Received: 27 March 2017 | Accepted: 12 May 2017

DOI: 10.1111/liv.13478

METABOLIC LIVER DISEASE

WILEY Liver INTERNATIONAL

Incidence and risk factors for non-alcoholic fatty liver disease: A 7-year follow-up study among urban, adult Sri Lankans

Madunil A. Niriella[1] | Arunasalam Pathmeswaran[1] | Shamila T. De Silva[1] | Anuradhani Kasturiratna[1] | Ruwan Perera[2] | Chamila E. Subasinghe[2] | Kuleesha Kodisinghe[2] | Chathura Piyaratna[2] | Vithiya Rishikesawan[2] | Anuradha S. Dassanayaka[1] | Arjuna P. De Silva[1] | Rajitha Wickramasinghe[1] | Fumihiko Takeuchi[3] | Norihiro Kato[3] | Hithanadura J. de Silva[1]

[1]Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

[2]University Medical Unit, Colombo North Teaching Hospital, Ragama, Sri Lanka

[3]National Center for Global Health and Medicine, Toyama, Shinjuku-ku, Tokyo, Japan

Correspondence
Madunil Anuk Niriella, Department of Medicine, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka.
Email: maduniln@yahoo.co.uk

Funding information
Grants from the Ministry of Higher Education of Sri Lanka and National Center for Global Health and Medicine, Tokyo, Japan.

Handling Editor: Luca Valenti

Abstract

Background: This study investigated incidence and risk factors for NAFLD among an adult cohort with 7-year follow-up.

Methods: The study population (age-stratified random sampling, Ragama MOH area) was screened initially in 2007 (aged 35-64 years) and re-evaluated in 2014 (aged 42-71 years). On both occasions assessed by structured interview, anthropometric measurements, liver ultrasound, biochemical and serological tests. NAFLD was diagnosed on ultrasound criteria, safe alcohol consumption and absence of hepatitis B/C markers. Non-NAFLD controls did not have any ultrasound criteria for NAFLD. An updated case–control genetic association study for 10 selected genetic variants and NAFLD was also performed.

Results: Out of 2985 of the original cohort, 2148 (72.0%) attended follow-up (1238 [57.6%] women; mean-age 59.2 [SD-7.6] years) in 2014, when 1320 (61.5%) were deemed NAFLD subjects. Out of 778 who initially did not have NAFLD and were not heavy drinkers throughout follow-up, 338 (43.4%) (221 [65.4%] women, mean-age 57.8 [SD-8.0] years) had developed NAFLD after 7-years (annual incidence-6.2%). Central obesity (OR=3.82 [95%-CI 2.09-6.99]), waist increase >5% (OR=2.46 [95%-CI 1.20-5.05]) overweight (OR=3.26 [95%-CI 1.90-5.60]), weight gain 5%-10% (OR=5.70 [95%-CI 2.61-12.47]), weight gain >10% (OR=16.94 [95%-CI 6.88-41.73]), raised plasma triglycerides (OR=1.96 [95%-CI 1.16-3.29]) and diabetes (OR=2.14 [95%-CI 1.13-4.06]), independently predicted the development of incident NAFLD in multivariate analysis. The updated genetic association study (1362-cases, 392-controls) showed replicated association (P=.045, 1-tailed) with NAFLD at a candidate locus: *PNPLA3* (rs738409).

Abbreviations: ALT, alanine aminotransferase activity; Anti-HCV, anti-hepatitis C virus antibodies; BMI, Body mass index; BP, Blood pressure; CLD, chronic liver disease; GWAS, genome-wide association study; HbA1c, glycosylated hemoglobinA1c; HBsAg, Hepatitis B surface antigen; HDL, high density lipoprotein; IR, Insulin resistance; MetS, metabolic syndrome; MOH, Medical Officer of Health; NAFLD, Non-alcoholic fatty liver disease; NAFL, Non-alcoholic fatty liver; NASH, Non alcoholic steatohepatitis; PA, Physical activity; *PNPLA3*, patatin-like phospholipase domain containing 3; RHS, Ragama Health Study; SNP, single nucleotide polymorphisms; ST, Sedentary time; T2DM, Type 2 diabetes mellitus; TBF, Total body fat; TG, triglyceride; VFP, Visceral fat percentage; WC, Waist circumference; Wt, weight.

