly increased in adult NAFLD patients (SMD=0.48, 95% CI: 0.24–0.72,  $p<0.001$ ) and not in pediatric/adolescent patients compared to controls. Circulating fetuin-A level was markedly increased in ultrasound-proven NAFLD pediatric/adolescent patients (SMD=0.42, 95% CI: 0.12–0.72,  $p=0.007$ ), other than in the liver biopsy-proven NAFLD pediatric/adolescent patients. Body mass index might be the influencing factor to the heterogeneity in adult patients. Circulating fetuin-A level was not associated with the classification of NAFL vs. nonalcoholic steatohepatitis (NASH). Whether the circulating fetuin-A level was associated with the development of fibrosis remains controversial. **Conclusions:** Circulating fetuin-A level was significantly higher in NAFLD patients and was not associated with the classification of NAFL vs. NASH. Whether the circulating fetuin-A level was associated with the development of fibrosis remains controversial.

**Keywords:** Nonalcoholic fatty liver disease; Fetuin-A; Meta-analysis; Fibrosis.

**Abbreviations:** ARHQ, Agency for Healthcare Research and Quality; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; NAFL, non-alcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NOS, Newcastle-Ottawa scale; SD, standard deviation; SMD, standardized mean differences.

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<sup>#</sup>These two authors contributed equally to this work.

\*Correspondence to: Yongning Xin, Department of Infectious Disease, Qingdao Municipal Hospital, 1 Jiaozhou Road, Qingdao, Shandong 266011, China. Tel: +86-532-82789463, Fax: +86-532-85968434, E-mail: xinyongning@163.com

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## Introduction

Nonalcoholic fatty liver disease (NAFLD) has become one of the most common chronic liver diseases in recent years, and the overall prevalence of NAFLD is approximately 25% in the world.<sup>1,2</sup> There is a broad spectrum of NAFLD, which ranges from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC).<sup>3,4</sup> NAFLD is the hepatic manifestation of metabolic syndrome and is affected by many risk factors, such as obesity, hyperglycemia, type 2 diabetes, and hypertriglyceridemia usually.<sup>5</sup> However, under certain conditions, mostly during genetically-determined NAFLD (such as in carriers of the *TM6SF2* E167K or *PNPLA3* I148M gene polymorphism), NAFLD does not show association with the metabolic syndrome and an increased risk of cardiovascular disease.<sup>6</sup>

Liver biopsy remains the gold standard for diagnosis and histological assessment of NAFLD, but the obvious defects (e.g., invasiveness, inter-observer differences, sampling error) cannot be ignored.<sup>7,8</sup> In clinical practice, imaging methods such as ultrasonography, computed tomography, controlled attenuation parameter and magnetic resonance have been used widely for diagnosing NAFLD.<sup>9–13</sup> In addition, many studies have been conducted to explore the valuable serum biomarkers for early diagnosis and progression of NAFLD. Several serum biomarkers, such as alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltranspeptidase, cytokeratin-18 and fibroblast growth factor 21, have been researched in some studies and their potential to serve as the biomarkers in clinical diagnosing of NAFLD have been mentioned.<sup>14–18</sup>

Fetuin-A, also known as the 2-Herefman-Schmid glycoprotein, is a phosphorylated glycoprotein and a member of the fetuin group of serum binding proteins that are synthesized primarily by hepatocytes.<sup>19</sup> As an endogenous inhibitor of tyrosine kinase, fetuin-A can trigger insulin resistance in the target tissues, such as liver and skeletal muscle.<sup>20,21</sup> Pal *et al.*<sup>22</sup> reported that fetuin-A acts as an endogenous ligand for toll-like receptor 4 and could enhance both insulin resistance and inflammation response. High serum fetuin-A was also found to strongly interact with the high levels of free fatty acids to induce insulin resistance in rodents,

which was then observed in large human studies; moreover, the relationships of fetuin-A with fatty acids, to determine insulin resistance, were particularly strong in patients with NAFLD.<sup>23,24</sup> Fetuin-A is also known to inhibit transforming growth factor- $\beta$ 1 signaling, which promotes fibrotic changes in many tissues, including liver and arteries; therefore, fetuin-A could prevent fibrotic changes in organs.<sup>25,26</sup> Recently, fetuin-A has been regarded as a potential link molecule between obesity, insulin resistance, and coronary heart disease,<sup>27–31</sup> and was found to be strongly related to several parameters such as circulating lipid levels (non-esterified free fatty acids and triglycerides), glucose tolerance, circulating pro-inflammatory and anti-inflammatory factors, and interleukin-6.<sup>32</sup> Fetuin-A can also induce low-grade inflammation and repress adiponectin production in animals and humans, playing an important role in the pathogenesis of insulin and possessing a pro-adiposity effect.<sup>33,34</sup> Accumulated lines of evidence have reported the significant association between circulating fetuin-A level and the development and progression of NAFLD, but the results have been inconsistent. Additionally, there has been no definite conclusion as to whether circulating fetuin-A can reflect the grading of NAFL vs. NASH and advanced fibrosis.

The aim of this study was to investigate the importance of fetuin-A in the development and classification of NAFLD.

## Methods

### Search strategy

This systematic review and meta-analysis was conducted following a priori established protocol and was reported according to PRISMA guidelines.<sup>35</sup> Two independent observers (Shousheng Liu and Jianhan Xiao) performed a systematic search of the PubMed, EMBASE, and the Cochrane Library databases up to May 2020 and with English language restrictions. The first step for information retrieval was to gain the subject term of fetuin-A, NAFLD or NASH in the MeSH database of PubMed; meanwhile, we gained the entry terms of them, respectively. The combined results of fetuin-A, NAFLD or NASH in the three databases were obtained based on the search method of "subject term + entry terms". In addition, we examined the reference lists in relevant original research and review articles to search additional potentially eligible studies.

