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Non-alcoholic fatty liver disease and risk of type 2 diabetes

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Non-alcoholic fatty liver disease (NAFLD) covers a spectrum of liver disease from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. NAFLD is commonly associated with features of the metabolic/insulin resistance syndrome ('Metabolic/Obese NAFLD') and may therefore predict type 2 diabetes (T2DM). For this review, we searched for prospective studies examining whether NAFLD predicts T2DM, and if so, whether this occurs independently of factors such as age and obesity. These studies included NAFLD diagnosed by ultrasonography (n = 6) or liver enzymes (n = 14). All ultrasonography studies found NAFLD to predict the risk of T2DM independently of age, and in 4 out of 6 studies NAFLD was also a predictor independently of BMI. NAFLD was a predictor of T2DM in all 14 studies where NAFLD was diagnosed by liver enzymes. In 12 of these studies, ALT or AST or GGT were significant predictors of T2DM risk, independently of age and BMI. NAFLD, however, is heterogeneous and may also be caused by common genetic variants. The I148M variant in *PNPLA3* and the E167K variant in *TM6SF2* are both associated with increased liver fat content, but not features of the metabolic/insulin resistance syndrome. These genetic forms of NAFLD predict NASH and cirrhosis but not T2DM. Taken together these data imply

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; GGT, Gamma-glutamyltransferase; HCC, Hepatocellular carcinoma; HDL, High-density lipoprotein; ^{1}H-MRS, Proton magnetic resonance spectroscopy; HOMA-IR, Homeostasis model assessment for insulin resistance; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; OGTT, Oral glucose tolerance test; PNPLA3, Patatin-like phospholipase domain-containing 3; T2DM, Type 2 diabetes; TM6SF2, Transmembrane 6 superfamily member 2; VLDL, Very low-density lipoprotein.

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that 'Metabolic/Obese NAFLD' predicts T2DM independently of age and obesity and support the role of hepatic insulin resistance in the pathogenesis of this disease.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as hepatic steatosis not caused by excess use of alcohol (>20 g/day in women, >30 g/day in men), viruses such as hepatitis B or C, autoimmune hepatitis, use of hepatotoxic drugs or other compounds, or rare genetic forms [1]. It covers a range of conditions from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. NAFLD is currently the most common liver disorder with an estimated worldwide prevalence of 25% [2]. Depending on the method of diagnosis, 65–87% of patients with type 2 diabetes (T2DM) have NAFLD [3,4]. NAFLD is the second most common cause of being on a waiting list for a liver transplant in the US [5] and the most common cause of hepatocellular carcinoma (HCC) in both US [6] and UK [7].

The metabolic/insulin resistance syndrome is a well-established predictor of T2DM, although overt hyperglycemia only develops in those whose beta-cells fail to sustain hyperinsulinemia in the face of insulin resistance [8]. The liver is the site of production of glucose and very low-density lipoprotein (VLDL) -triglycerides. In subjects with 'metabolic/obese NAFLD', the liver is insulin resistant leading to overproduction of both glucose and VLDL [8]. Glucose in turn stimulates insulin secretion thereby inducing hyperinsulinemia. The increase in VLDL leads to lowering of the concentration of high-density lipoprotein (HDL) cholesterol. These changes are often observed in obese subjects, but are also observed independently of obesity [9]. NAFLD is thus closely linked to the pathogenesis of the metabolic syndrome raising the possibility that NAFLD predicts T2DM, even independently of obesity.

In addition to the association of NAFLD with the metabolic/insulin resistance syndrome, two common genetic variants increase the risk of NAFLD. A variant in the patatin-like phospholipase domain-containing 3 (*PNPLA3*) (rs738409[G], encoding I148M) confers to NAFLD susceptibility by increasing liver fat content, risk of inflammation, and fibrosis ('PNPLA3 NAFLD') [10,11]. Genetic variation in the transmembrane 6 superfamily member 2 (*TM6SF2*) (rs58542926[T], encoding E167K) is also associated with liver fat accumulation and increased risk of NASH ('TM6SF2 NAFLD') [12,13]. Insulin resistance is not a characteristic of these two conditions [14], although genetic and metabolic causes of NAFLD may both exist in the same person [15].

The ensuing discussion is focused on reviewing studies which have examined whether NAFLD, diagnosed either by liver enzymes, ultrasonography, other imaging techniques, or by liver biopsy, predicts T2DM, and if so, whether this is observed independently of obesity and other established predictors of T2DM. We will also briefly comment on whether and why NAFLD should be screened for in the diabetes clinic.