Conclusions: In this community cohort study, the annual incidence of NAFLD was 6.2%. Incident NAFLD was associated with general and central obesity, raised triglycerides and diabetes, and showed a tendency of association with *PNPLA3* gene polymorphisms.

KEYWORDS
fatty liver, incidence, non-alcoholic fatty liver disease, risk factors, Sri Lanka

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as hepatic steatosis detected either on imaging or histology, in the absence of secondary causes.[1] It is an umbrella term covering a spectrum of diseases ranging from simple non-alcoholic fatty liver (NAFL; ie, fat deposition with no or mild inflammation, but no fibrosis), to non-alcoholic steatohepatitis (NASH; ie, fat deposition with inflammation and hepatocellular injury, with or without fibrosis) to cirrhosis.[1] Most NAFLD subjects are likely to have one or more features of metabolic syndrome (MetS) associated with insulin resistance (IR), such as obesity, type2 diabetes mellitus (T2DM), hypertension and dyslipidemia.[2] Genome-wide association studies (GWAS) have successfully identified loci associated with susceptibility to NAFLD, with the most consistent association being at the patatin-like phospholipase domain containing 3 (PNPLA3) gene rs738409 polymorphism.[3]

NAFLD is already the commonest liver disorder in developed countries with 20%-30% prevalence.[4] The reported prevalence of NAFLD in Asian populations ranges from 5% to 40% depending on the population studied and the assessment methods used.[5] It is believed that with increasing obesity, NAFLD may well become the commonest form of chronic liver disease (CLD) which can progress to cirrhosis.[6]

Sri Lanka has a unique pattern of CLD. Hepatitis B and hepatitis C prevalence is very low even among presumed "high risk" populations.[7] Chronic viral hepatitis related CLD is also rare (<2% for HBV and <1% for HCV related cirrhosis).[8] Instead, alcoholic and cryptogenic or NASH-related forms of cirrhosis predominate.[8] We previously reported that 63% of cases referred for liver transplantation were related to NASH-cirrhosis.[9] We also reported that 59% of the hepatocellular carcinomas were secondary to cryptogenic or NASH-cirrhosis.[10] Large percentages (45%) of our liver donors are rejected as a result of the presence of NAFLD.[11] Therefore, it is evident that NALFD related CLD burden predominates in Sri Lanka.

The Ragama Health Study (RHS) is a large community-based cohort study on non-communicable diseases.[12] It is a collaborative study between the National Centre for Global Health and Medicine, Tokyo, Japan and the Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka. As part of this study, using stringent ultrasound criteria, we previously reported a community prevalence of 32.6% for NAFLD in an urban, adult Sri Lankan population.[12] We also found a significant association between *PNPLA3* gene rs738409 polymorphism and susceptibility to NAFLD in this population, after testing 10 selected single nucleotide polymorphisms (SNPs) in a case–control study.[13]

Key points

- There are no Asian community based prospective studies reporting the incidence of NAFLD.
- This study investigated incidence and risk factors for NAFLD among an urban, adult cohort with 7-year follow-up.
- In this community cohort study, the annual incidence of NAFLD was 6.2%.
- Incident NAFLD was associated with general and central obesity, raised triglycerides and diabetes, and showed a tendency of association with *PNPLA3* gene polymorphisms.

The community incidence of NAFLD has been poorly studied. Although there have been reports on incidence of NAFLD from Asia, they are studies either in hospital or employment setting and not prospective community cohort studies.[14,15] Therefore, the aim of this study was to assess the incidence and risk factors for NAFLD in an urban, adult, Sri Lankan community-based cohort (the RHS cohort) after 7 years of follow-up.