### Inclusion and exclusion criteria

Studies that investigated the circulating fetuin-A level in patients with NAFLD were eligible for review. Studies were included in this systematic review and meta-analysis if they met the following criteria: (1) original full-text publications; (2) comparison of circulating fetuin-A level between NAFLD patients and healthy controls; (3) investigations of the effect of circulating fetuin-A level on the classification of NAFL vs. NASH or fibrosis. Studies were excluded according to the following criteria: (1) patients with other causes of liver disease (*e.g.*, viral or autoimmune hepatitis, alcoholic fatty liver disease, HCC, coronary artery disease) or in whom NAFLD co-existed with another liver disease; (2) there was overlap of patients who were included in more than one study. Quality of case-control studies were evaluated use the Newcastle-Ottawa scale (NOS) scoring system and the quality of cross-sectional studies were evaluated use the Agency for Healthcare Research and Quality (AHRQ) scoring system.<sup>36</sup> (3) Studies of low methodological quality, as defined by a NOS score  $\leq 2$  or AHRQ score  $\leq 3$ , were excluded.

ed. Finally, (4) reviews, editorials, case reports, conference abstracts, letters to the editor, hypotheses, book chapters, and studies on animals or cell lines were excluded.

### Data extraction

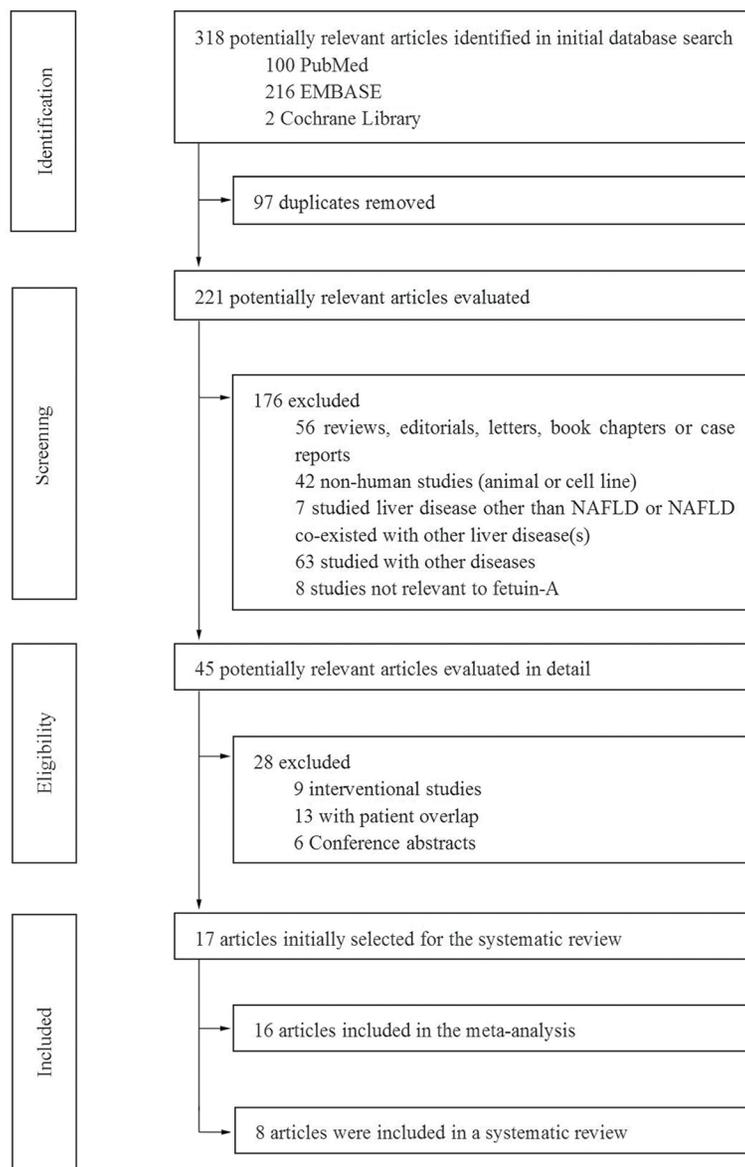
Available data were extracted from the full text and corresponding supplemental information by two investigators working independently (Shousheng Liu and Jianhan Xiao) and confirmed by a third reviewer (Zhenzhen Zhao). Disagreement was resolved by discussion among all researchers. The following information of each selected publication was extracted: (1) general characteristics, such as first author's name, year of publication, country where the study was carried out, study design, diagnostic methods of NAFLD, type of samples (*e.g.*, serum, plasma, blood); (2) subjects' characteristics, such as age, gender, body mass index (BMI); and (3) effect of circulating fetuin-A on the grading of NAFL vs. NASH or fibrosis. When the same population was published in several journals, we retained only the most informative article or complete study, to avoid duplication. If some necessary data were not offered in the article, the corresponding author would be contacted for the data. If no response, the following methods would be carried out: (1) data from the graphical plots were extracted to calculate the circulating fetuin-A levels by using WebPlotDigitizer (version 4.1.0, <https://apps.automeris.io/wpd/>);<sup>37</sup> (2) circulating fetuin-A levels which were expressed as median (mix-mix) or median (25–75 quartile) were transformed into the standard form of mean, according to the Cochrane book or method (Hozo, Stela Pudar *et al.*)<sup>38</sup>.

### Quality assessment

The included studies in the systematic review and meta-analysis were independently assessed by two investigators (Shousheng Liu and Jianhan Xiao). We assessed the quality of included case-control studies based on the NOS scoring system, and cross-sectional studies based on the AHRQ scoring system. The full NOS score was 9 stars; a study that met 7 or more stars was defined as a high-quality study, less than 3 stars as low-quality and other studies were defined as moderate quality. Article quality by AHRQ was assessed as follows: low quality: 0–3; moderate quality: 4–7; and high quality: 8–11.