Methods

Data sources and searches

This systematic review was performed as suggested by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) group [16]. We searched MEDLINE using the terms "fatty liver", "diabetes", and "ultrasound" for publications with NAFLD diagnosed by ultrasonography, "magnetic resonance spectroscopy" for those with NAFLD diagnosed by ^{1}H-MRS, and "biopsy" for those with NAFLD diagnosed by biopsy. In addition, we used the terms "fatty liver", "steatosis", "liver enzymes", "transaminases", "alanine aminotransferase" (ALT), "aspartate aminotransferase" (AST), "gamma-glutamyltransferase" (GGT), and "diabetes incidence" while searching for publications in which NAFLD had been diagnosed by using liver enzymes. All searches were performed by the end of February 2016.

Study selection

Inclusion criteria

We included prospective longitudinal cohort studies investigating whether baseline NAFLD predicts the development of T2DM. We included only publications in English.

Exclusion criteria

Since serum or plasma liver enzyme concentrations and liver fat content may increase due to other reasons than NAFLD [17,18], we excluded studies which did not mention exclusion of subjects with hepatitis B or C, cirrhosis or malignancy, and those in which alcohol consumption was not assessed or the analyzes were not adjusted for alcohol consumption.

Results

Study selection

The searches resulted in 1718 potentially relevant citations; NAFLD was defined using liver enzymes in 810, ultrasonography in 605, ^{1}H-MRS in 247, and liver biopsy in 56. After screening the titles and abstracts of these citations, 54, 30, one and four, respectively, remained for further evaluation. Based on full-text judgment, we excluded i) 30 articles which were not prospective cohort studies, ii) 22 articles which did not adjust their analyzes for alcohol use or exclude other liver diseases, iii) 15 articles in which the above listed methods were not used to diagnose NAFLD, and iv) two citations which only had an abstract available. Thus, we included a total of 20 publications which are listed in Tables 1 and 2. We did not identify any longitudinal studies investigating whether ^{1}H-MRS-determined liver fat content or liver histology predict T2DM.

Study characteristics

Table 1 shows details of the six studies that used ultrasonography to diagnose NAFLD. All except one small study included subjects of Asian origin (Table 1). The mean age of study subjects ranged from 37 to 49 years and BMI from 23 to 27 kg/m^2. Duration of follow-up varied from three to 10 years. In four of the six studies [19–22], T2DM was diagnosed based on a fasting plasma or serum glucose $\geq$7.0 mmol/L, HbA_{1c} $\geq$6.5%, a 2-hour glucose concentration $\geq$11.1 mmol/L during a 75 gr oral glucose tolerance test (OGTT), and/or use of glucose-lowering therapy. Okamoto et al. defined hyperglycemia as a fasting plasma glucose concentration >6.1 mmol/L or HbA_{1c} >6.4% [23], whilst Zelber-Sagi et al. used cut-offs of $\geq$5.6 mmol/L and $\geq$5.7%, respectively [24].

Table 2 shows details of the 14 studies in which used liver enzymes to diagnose NAFLD. The mean age of study subjects ranged from 44 to 61 years and BMI from 23 to 28 kg/m^2. The duration of follow-up ranged from two to 20 years. Half of the studies included only men. T2DM was defined as a fasting glucose $\geq$7.0 mmol/L or use of diabetes medication in nine studies [25–33]. An OGTT was performed in three studies [29,32,34] and HbA_{1c} measured in one study [33]. Questionnaires or patient records were used for diagnosis of T2DM in four studies [35–38]. Most of the studies divided the subjects at baseline by quartiles or quintiles of liver enzymes and compared the risk of diabetes in highest quarter/fifth with the lowest quarter/fifth. Two studies with slightly different study designs included subjects from the same cohort [26,27].

Ultrasonography-diagnosed NAFLD and T2DM

Each of the six studies using ultrasonography to diagnose NAFLD showed that NAFLD predicted T2DM independently of baseline age. NAFLD also remained a significant predictor of T2DM in four out of the five studies, after adjustment for BMI (Table 1). NAFLD also predicted T2DM independently of several other factors such as sex, family history of diabetes, HDL-cholesterol, triglycerides, high-sensitivity C-reactive protein, insulin resistance by Homeostasis Model Assessment for insulin resistance (HOMA-IR), physical activity, and smoking status (Fig. 1).

Table 1
NAFLD as a predictor of type 2 diabetes diagnosed by ultrasonography in prospective studies.