2 | METHODS

This study was part of an on-going large community-based cohort follow-up: the RHS.[12] Ethical approval for the study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Kelaniya. Informed written consent was obtained from all participants. This study was conducted in the Ragama Medical Officer of Health (MOH) administrative area situated 18 km north of the capital, Colombo. It has urban characteristics and a multi-ethnic population. The study population consisted of 35-64-year-old adult residents, originally selected by age-stratified random sampling from electoral lists in 2007. The target population screened initially in 2007 was invited back after 7 years of follow-up for re-evaluation in 2014.

On both occasions all participants were assessed using a structured interview, clinical and anthropometric measurements, liver ultrasound, and biochemical and serological tests. Details of screening of the inception cohort are described elsewhere.[12] For re-evaluation, the follow-up cohort was interviewed by trained personnel to obtain

information on socio-demographic variables, lifestyle habits with special emphasis on identification of alcohol consumption above the safe limit, dietary habits and physical activity. Details regarding the type and amount of alcohol consumed and duration of drinking were obtained by direct questioning of participants by trained research assistants using a structured questionnaire. All past medical records of the subjects were scrutinized and recorded. Blood pressure (BP) and anthropometric measurements including height, weight (Wt) and waist circumference (WC) were made. Normal cut-off values were based on the revised Adult Treatment Panel III criteria for metabolic syndrome for Asians.[2] Total body fat (TBF) and visceral fat percentage (VFP) were measured only at follow-up in 2014, using a body composition monitor using proven bioelectrical impedance method (Omron HBF-362 body composition monitor, Omron Healthcare, Lake Forrest, IL, USA). Change in WC was classified as reduction >5%, between reduction ≤5% and increase <5% (no change) and increase ≥5%. Change in Wt was classified as loss >5%, loss ≤5% and gain <5% (no change), gain ≥5% and gain ≥10%. Abnormal TBF definition for females was >32% and for males >25% while abnormal VFP for both females and males was defined as >10%.[16] Physical activity (PA) was categorized as no-PA, some-PA (below recommendation [duration of exercise<150 min/wk]) and recommended-PA [at or above recommendation [duration of exercise≥150 min/wk]) while sedentary time (ST) was categorized as with ST<1, 1-4, 4-6, >6 hours. Inadequate physical activity was no-PA or some PA coupled with ST>4 hours or more. A 10-mL sample of venous blood was obtained from each subject. This was used to determine glycosylated hemoglobinA1c (HbA1c), fasting serum triglycerides (TG) and high density lipo-proteins (HDL), serum alanine aminotransferase activity (ALT) and hepatitis B and C serology (hepatitis B surface antigen [HBsAg] and anti-hepatitis C virus antibodies [anti-HCV] using CTK Biotech ELISA kits). All subjects underwent ultrasonography of the liver with a 5-MHz 50 mm convex probe (MindrayDP-10 Ultrasound Diagnostic Systems, Mindray Medical International Limited, Shenzhen, China). Ultrasonographic examination was carried out by five doctors with special training in liver ultrasonography.

NAFLD was diagnosed on established ultrasound criteria for fatty liver (two out of the following three criteria: increased echogenecity of the liver compared to kidney and spleen, obliteration of the vascular architecture of the liver and deep attenuation of the ultrasonic signal), safe alcohol consumption (Asian standards: <14 units/wk for men, <7 units/wk for females) and absence of HbsAg and anti-HCV.[17] Non-NAFLD controls were defined as those who did not have any of the three ultrasound criteria for NAFLD.

Data were entered in Epi Info 7 (Centres for Disease Control and Prevention, Atlanta, GA, USA) and logical and random checks were done. Statistical analysis was done using Stata 14.1 (StataCorp, College Station, TX, USA). Continuous and categorical data were described using mean and standard deviations and percentages respectively. Bivariate analysis was done using the Chi squared test. Multivariate analysis was done using binary logistic regression. $P<.05$ was considered as significant.