### Statistical analysis

All statistical analyses were performed using Stata12.0 (StataCorp LP, College Station, TX, USA). The effect sizes were generated by sample sizes, mean circulating fetuin-A levels, and the standard deviation (SD), and presented as standardized mean differences (SMD) and 95% confidence intervals (95% CIs) for circulating fetuin-A levels in comparisons between groups. Given the expected heterogeneity of the outcome, a random-effect inverse-variance model was chosen for this meta-analysis.<sup>39</sup> The heterogeneity between the results of different studies was evaluated using the  $I^2$  statistic, values of  $I^2 > 50\%$  were considered to represent substantial heterogeneity. The potential moderating effects of continuous variables on between-study heterogeneity were evaluated by meta-regression analyses and subgroup-analysis. Subgroup-analysis were first conducted according to age, region, and diagnostic method of NAFLD, then the sex distribution (number of males), mean age, BMI and HOMA-IR of NAFLD patients were regarded as the potential moderators for the adult outcome of the



**Fig. 1. Flowchart presenting the literature search process, according to the PRISMA statement.**

meta-analysis when a high heterogeneity of adult NAFLD was observed. Sensitivity analysis was performed to investigate the influence of each study on the pooled measures by omitting a study each time to assess the stability of our results. A *p*-value of <0.05 was considered to indicate a statistical difference. Publication bias was assessed by funnel plot and Begg’s and Egger’s tests.

## Results

### Literature search

A total of 318 studies were retrieved initially from the three databases. After removing duplications (*n*=97), 221 studies remained for evaluation. In all, 204 studies were excluded for representing reviews, editorials, letters, book chapters

or case reports, animal or cell experiments, other liver diseases, patient overlap, conference abstracts, and so on (Fig. 1). The final dataset for the systematic review and meta-analysis comprised 17 full-text studies.<sup>25,27,28,40-53</sup> Among them, 16 studies were selected to conduct the meta-analysis and 8 studies were selected to investigate the relationship between circulating fetuin-A level and the classification of NAFL vs. NASH.

### Characteristics of included studies

A total of 1,755 NAFLD patients and 2,010 healthy controls were included in the 17 studies, the main characteristics of these studies are shown in Table 1. Among these studies, two were conducted with the same participants, but the former investigated the association of plasma fetuin-A level with NAFLD;<sup>44</sup> however, the latter study not only investi-

Table 1. Characteristics of included studies focused on circulating levels of fetuin-A in this meta-analysis and systematic review

Study (Year)	Country	Region	Study design	Age	Sample size	Tissue type	Fetuin-A measurement method	NAFLD diagnosis method	NOS, 0-9 score	AHRQ, 0-11 score	Additional information
Reinehr et al. (2008)	Germany	European	Cross-sectional	Paediatric/ Adolescent	48	Serum	ELISA	Ultrasound	6		
Yilmaz et al. (2010)	Turkey	Eurasian	Case-control	Adult	174	Serum	ELISA	Liver biopsy	7		
Haukeland et al. (2012)	Norway	European	Cross-sectional	Adult	242	Plasma	ELISA	Liver biopsy	4		
Ou et al. (2012)	Chinese	Asian	Cross-sectional	Adult	510	Serum	ELISA	Ultrasound	6		(1)
Ballestri et al. (2013)	Italy	European	Cross-sectional	Adult	70	Serum	ELISA	Ultrasound	5		(2)
Dogru et al. (2013)	Turkey	Eurasian	Cross-sectional	Adult	189	Plasma	ELISA	Liver biopsy	5		(3)
Kahraman et al. (2013)	Germany	European	Cross-sectional	Adult	118	Serum	ELISA	Liver biopsy	4		
Lebensztejn et al. (2014)	Poland	European	Cross-sectional	Paediatric/ Adolescent	45	Serum	ELISA	Ultrasound	5		
Rametta et al. (2014)	Italy	European	Cross-sectional	Adult	397	Serum	ELISA	Liver biopsy	7		
Sato et al. (2015)	Japanese	Asian	Cross-sectional	Adult	295	Serum	ELISA	Ultrasound	5		
Wong et al. (2015)	Chinese	Asian	Case-control	Adult	920	Serum	ELISA	Ultrasound	5		
Celebi et al. (2015)	Turkey	Eurasian	Cross-sectional	Adult	/	Plasma	ELISA	Liver biopsy	5		(3)
Cui et al. (2017)	Chinese	Asian	Case-control	Adult	158	Serum	ELISA	Ultrasound	5		
Siraz et al. (2017)	Turkey	Eurasian	Cross-sectional	Paediatric/ Adolescent	80	Serum	ELISA	Ultrasound	6		
Pampanini et al. (2018)	Italy	European	Cross-sectional	Paediatric/ Adolescent	183	Serum	ELISA	Ultrasound	6		(1)
Mondal et al. (2018)	India	Asian	Cross-sectional	Adult	188	Serum	ELISA	Ultrasound	6		(2)
Nascimbeni et al. (2018)	Italy	European	Cross-sectional	Adult	149	Serum	ELISA	Ultrasound Liver biopsy	6		(4)

(1) All participants were prediabetic. (2) All participants were suspected cases of coronary heart disease. (3) The two studies were conducted on the same subjects, with the former study investigating the circulating fetuin-A levels between NAFLD patients and healthy controls, and the latter study investigating the circulating fetuin-A levels between NASH and NAFL patients, and fibrosis and no fibrosis patients. (4) The study investigated fetuin-A levels in 81 obese children with NAFLD diagnosed by biopsy, 79 obese children with NAFLD defined by liver ultrasonography, and 23 lean subjects. In order to make a better analysis, we made two groups: one was for comparison between obese NAFLD and non-NAFLD patients verified by ultrasound; the other one was for comparison between obese NAFLD verified by liver biopsy and characterized as lean healthy. Abbreviation: ELISA, enzyme-linked immunosorbent assay.

gated the relationship between plasma fetuin-A level and NASH or NAFL but also investigated the association of plasma fetuin-A level and fibrosis.<sup>47</sup> As such, 16 studies were selected to perform the meta-analysis.

Studies in this meta-analysis included three case-control and thirteen cross-sectional designs. Enzyme-linked immunosorbent assay was used to test the serum/plasma fetuin-A level in all the studies. Liver biopsy was performed to determine the NAFLD in six studies, and ultrasound was used in other ten. Another study, conducted by Pampanini *et al.*,<sup>27</sup> diagnosed NAFLD with both liver biopsy and ultrasound in different groups; so, we regarded this study as two individual studies. Among these studies, six performed the comparison of circulating fetuin-A level between NAFL and NASH patients and eight determined the relationship of circulating fetuin-A level with liver fibrosis (Table 2).