Cohort and year	N (men, %)	Age (years)	BMI (kg/m^2)	Follow up (years)	Outcome	Independent of BMI	Independent of other confounders	Effect estimate (95% CI)
Japanese 2003 [23]	840 (55.6%)	42.7	22.5	10	T2DM +IFG	No	No (age, gender, FPG, HbA_{1c}, alcohol, family history of T2DM)	OR 2.62 (1.58–4.34)[a]
Japanese 2007 [19]	3189 (100.0%)	48.0	23.1	4	T2DM	Yes	Age	HR 4.8 (3.3–7.1)
Koreans 2013 [20]	25,232 (100.0%)	42.5	24.2	3.8	T2DM	–	Age, WC, TG, HDL, systolic BP, hsCRP, HOMA, creatinine, family history T2DM, exercise, MetS	mild NAFLD HR 1.09 (0.81–1.48) moderate/severe NAFLD HR 1.73 (1.00–3.01)
Sri Lankans 2013 [21]	1842 (2880[b]; 43.2%)	–(52.5)	–(24.0)	3	T2DM	Yes	Age, gender, WC, ALT, family history of T2DM, hypertension	HR 1.64 (1.20–2.23)
Koreans 2013 [22]	38,291 (62.5%)	36.8	23.3	5.1	T2DM	Yes	Age, gender, smoking, alcohol, exercise, family history of T2DM, cholesterol, TG, HDL, HOMA, hsCRP	Low NFS HR 1.81 (1.61–2.04) High NFS HR 3.84 (2.93–5.02)
Israelis 2013 [24]	141 (50.4%)	48.8	26.6	6.8	T2DM +IFG	Yes	Age, gender, family history of T2DM, fS-insulin, adiponectin, fS-glucose, physical activity	Normal US OR 2.95 (1.03–8.44) HRI OR 7.77 (1.82–33.26)

ALT, alanine transferase; BP, blood pressure; DM, diabetes mellitus, fS, fasting serum; FPG, fasting plasma glucose; HbA_{1c}, glycosylated hemoglobin A_{1c}, HDL, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment for insulin resistance; HR, hazard ratio; HRI, hepato-renal ultrasound index; hsCRP, high-sensitivity C-reactive protein; IFG, impaired fasting glucose; MetS, metabolic syndrome; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD fibrosis score; OR, odds ratio; T2DM, type 2 diabetes; TG, triglyceride; US, ultrasonography; WC, waist circumference.

[a] Unadjusted estimate.

[b] More subjects at baseline than at follow up.

Table 2
NAFLD diagnosed by liver function tests as a predictor of T2DM. Prospective studies.

Cohort, study and year	N (men, %)	Age (years)	BMI (kg/m2)	Follow up (years)	Predictive liver function test	Independent of BMI	Independent of other confounders	Effect estimate (95% CI)[a]
British men BRHS, UK, 1998 [35]	7458 (100.0%)	40–59	–	12.8	GGT	Yes	Age, physical activity, alcohol, smoking, prevalent CHD	Quintiles Q5 vs. Q1 RR 4.7 (2.4–9.4)
Korean men 2003 [25]	4088 (100.0%)	25–55	–	4	GGT, ALT	Yes	Age, smoking, exercise, family history of T2DM, FPG, alcohol	Highest vs. lowest concentration group with alcohol <90 g/week GGT RR 3.6 (1.1–2.0)
Japanese men 2003 [26]	2918 (100.0%)	46.5	23.3	7	GGT	Yes	Age, family history of T2DM, alcohol, smoking, physical activity, systolic BP, cholesterol, TG, FPG, white blood count	Quartiles Q4 vs. Q1 RR 3.44 (1.69–6.70)
Japanese men 2004 [27]	3260 (100.0%)	–	–	7	GGT, ALT	Yes	Age, family history of T2DM, alcohol, smoking, physical activity, FPG, white blood count, other liver enzymes	Quintiles Q5 vs. Q1 GGT HR 2.44 (1.34–4.46)
Scotsmen with hypercholesterolemia WOSCOPS, Scotland 2004 [28]	5974 (100.0%)	55.4	26.0	4.9	ALT	Yes	Age, smoking, systolic BP, cholesterol/HDL ratio, TG, alcohol, FPG	Quartiles Q4 vs. Q1 HR 2.04 (1.16–3.58)
Mexicans Mexico City Diabetes Study, 2005 [29]	1441 (38.8%)	47.1	28.0	7	AST	Yes	Age, gender, WC, alcohol, FSI	Quartile Q4 vs. Q1–3 OR 1.67 (1.06–2.64)
British men BRHS, UK, 2005 [36]	3500 (100.0%)	60–79	–	5	GGT, ALT	Yes	Age, social class, physical activity, smoking, alcohol, preexisting CHD/stroke, use of statins	Quartiles: Q4 vs. Q1 GGT RR 3.68 (1.68–8.04)
Koreans 2007 [34]	8750 (46.6%)	51.8	24.4	2	ALT, GGT	Yes	Age, systolic BP, family history of T2DM, smoking, alcohol, exercise, FPG, TG, HDL, HOMA, high-sensitivity CRP	Quartiles Q4 vs. Q1 ALT RR 2.20 (1.28–3.73) in men RR 1.97 (1.03–3.77) in women
Framingham Offspring Heart Study, US, 2008 [30]	2812 (44.4%)	44.0	25.6	20	ALT, AST	Yes	Age, gender, smoking, menopause, alcohol	Per +1 SD in logALT ALT OR 1.48 (1.30–1.69)
Germans 2008 [37]	2298 (38.3%)	49.5	26.0	7.0	GGT, ALT	Yes	Age, gender, education, smoking, alcohol, physical activity, WC, systolic BP, cholesterol, HDL, CRP, FPG	Quintiles Q5 vs. Q1 GGT HR 2.61 (1.59–4.28)
Italians FIBAR, IT 2009 [38]	2662 (42.9%)	54.3	25.9	3.3	GGT, ALT, AST	–	Age, gender, alcohol, smoking	Per +10 U/l GGT HR 1.09 (1.04–1.15)