To investigate associations of selected genetic variants with incident NAFLD, we also performed an updated case–control study using NAFLD cases and non-NAFLD control subjects after the 7-year follow-up. Of the 10 selected SNPs, 5 SNPs had been previously identified to be associated with NAFLD and related phenotypes at a genome-wide significance level ($P\leq5\times10^{-8}$) in European GWAS (*PNPLA3* [rs738409], *LYPLAL1* [rs12137855], *GCKR* [rs780094], *PPP1R3B* [rs4240624] and *NCAN* [rs2228603]), and the remaining 5 SNPs were derived from 3 previously documented candidate genes for NAFLD (*APOC3* [rs2854117, rs2854116], *ADIPOR2* [rs767870] and *STAT3* [rs6503695, rs9891119]).[13] The genotype distribution of all tested SNPs was in Hardy-Weinberg equilibrium ($P>10^{-3}$).

3 | RESULTS

There were 3012 participants in the initial study of whom 2985 (99.1%) had complete data for analysis (Ethnic breakdown: Sinhalese 96.2%, Tamil 1.3%, Muslim 1.3%, Burgher 1.3%). This included 1636 women (54.8%). The mean age (SD) was 54.2 (7.8) years. Of them, 2148 (72.0%) including 1238 (57.6%) women participated in the follow-up assessment. Except for fewer males attending follow-up, rest of the characteristics were similar among initial and follow-up cohorts (Table 1). 1320 (61.5%) including 840/1238 (67.9%) women

TABLE 1 Profile of the participants in 2007 and 2014

	Initial cohort 2007 (n=2985)	Attended follow-up 2014 (n=2148)	Did not attend follow-up 2014 (n=837)
Males (%)	1349 (45.2)	910 (42.4)*	439 (52.4)*
Mean age (SD)	52.4 (7.8)	52.4 (7.7)	52.5 (8.1)
Mean BMI (SD)	24.1 (4.2)	24.3 (4.1)	23.7 (4.4)
Mean waist-hip ratio (SD)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
DM (raised FBS) (%)	709 (23.8)	477 (22.2)	232 (27.7)
HBP (SBP>140, DBP>90) (%)	1820 (60.4)	1298 (60.4)	522 (62.3)
Mean TG (SD)	131.6 (68.2)	130.8 (68.0)	133.5 (68.6)
Mean HDL (SD)	49.6 (4.5)	49.6 (4.5)	49.6 (4.4)
Mean LDL (SD)	136.2 (37.8)	136.5 (37.7)	135.3 (38.0)

*$Z=4.97$; $P<.001$ (Z test comparing two proportions).