**Quality of included studies**

The qualities of included case-control or cohort studies were assessed based on the NOS, and the cross-sectional studies were assessed based on the ARHQ methodology checklist.<sup>36</sup> The detailed quality scores of each study are shown in Table 1. All the studies were assessed as moderate quality. No study was eliminated due to low quality (NOS score ≤2 or AHRQ score ≤3).

**Effect of circulating fetuin-A level on NAFLD**

A random-effect meta-analysis was performed to investigate the effect of circulating fetuin-A level on the development of NAFLD. As the results show in Fig. 2A, the circulating fetuin-A level in NAFLD patients was significantly higher than in healthy controls, with a summarized SMD of 0.43 (95%CI: 0.22–0.63, *p*<0.001). A striking heterogeneity among included studies was observed in the comparison of circulating fetuin-A level in NAFLD patients and healthy controls; the *I*<sup>2</sup> value was 85.7% (*p*<0.001).

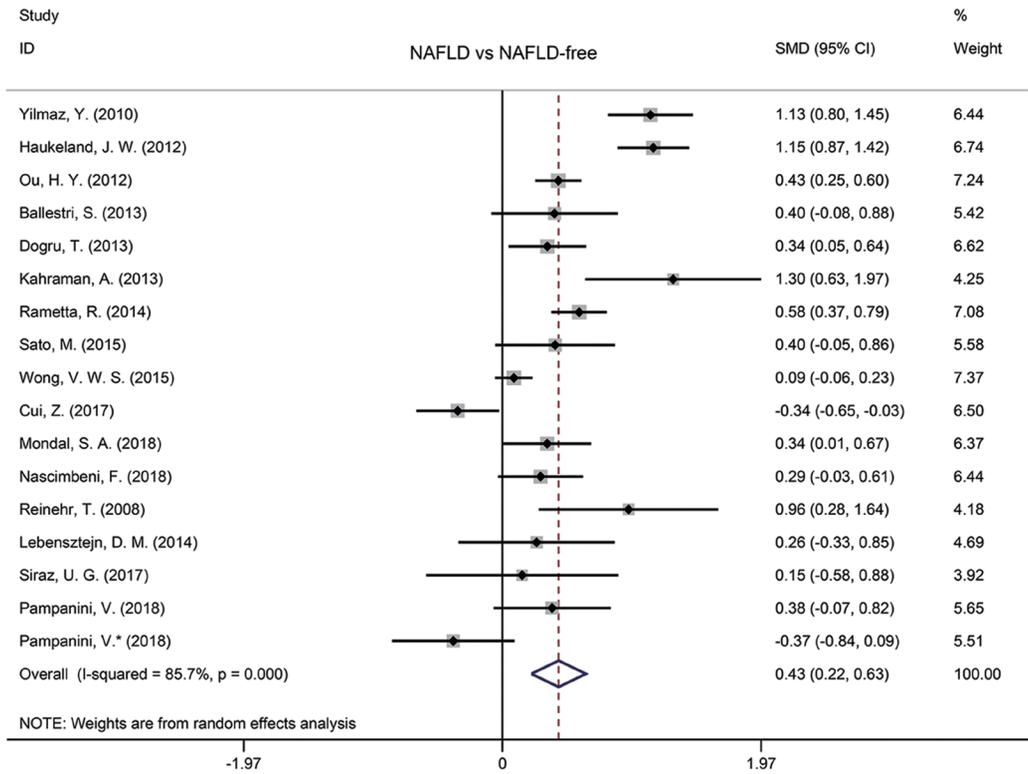
Subgroup analysis based on age implied that the circulating fetuin-A level of NAFLD patients was significantly elevated in adults (SMD=0.48, 95% CI: 0.24–0.72, *p*<0.001) and no obvious difference was observed in pediatric/adolescent patients (SMD=0.25, 95% CI: -0.18–0.67, *p*=0.256) (Fig. 2B). Based on age, we also performed subgroup analysis according to the region of subjects and diagnostic method of NAFLD, respectively. As shown in Fig. 3A, the circulating fetuin-A level in adult NAFLD patients was increased among Europeans (SMD=0.71, 95% CI: 0.35–1.07, *p*<0.001), and no significant differences were observed in the Eurasians (SMD=0.73, 95% CI: -0.04–1.50, *p*=0.062) nor Asians (SMD=0.18, 95% CI: -0.08–0.43, *p*=0.174). In the pediatric/adolescent group, there was no significant difference of circulating fetuin-A level between NAFLD patients and controls of European populations (SMD=0.27, 95% CI: -0.25–0.79, *p*=0.303) and Eurasian populations (SMD=0.15, 95% CI: -0.58–0.88, *p*=0.688). Subgroup analysis results according to NAFLD diagnosis method (ultrasound vs. liver biopsy) are shown in Fig. 3B. In adults, the level of circulating fetuin-A was higher in both ultrasound-proven NAFLD patients and liver biopsy-proven NAFLD patients than in healthy controls (SMD=0.21, 95% CI: 0.01–0.42, *p*<0.001; SMD=0.86, 95% CI: 0.51–1.21, *p*<0.001, respectively). Interestingly, the circulating fetuin-A level in ultrasound-proven NAFLD pediatric/adolescent patients was significantly increased compared to pediatric/adolescent controls (SMD=0.42, 95% CI: 0.12–0.72, *p*=0.007), but no difference was observed between the liver biopsy-proven NAFLD pediatric/adolescent patients and healthy

Table 2. Relationship of circulating fetuin-A levels with the progression of NAFLD