(*continued on next page*)

Table 2 (*continued*)

Cohort, study and year	N (men, %)	Age (years)	BMI (kg/m2)	Follow up (years)	Predictive liver function test	Independent of BMI	Independent of other confounders	Effect estimate (95% CI)[a]
Australians 2009 [31]	358 (68.4%)	59.9	27.1	11.1	ALT	–	No (age, WC, HOMA, HDL, TG)	ALT >40 vs. <40 U/l RR 3.1 unadjusted
Asian Indian men with IFG 2014 [32]	537 (100.0%)	46.0	25.8	2	GGT	Yes	Age, family history of T2DM, smoking, alcohol, ALT, OGTT, FPG, HbA_{1c}, TG, HOMA	Above vs. below median GGT HR 1.78 (1.17–2.68)
Koreans 2014 [33]	6926 (37.6%)	61.4	24.3	4.2	GGT, ALT	Yes	Age, WC, cholesterol, HDL, TG, alcohol, smoking, physical activity, follow-up time, CRP	Quartiles Q4 vs. Q1 GGT OR 2.13 (1.33–3.41) in men OR 2.69 (1.86–3.89) in women

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; CHD, coronary heart disease; FG, fasting plasma or serum glucose; FSI, fasting serum insulin; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment for insulin resistance; HR, hazard ratio; IFG, impaired fasting glucose; NAFLD, non-alcoholic fatty liver disease; OGTT, 2-hour oral glucose tolerance test; OR, odds ratio; RR, risk ratio; T2DM, type 2 diabetes; TG, triglyceride; US, ultrasonography; WC, waist circumference.

[a] The column represents the effect estimate of the best predictor of type 2 diabetes in the respective study.

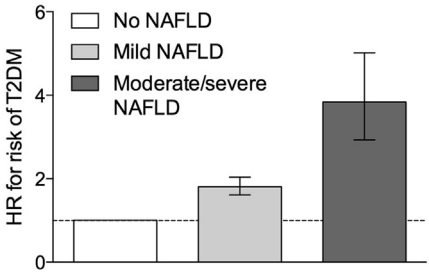

Fig. 1. Risk of T2DM in the mild and moderate/severe NAFLD compared to the non-NAFLD group. NAFLD was diagnosed by ultrasonography. Severity of NAFLD was defined based on NAFLD fibrosis score using the cut-off point <-1.455 for mild and ≥ -1.455 for moderate/severe NAFLD [68]. Analyzes are adjusted for age, sex, BMI, smoking, alcohol intake, exercise, family history of T2DM, total cholesterol, triglycerides, HDL-cholesterol, HOMA-IR, high-sensitive CRP. Data are obtained from the study written by Chang et al. [22] and shown as the hazard ratios and 95% confidence intervals. *P*-value for a trend was <0.001. HR, hazard ratio; NAFLD, non-alcoholic fatty liver disease; T2DM, type 2 diabetes.