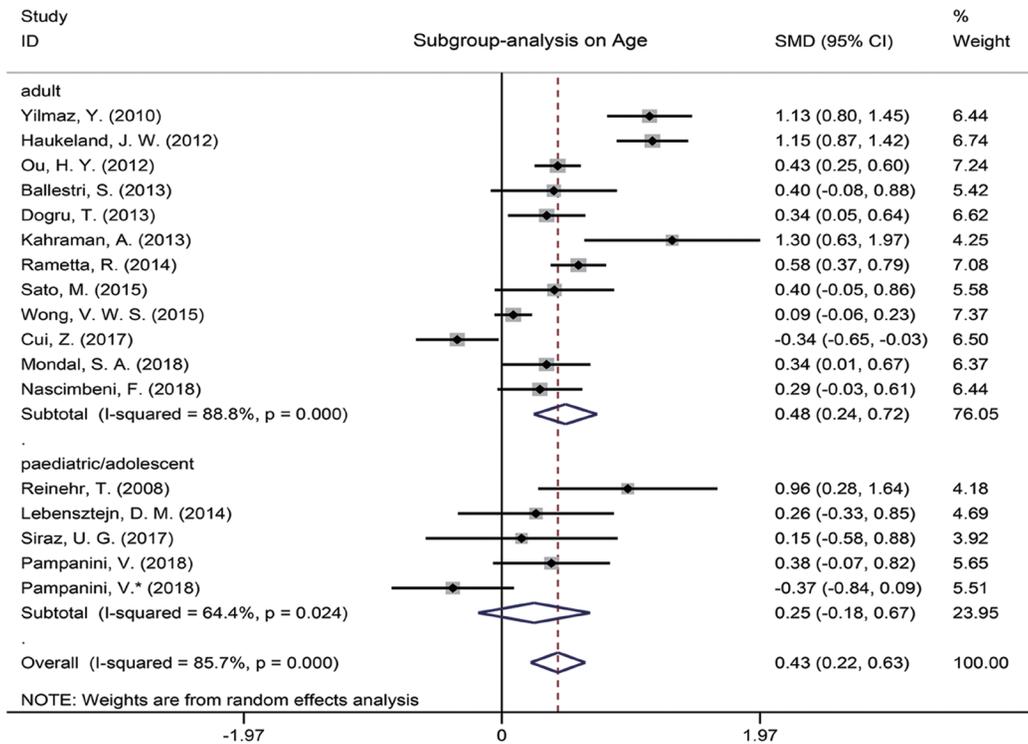
Studies	NASH vs. NAFL				Fibrosis vs. No fibrosis			
	Sample sizes	Correlation	Diagnosis method	Data	Correlation	Diagnosis method	Data	Logistic regression analysis
Yilmaz <i>et al.</i> (2010)	99	Ns	Liver biopsy	Not shown	Positive correlation	Liver biopsy	Graphic results	Consistent
Haukeland <i>et al.</i> (2012)	111	Ns	Liver biopsy	Graphic results	Ns	Liver biopsy	Not shown	/
Kahraman <i>et al.</i> (2013)	109	Ns	Liver biopsy	Detailed data	Negative correlation	Liver biopsy	Not shown	/
Rametta <i>et al.</i> (2014)	137	Ns	Liver biopsy	Graphic results	Ns	Liver biopsy	Not shown	/
Celebi <i>et al.</i> (2015)	105	Ns	Liver biopsy	Detailed data	Ns	Liver biopsy	Detailed data	/
Sato <i>et al.</i> (2015)	275	/	/	/	Negative correlation	serology	Detailed data	Consistent
Pampanini <i>et al.</i> (2018)	81	Ns	Liver biopsy	Detailed data	Ns	Liver biopsy	Graphic results	
Mondal <i>et al.</i> (2018)	46	/	/	/	Positive correlation	FibroScan	Detailed data	Consistent

Abbreviation: Ns, not significant.

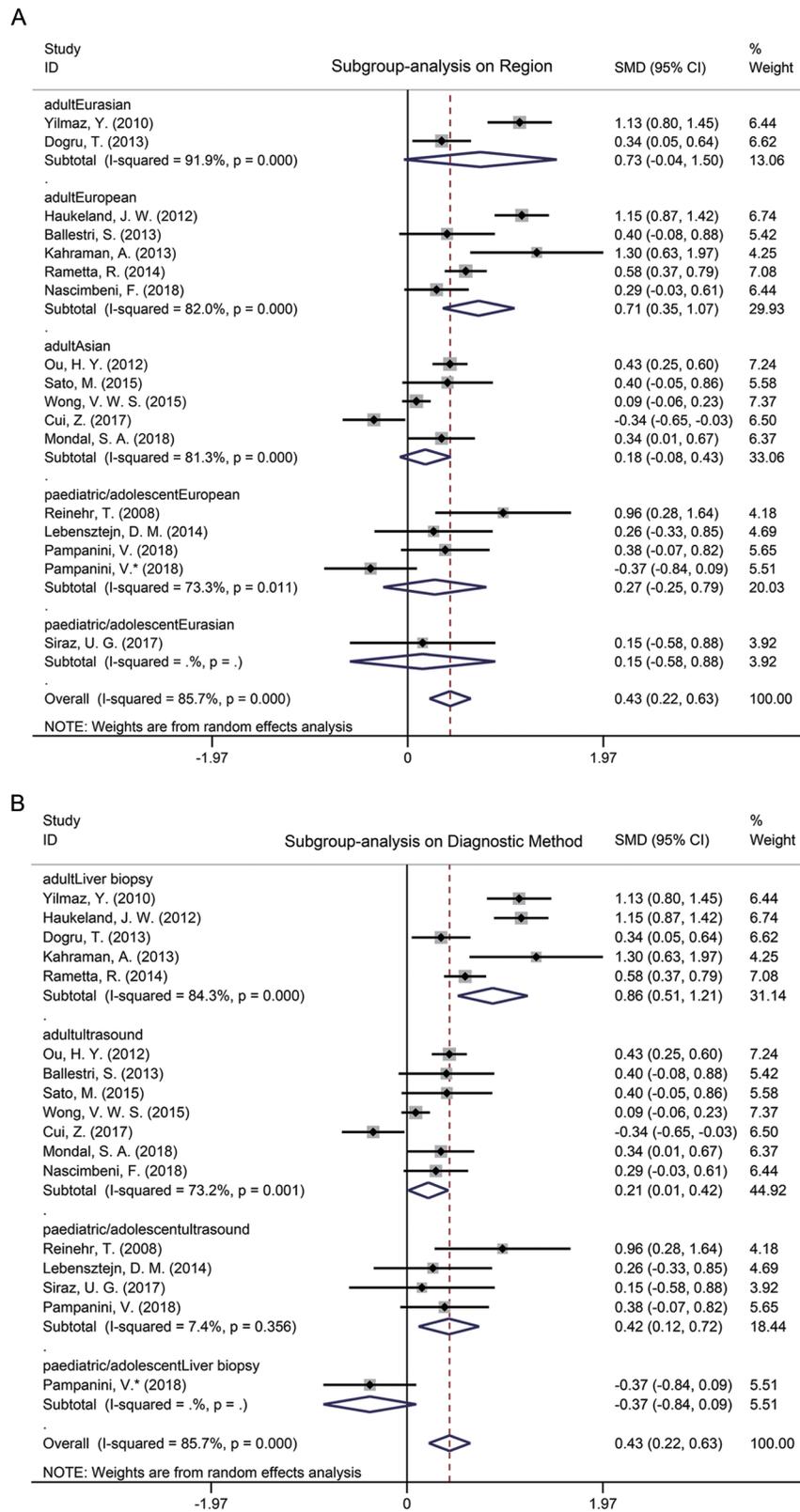
A



B



**Fig. 2. Meta-analysis and influence of age.** (A) Meta-analysis of the circulating fetuin-A levels in the included NAFLD patients compared to healthy controls. (B) Subgroup analysis on the difference of circulating fetuin-A levels between the included NAFLD patients and healthy controls based on age.



**Fig. 3. Influence of region and diagnostic method.** (A) Subgroup analysis on the difference of circulating fetuin-A levels between the included NAFLD patients and healthy controls based on region. (B) Subgroup analysis on the difference of circulating fetuin-A levels between the included NAFLD patients and healthy controls based on diagnostic method.

**Table 3. Demographic and clinical data of patients with NAFLD and healthy controls among the adults**

Studies	Group	Size, n	Males, %	Age in years, mean (SD)	BMI in kg/m <sup>2</sup> , mean (SD)	HOMA-IR
Yilmaz et al. (2010)	NAFLD	99	46.0	47.0 (9.0)	30.7 (4.9)	3.80 (0.40)
	Control	75	51.0	47.0 (8.0)	27.5 (4.3)	1.40 (0.30)
Haukeland et al. (2012)	NAFLD	111	60.0	46.5 (11.6)	30.5 (4.3)	2.21 (1.14)
	Control	131	44.0	43.3 (3.0)	23.9 (3.0)	1.40 (0.77)
Ou et al. (2012)	NAFLD	255	56.0	61.1 (10.3)	26.7 (3.1)	1.21 (0.12)
	Control	255	60.0	62.1 (11.3)	23.3 (2.8)	0.58 (0.16)
Ballestri et al. (2013)	NAFLD	29	69.0	64.5 (10.5)	29.2 (5.0)	1.50 (0.325)
	Control	41	68.3	70.6 (12.7)	25.8 (3.1)	1.40 (0.350)
Dogru et al. (2013)	NAFLD	115	100.0	31.0 (5.2)	28.4 (2.97)	3.35 (2.18)
	Control	74	100.0	28.0 (5.2)	24.0 (2.65)	1.22 (0.62)
Kahraman et al. (2013)	NAFLD	108	23.0	41.9 (0.9)	53.3 (1.1)	Na
	Control	10	50.0	32.5 (5.5)	23.9 (1.2)	Na
Rametta et al. (2014)	NAFLD	137	77.4	49.7 (12.1)	26.9 (3.4)	2.50 (2.80)
	Control	260	80.0	47.7 (12.1)	25.1 (2.8)	1.30 (0.20)
Sato et al. (2015)	NAFLD	275	55.0	56.4 (6.9)	26.5 (3.6)	Na
	Control	20	65.0	61.0 (7.0)	22.2 (2.6)	Na
Wong et al. (2015)	NAFLD	263	54.0	51.0 (9.0)	25.3 (4.0)	2.50 (0.37)
	Control	657	37.4	47.0 (11.0)	21.3 (3.1)	1.10 (0.15)
Cui, Xuan, and Yang (2017)	NAFLD	79	73.0	42.0 (10.8)	26.0 (3.0)	3.27 (2.18)
	Control	79	73.0	40.0 (12.0)	22.0 (2.0)	1.81 (1.80)
Mondal et al. (2018)	NAFLD	46	57.0	49.5 (12.2)	27.5 (6.2)	1.10 (0.26)
	Control	142	66.0	46.2 (12.7)	25.7 (4.8)	1.10 (0.10)
Nascimbeni et al. (2018)	NAFLD	80	79.0	70.0 (7.0)	28.0 (3.8)	1.80 (3.05)
	Control	69	77.0	73.0 (8.2)	25.0 (2.5)	1.40 (1.87)
R <sup>2</sup> (%)			15.95	-10.90	41.60	-13.12
β			0.297	0.995	1.058	0.041
p value			0.097	0.693	0.023	0.812

Abbreviation: NA, not applicable.

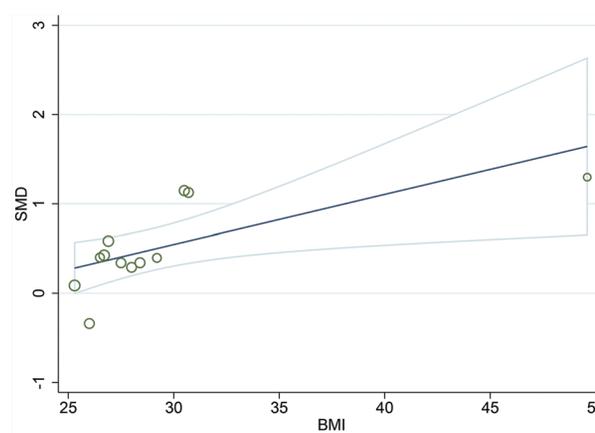
controls (SMD=-0.37, 95% CI: -0.84-0.09,  $p=0.116$ ). In addition, heterogeneity in the pediatric/adolescent patients with ultrasound diagnosis was markedly lower ( $I^2=7.4%$ ,  $p=0.356$ ) than the overall heterogeneity in the remaining pediatric/adolescent patients ( $I^2=64.4%$ ,  $p=0.024$ ) (Figs. 2B and 3B).