Liver enzymes and T2DM

In 12 of the 14 studies, either GGT or ALT or AST or a combination of these enzymes was a significant predictor of T2DM independently of age, BMI and alcohol consumption (Table 2). GGT was a significant predictor in 10 of 11, ALT in 10 of 13 and AST in three of seven studies (Table 2). GGT and ALT predicted T2DM even in the normal range (Fig. 2).

Discussion

The data from the multiple available large studies document that NAFLD, diagnosed either by ultrasonography or elevated liver enzymes, predicts an increased risk of T2DM independently of age and obesity. These results raise the question as to whether the presence of NAFLD should be used in clinical practice to identify patients at risk for T2DM.

Ultrasonography-diagnosed NAFLD predicted T2DM in all studies, and in most studies after adjustment for potential confounders. These studies were, however, performed in Asian subjects, with the exception of one small study in Israelis (Table 1). Asian subjects, both those with NAFLD [39] and those with T2DM [40,41] are leaner than Europid or American subjects. It is therefore uncertain whether these data apply to non-Asian subjects. Since ultrasonography is unreliable and difficult to use in obese subjects [42], it is possible that it is more sensitive to detecting T2DM risk in Asian subjects. Ultrasonography is also inaccurate at quantifying liver fat percentages below 20–30% [43] which may influence estimation of disease risk. On the other hand, ultrasonography is widely available and can detect focal lesions in addition to providing a semi-quantitative estimate of steatosis.

Of the liver enzymes, it is well established that GGT is more sensitive to alcohol intake than ALT [44,45]. Nevertheless, both GGT and ALT predicted T2DM even in studies which excluded excessive alcohol use already at baseline [29–31], as well as in those statistically adjusting for alcohol intake [25–30,32–38]. The relationship between ALT and risk of T2DM was linear and observed within the normal range of ALT (Fig. 2B). Although ALT and GGT predicted T2DM on average, the correlation between liver fat quantified by ^{1}H-MRS, the state-of the art technique, and ALT is sex-dependent and weaker than between liver fat and fasting serum insulin [46]. From the relationship between liver fat and ALT, we could calculate that ALT is normal in 48% of subjects with NAFLD diagnosed by ^{1}H-MRS (liver fat content equal or greater than 5.56%), and 23% of subjects without NAFLD have increased ALT (ALT >30 U/L in women, >40 U/L in men) [46]. This implies that ALT has major limitations as a predictor of T2DM for individuals. AST is not very helpful either as it is less liver specific than ALT [47]. Consistent with the present data (Fig. 2B and C), meta-analysis of prospective longitudinal studies found ALT but not AST to increase the risk of T2DM [48].