To further investigate the cause of heterogeneity in adult NAFLD patients, we performed univariate, random-effects meta-regression analysis to test whether the continuous variables, including sex distribution (percentage of males), mean age, BMI and HOMA-IR of NAFLD patients, could explain the high heterogeneity among studies. As the results show in Table 3 and Fig. 4, BMI was the significant influencing factor of the meta-analysis ( $R^2=41.60$ ,  $\beta=1.058$ ,  $p=0.023$ ), and the other tested variables did not show moderating effects.

### Sensitivity and publication bias analyses

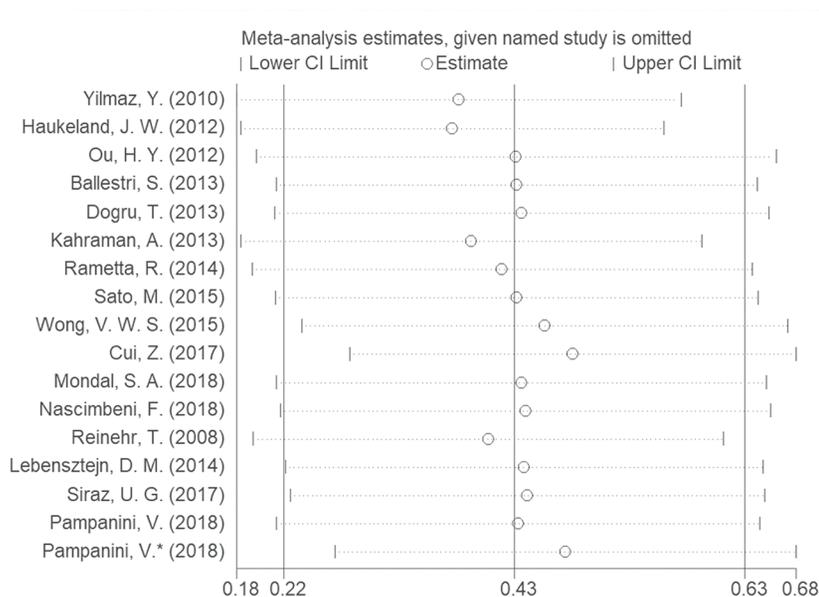
A leave-one-out sensitivity analysis was conducted to evaluate the stability of this meta-analysis (Fig. 5A). Each study included in our meta-analysis was evaluated one-by-one, to reflect the effect of pooled SMDs. The overall statistical significance did not change when any single study was omitted at one time. Therefore, the data presented in this meta-analysis is relatively stable and credible. Publication bias in this meta-analysis was confirmed by Egger's test, the

results showed no significant publication bias ( $p=0.152$ ). In addition, no significant publication biases were observed in the adult population Egger's test ( $p=0.275$ ) and in the pediatric/adolescent population Egger's test ( $p=0.450$ ). Funnel plots of effect size vs. standard error were symmetrical ( $p>0.05$ ) (Fig. 5B).

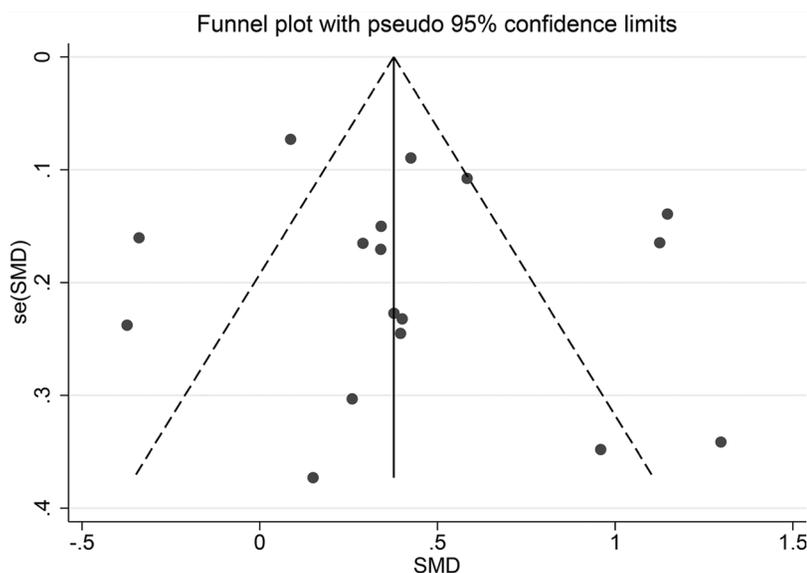


**Fig. 4. Meta-regression analysis for the effect of BMI on the NAFLD patients and healthy controls.**

A



B



**Fig. 5. Sensitivity analysis and forest plotting.** (A) Sensitivity analysis of the summary odds ratio coefficients on the difference of circulating fetuin-A levels between NAFLD patients and healthy controls. (B) Forest plots on the difference of circulating fetuin-A levels between NAFLD patients and healthy controls.

**Effect of fetuin-A levels on grading of NAFLD**

In order to investigate whether circulating fetuin-A level can be used as a potential diagnostic biomarker for the classification of NAFLD, all the available information of circulating fetuin-A level on the classification of NAFLD were collected. As shown in Table 2, there were no significant differences of circulating fetuin-A level in the classification of NAFL vs. NASH in liver biopsy-proven NAFLD patients. In the liver biopsy-proven fibrosis patients, results from four studies suggested there was not association between circulating fetuin-A level and the development of fibrosis, two stud-

ies showed that circulating fetuin-A levels were negatively correlated with fibrosis, and one study showed a positive correlation. In addition, one study found that circulating fetuin-A level was negatively correlated with serology-proven fibrosis, and another one study showed positive correlation in FibroScan-proven fibrosis (Table 2).