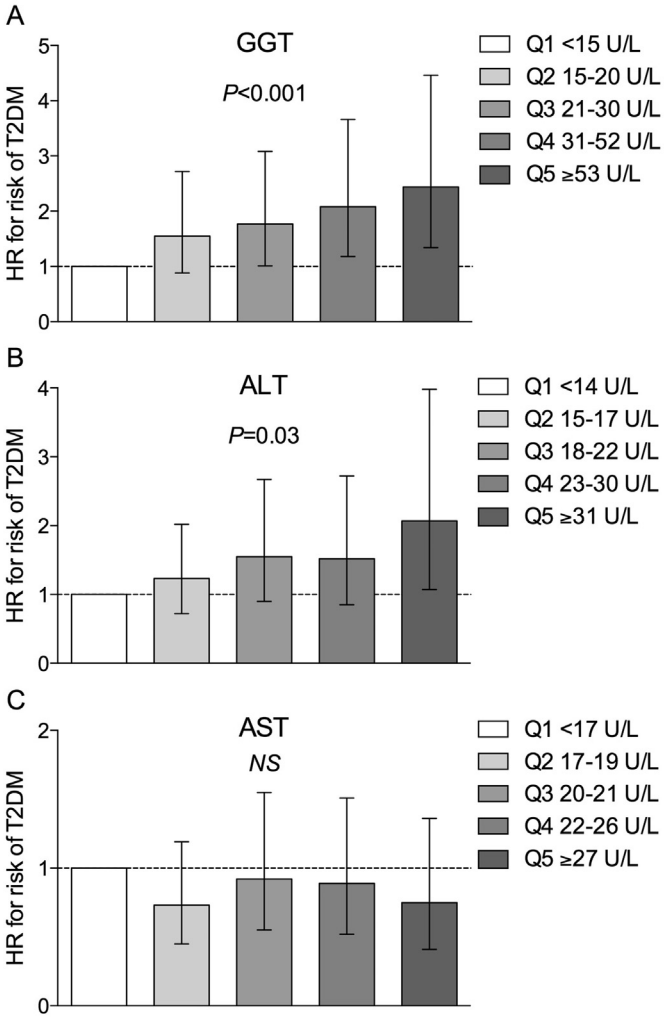

Fig. 2. Risk of T2DM according to quintiles of liver enzymes GGT (panel A), ALT (panel B) and AST (panel C). Analyzes are adjusted for age, BMI, smoking, alcohol intake, physical activity, family history of T2DM, fasting plasma glucose, white blood cell count, the other liver enzymes including also alkaline phosphatase. Data are obtained from the study written by Nakanishi et al. [27] and shown as the hazard ratios and 95% confidence intervals. *P*-value is presented for a trend. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; HR, hazard ratio; NS, non-significant; Q, quintile; T2DM, type 2 diabetes.

NAFLD is closely linked to the pathogenesis of the metabolic/insulin resistance syndrome but it can also be caused by genetic variations in *PNPLA3* [10] and *TM6SF2* [12]. Neither 'PNPLA3 NAFLD' [10,49–57] nor 'TM6SF2 NAFLD' [12,58–60] are associated with insulin resistance. We did not identify any studies, which examined whether NAFLD caused by these gene variants influenced the future risk of T2DM. These variants do predict NASH, cirrhosis and HCC worldwide [14,61], but have not turned out to be significant predictors of T2DM in the multiple genome-wide association studies searching for genetic risk markers for T2DM [62].

In conclusion, abundant longitudinal studies especially in Asian populations using ultrasonography have shown NAFLD to predict T2DM independently of confounders such as age and obesity. Liver enzymes, especially ALT and GGT are also independent predictors of T2DM. While these studies

support the view that hepatic insulin resistance is an important feature of the pathogenesis of T2DM, they are not particularly useful in the clinic because of limitations in both ultrasonography and liver enzymes as diagnostic tools, and because of the heterogeneity of NAFLD. Indeed, established methods such measurement of features of the metabolic syndrome and assessment of family history [63] remain the gold standard for predicting risk of T2DM. On the other hand, physicians treating T2DM should not forget to think of the liver and should measure at least liver enzymes in all patients as patients with T2DM have a markedly increased risk of developing NASH [64] and cirrhosis [65] and even HCC [66,67].

Practice points

- Non-alcoholic fatty liver disease (NAFLD) is defined as hepatic steatosis not caused by excess use of alcohol (>20 g/day in women, >30 g/day in men), viruses such as hepatitis B or C, autoimmune hepatitis, use of hepatotoxic drugs, or rare genetic forms.
- NAFLD is currently the most common liver disorder with a prevalence of 25%
- NAFLD closely linked to the metabolic/insulin resistance syndrome is often observed in obese subjects, but also independently obesity.
- Insulin resistance is not a characteristic of NAFLD due to the common genetic variations in *PNPLA3* or *TM6SF2*.
- 'Metabolic/Obese NAFLD' predicts T2DM independently of age and obesity and support the role of hepatic insulin resistance in the pathogenesis of T2DM.

Research agenda

- Cost-effectiveness of imaging the liver in patients with the metabolic syndrome or type 2 diabetes
- Cost-effectiveness of genotyping for *PNPLA3* and *TM6SF2* variants in the identification of subjects at risk for advanced liver disease in the clinic

Conflict of interest

None.

Acknowledgments

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References

[1] European Association for the Study of the Liver (EASL). European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388–402.

[2] Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of non-alcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence and outcomes. Hepatology 2016;64:73–84.
*[3] Doycheva I, Cui J, Nguyen P, et al. Non-invasive screening of diabetics in primary care for NAFLD and advanced fibrosis by MRI and MRE. Aliment Pharmacol Ther 2016;43:83–95.
[4] Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic fatty liver disease and type 2 diabetes: common pathophysiologic mechanisms. Curr Diabet Rep 2015;15. 607,015-0607-4.
[5] Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 2015;148:547–55.
[6] Yu J, Shen J, Sun TT, et al. Obesity, insulin resistance, NASH and hepatocellular carcinoma. Semin Cancer Biol 2013;23:483–91.
[7] Dyson J, Jaques B, Chattopadyhay D, et al. Hepatocellular cancer: the impact of obesity, Type 2 diabetes and a multidisciplinary team. J Hepatol 2014;60:110–7.
[8] Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014;2:901–10.
*[9] Kotronen A, Westerbacka J, Bergholm R, et al. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab 2007;92:3490–7.
*[10] Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40:1461–5.
*[11] Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–17.
*[12] Kozlitina J, Smagris E, Stender S, et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014;46:352–6.
[13] Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.
[14] Anstee QM, Day CP. The genetics of nonalcoholic fatty liver disease: spotlight on PNPLA3 and TM6SF2. Semin Liver Dis 2015;35:270–90.
*[15] Luukkonen PK, Zhou Y, Sadevirta S, et al. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. J Hepatol 2016;64:1167–75.
[16] Moher D, Liberati A, Tetzlaff J, et al. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009;339. b2535.
[17] Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American association for the study of liver diseases, American college of gastroenterology, and the American gastroenterological association. Am J Gastroenterol 2012;107:811–26.
[18] Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol 2003;98:960–7.
[19] Shibata M, Kihara Y, Taguchi M, et al. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. Diabetes Care 2007;30:2940–4.
[20] Park SK, Seo MH, Shin HC, et al. Clinical availability of nonalcoholic fatty liver disease as an early predictor of type 2 diabetes mellitus in Korean men: 5-year prospective cohort study. Hepatology 2013;57:1378–83.
[21] Kasturiratne A, Weerasinghe S, Dassanayake AS, et al. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. J Gastroenterol Hepatol 2013;28:142–7.
*[22] Chang Y, Jung HS, Yun KE, et al. Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. Am J Gastroenterol 2013;108:1861–8.
[23] Okamoto M, Takeda Y, Yoda Y, et al. The association of fatty liver and diabetes risk. J Epidemiol 2003;13:15–21.
[24] Zelber-Sagi S, Lotan R, Shibolet O, et al. Non-alcoholic fatty liver disease independently predicts prediabetes during a 7-year prospective follow-up. Liver Int 2013;33:1406–12.
[25] Lee DH, Ha MH, Kim JH, et al. Gamma-glutamyltransferase and diabetes–a 4 year follow-up study. Diabetologia 2003;46:359–64.
[26] Nakanishi N, Nishina K, Li W, et al. Serum gamma-glutamyltransferase and development of impaired fasting glucose or type 2 diabetes in middle-aged Japanese men. J Intern Med 2003;254:287–95.
[27] Nakanishi N, Suzuki K, Tatara K. Serum gamma-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. Diabetes Care 2004;27:1427–32.
[28] Sattar N, Scherbakova O, Ford I, et al. West of Scotland coronary prevention study. Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. Diabetes 2004;53:2855–60.
[29] Nannipieri M, Gonzales C, Baldi S, et al. Mexico City diabetes study. Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City diabetes study. Diabetes Care 2005;28:1757–62.
*[30] Goessling W, Massaro JM, Vasan RS, et al. Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. Gastroenterology 2008;135. 1935,44, 1944.e1.
[31] Adams LA, Waters OR, Knuiman MW, et al. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. Am J Gastroenterol 2009;104:861–7.
[32] Nanditha A, Jagannathan R, Sundaram S, et al. Combining fasting plasma glucose with gamma-glutamyl transferase improves the sensitivity to predict incident diabetes in asian indian men with impaired glucose tolerance. J Assoc Physicians India 2014;62:18–22.
[33] Ahn HR, Shin MH, Nam HS, et al. The association between liver enzymes and risk of type 2 diabetes: the Namwon study. Diabetol Metab Syndr 2014;6(14). 5996-6-14.
[34] Cho NH, Jang HC, Choi SH, et al. Abnormal liver function test predicts type 2 diabetes: a community-based prospective study. Diabetes Care 2007;30:2566–8.
[35] Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care 1998;21:732–7.