**Discussion**

NAFLD has become one of the most prevalent chronic liver diseases in the world and the major cause of liver-related

morbidity and mortality.<sup>1</sup> Up to now, liver biopsy remains the recognized gold standard for the diagnosis of NAFLD. In consideration of the defects of biopsy, several imaging and serological diagnosis methods have been developed. Fetuin-A is a member of the fetuin group of serum binding proteins that are primarily synthesized by the hepatocytes.<sup>54</sup> Some studies have investigated the circulating fetuin-A level between NAFLD patients and healthy controls in adult or pediatric/adolescent populations, but the results have been inconsistent. In this study, we conducted a meta-analysis to summarize the circulating fetuin-A levels in NAFLD patients and healthy controls, and a systematic review to determine the correlation of circulating fetuin-A level with the classification of NAFLD. As the results show, the circulating levels of fetuin-A in patients with NAFLD were significantly higher than in healthy controls for the included subjects. There was no difference of circulating fetuin-A level between NAFL and NASH patients. In addition, the relationship of circulating fetuin-A level with fibrosis remains unclear. Sensitivity analysis suggested that our meta-analysis was stable, and no significant publication bias was observed.

In this meta-analysis, the included studies were carried out from a different region in adult and pediatric/adolescent patients, and the diagnostic methods of NAFLD were also different. A marked heterogeneity was observed ( $I^2=85.7$ ,  $p<0.001$ ) when the circulating fetuin-A levels were analyzed for the patients with NAFLD and healthy controls. In order to explore the potential factors which contribute to the heterogeneity, subgroup analysis based on the different variables was conducted. In the adults, circulating fetuin-A level was significantly increased in patients with NAFLD compared to healthy controls, with a high heterogeneity ( $I^2=88.8$ ,  $p<0.001$ ). Subsequently, the region of subjects and diagnostic methods of NAFLD were considered for analysis of the origin of heterogeneity. In the adults, circulating fetuin-A levels in Europeans were increased in patients with NAFLD but not in Eurasians and Asians, which suggested that region may be one of the influencing factors that contributes to the heterogeneity.

The circulating level of fetuin-A in patients with NAFLD did not vary according to the different diagnostic methods. Furthermore, meta-regression analysis suggested that BMI was the significant influencing factor to the heterogeneity. The reason why BMI contributes to the heterogeneity may be due to the incomplete correlation between BMI and the risk of NAFLD. Usually, subjects with metabolically healthy obesity, which is predominantly characterized by low liver fat content, may possess low risk of developing NAFLD, and the body fat distribution (more strongly than BMI) determines NAFLD in the general population; even patients with newly developed lipodystrophy can strongly develop NASH.<sup>55-57</sup>

The data of circulating fetuin-A level in NAFLD in pediatric/adolescent patients were relatively insufficient in that only four studies were included in this meta-analysis. We analyzed the pediatric/adolescent data using five individual studies, due to the study which was conducted by Pampanini *et al.*<sup>27</sup> that recruited two independent cohorts in which NAFLD was diagnosed with ultrasound and liver biopsy, respectively. There was no significance difference of circulating fetuin-A levels between patients with NAFLD and healthy controls. It is noteworthy that the high circulating fetuin-A level was observed in the ultrasound-diagnostic NAFLD in pediatric/adolescent patients. Notably, the heterogeneity of this subgroup analysis was very low ( $I^2=7.4\%$ ,  $p<0.001$ ). The circulating fetuin-A levels in biopsy-proven NAFLD and healthy controls in pediatric/adolescent patients was tested for the first time and no obvious difference of circulating fetuin-A levels was found between the two groups. The different fetuin-A levels in ultrasound-diagnostic NAFLD compared to biopsy-proven NAFLD in pediatric/adolescent patients may due to the difference of diagnostic results of

NAFLD. In other words, some ultrasound-diagnostic NAFLD may belong to healthy controls when diagnosed by biopsy. In order to illuminate this query, more studies should be conducted to investigate the circulating fetuin-A level in liver biopsy-proven NAFLD in pediatric/adolescent patients.

Fetuin-A possesses a pro-inflammatory role and is down-regulated during inflammation; expression of fetuin-A is also inversely correlated with the level of C-reactive protein, which is a well-known marker of systemic inflammation.<sup>33,47</sup> Sato *et al.*<sup>25</sup> reported that fetuin-A might promote insulin resistance and inhibit NAFLD progression, but whether circulating fetuin-A level is positively or negatively correlated with the classification of NAFLD remains controversial. Our results have suggested that circulating fetuin-A level is not related with the classification of NAFL vs. NASH, as no significant change of fetuin-A level was found between the two groups. In consideration of the previous controversial results, more studies should be conducted to investigate the relationship of fetuin-A with the markers of inflammation. Besides, there was not a definite conclusion as to whether circulating fetuin-A level was associated with fibrosis due to the positive, negative, and unrelated correlations that have been reported. In order to clarify the significant role of circulating fetuin-A level on the diagnosis of fibrosis, more attention should be paid to the relationship of circulating fetuin-A level with fibrosis in different regions and ethnicity groups.

Our study had several limitations. First, considerable heterogeneity among studies limits the reliability of the results. Although we performed subgroup and meta-regression analyses to investigate some potential sources of heterogeneity, the high levels of heterogeneity cannot be reasonably explained in adult NAFLD. Second, due to the lack of detailed data on fetuin-A in the classification of NAFLD in some studies, we just reviewed the change of fetuin-A rather than making a quantitative analysis. Finally, all these studies are the cross-sectional or case-control epidemiological design, and the dynamic changes of circulating fetuin-A level in NAFL or NASH patients were unclear.

## Conclusions

In summary, the circulating fetuin-A level was significantly higher in NAFLD patients than in healthy controls in adults and no difference was observed in pediatric/adolescent patients. BMI might be the risk factor that affects the stability of meta-analysis in adults. In pediatric/adolescent patients, ultrasound-proven NAFLD patients possess a markedly higher circulating fetuin-A level than healthy controls and there were no differences in biopsy-proven NAFLD patients. Our results suggest that circulating fetuin-A level could be regarded as a potential serum biomarker for the early diagnosis of NAFLD. Diagnostic values and differences of ultrasound and biopsy in pediatric/adolescent should be further studied to illuminate the significant effect of circulating fetuin-A level on pediatric/adolescent NAFLD. In addition, association of circulating fetuin-A level with fibrosis should be studied further.

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## Conflict of interest

The authors have no conflict of interests related to this publication.

## Author contributions

Study concept and design (SL, YX), data collection (SL, JX, ZZ, MW, and YW), analysis of data (SL, JX, and ZZ), drafting and writing of the manuscript (SL and JX), revision of the manuscript (YX).

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