[36] Wannamethee SG, Shaper AG, Lennon L, et al. Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes Care 2005;28:2913–8.
[37] Ford ES, Schulze MB, Bergmann MM, et al. Liver enzymes and incident diabetes: findings from the European prospective investigation into cancer and nutrition (EPIC)-Potsdam study. Diabetes Care 2008;31:1138–43.
[38] Monami M, Bardini G, Lamanna C, et al. Liver enzymes and risk of diabetes and cardiovascular disease: results of the firenze bagno a ripoli (FIBAR) study. Metabolism 2008;57:387–92.
[39] Wong RJ, Ahmed A. Obesity and non-alcoholic fatty liver disease: disparate associations among Asian populations. World J Hepatol 2014;6:263–73.
[40] Wong RJ, Chou C, Sinha SR, et al. Ethnic disparities in the association of body mass index with the risk of hypertension and diabetes. J Community Health 2014;39:437–45.
[41] Weston SR, Leyden W, Murphy R, et al. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. Hepatology 2005;41:372–9.
[42] Mottin CC, Moretto M, Padoin AV, et al. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. Obes Surg 2004;14:635–7.
[43] Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082–90.
[44] Sharpe PC, McBride R, Archbold GP. Biochemical markers of alcohol abuse. QJM 1996;89:137–44.
[45] Kotronen A, Yki-Jarvinen H, Mannisto S, et al. Non-alcoholic and alcoholic fatty liver disease – two diseases of affluence associated with the metabolic syndrome and type 2 diabetes: the FIN-D2D survey. BMC Public Health 2010;10(237). 2458-10-237.
[46] Kotronen A, Peltonen M, Hakkarainen A, et al. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 2009;137:865–72.
[47] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ 2005;172:367–79.
[48] Kunutsor SK, Apekey TA, Walley J. Liver aminotransferases and risk of incident type 2 diabetes: a systematic review and meta-analysis. Am J Epidemiol 2013;178:159–71.
[49] Romeo S, Sentinelli F, Dash S, et al. Morbid obesity exposes the association between PNPLA3 I148M (rs738409) and indices of hepatic injury in individuals of european descent. Int J Obes Lond 2010;34:190–4.
[50] Petit JM, Guiu B, Masson D, et al. Specifically PNPLA3-mediated accumulation of liver fat in obese patients with type 2 diabetes. J Clin Endocrinol Metab 2010;95:E430–6.
[51] Li Y, Xing C, Tian Z, et al. Genetic variant I148M in PNPLA3 is associated with the ultrasonography-determined steatosis degree in a chinese population. BMC Med Genet 2012;13(113). 2350-13-113.
[52] Li X, Zhao Q, Wu K, et al. I148M variant of PNPLA3 confer increased risk for nonalcoholic fatty liver disease not only in european population, but also in chinese population. Hepatology 2011;54:2275.
*[53] Sookoian S, Castano GO, Burgueno AL, et al. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. J Lipid Res 2009;50:2111–6.
[54] Valenti L, Alisi A, Galmozzi E, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. Hepatology 2010;52:1274–80.
[55] Krarup NT, Grarup N, Banasik K, et al. The PNPLA3 rs738409 G-allele associates with reduced fasting serum triglyceride and serum cholesterol in Danes with impaired glucose regulation. PLoS One 2012;7. e40376.
[56] Kitamoto T, Kitamoto A, Yoneda M, et al. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. Hum Genet 2013;132:783–92.
[57] Hyysalo J, Gopalacharyulu P, Bian H, et al. Circulating triacylglycerol signatures in nonalcoholic fatty liver disease associated with the I148M variant in PNPLA3 and with obesity. Diabetes 2014;63:312–22.
*[58] Zhou Y, Llaurado G, Oresic M, et al. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. J Hepatol 2015;62:657–63.
[59] Sookoian S, Castano GO, Scian R, et al. Genetic variation in transmembrane 6 superfamily member 2 and the risk of nonalcoholic fatty liver disease and histological disease severity. Hepatology 2015;61:515–25.
[60] Dongiovanni P, Petta S, Maglio C, et al. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. Hepatology 2015;61:506–14.
[61] Falleti E, Cussigh A, Cmet S, et al. PNPLA3 rs738409 and TM6SF2 rs58542926 variants increase the risk of hepatocellular carcinoma in alcoholic cirrhosis. Dig Liver Dis 2016;48:69–75.
[62] Prasad RB, Groop L. Genetics of type 2 diabetes-pitfalls and possibilities. Genes (Basel) 2015;6:87–123.
[63] Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;358:1240–9.
[64] Adams LA, Sanderson S, Lindor KD, et al. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005;42:132–8.
[65] Caldwell SH, Oelsner DH, Iezzoni JC, et al. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664–9.
[66] El-Serag HB, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. Clin Gastroenterol Hepatol 2006;4:369–80.
[67] Ratziu V, Bellentani S, Cortez-Pinto H, et al. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol 2010;53:372–84.
[68] Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846–54.