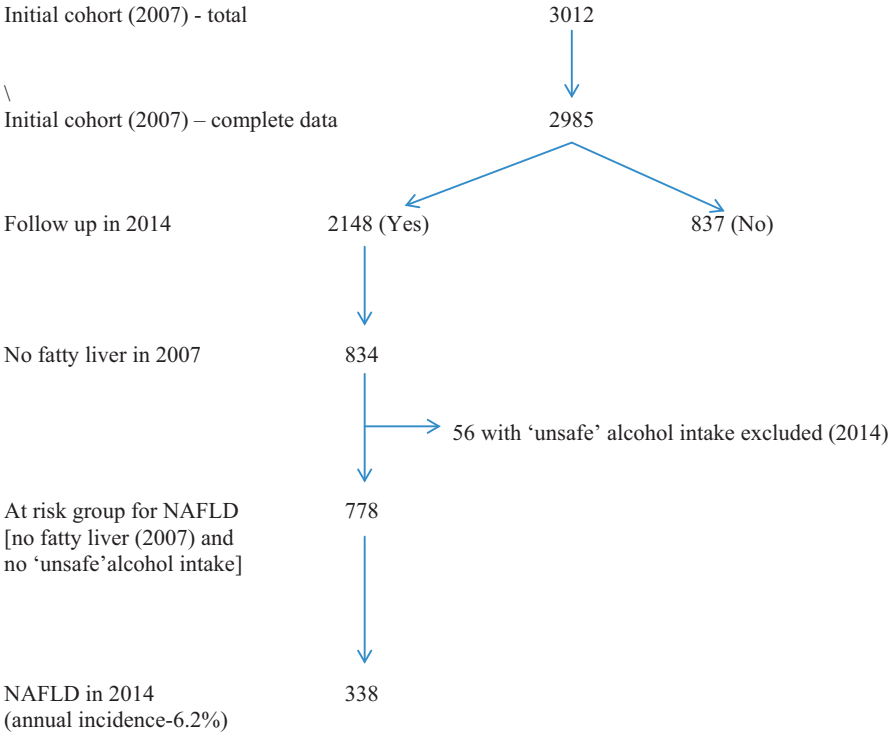

FIGURE 1 Study population

and 480/910 (52.7%) males had NAFLD in 2014. Of the 1238 women who attended follow-up, data on menopause were available for 897. 772/897 (86.1%) had reached menopause at follow-up. NAFLD was present in 69.0% of those who had reached menopause and 68.8% of those who had not.

Out of 778 who initially did not have NAFLD and were not heavy drinkers throughout follow-up, 338 (43.4%) had developed NAFLD after 7 years (annual incidence-6.2%; Figure 1). Of the population at risk, 49.6% (221/446) of the females and 35.2% (117/332) of the males had developed NAFLD over the 7 years. Among 373 persons who had a BMI<23 kg/m^2 at baseline 85 (22.8%) developed incident NAFLD after 7 years. 37 persons who were overweight or obese (BMI>23 kg/m^2) at baseline achieved normal BMI after 7 years. Of these 37, 10 (27.0%) developed incident NALFD after 7 years.

On bivariate analysis, central obesity (waist circumference >90 cm for males and >80 cm for females; *P*<.001), increase in waist circumference >5 cm (*P*<.001), over-weight (Body Mass Index [BMI]>23 kg/m^2; *P*<.001**)**, weight gain 5%-10% (*P*<.001), weight gain>10% (*P*<.001), hypertension (*P*<.001), raised plasma triglycerides (*P*<.001) and inadequate physical activity (*P*=.013) were risk factors for the development of incident NAFLD (Table 2). Abnormal high TBF percentage (*P*<.001) and high VFP (*P*<.001), assessed only at follow-up in 2014, were strong associated factors for incident NAFLD (Table 2). Consumption of tea, assessed on both occasions, was not a risk factor for incident NAFLD (*P*=.065 for >4 cups per day vs none). Consumption of coffee, only assessed at follow-up in 2014, did not show significant association with incident NAFLD (*P*=.523 for >4 cups per day vs none).

On multivariate logistical regression analysis, central obesity (OR=3.82 [95% CI 2.09-6.99]), waist increase >5% (OR=2.46 [95% CI 1.20-5.05]), overweight (OR=3.26 [95% CI 1.90-5.60]), weight gain 5%-10% (OR=5.70 [95% CI 2.61-12.47]), weight gain >10% (OR=16.94 [95% CI 6.88-41.73]), raised plasma triglycerides (OR=1.96 [95% CI 1.16-3.29]), and diabetes (OR=2.14 [95% CI 1.13-4.06]) remained independent risk factors for the development of incident NAFLD (Table 3).

In the updated association study between case (n=1360) involving incident NAFLD cases (n=664) and those who remained NAFLD from the inception cohort (n=696), and control subjects (n=391) who remained non-NAFLD after the 7-year follow-up, we found nominal association with NAFLD at one of the 10 candidate loci: rs738409 at *PNPLA3* (*P*=.045, one-tailed by logistic regression analysis), in accordance with the findings at baseline (Table 4).

4 | DISCUSSION

In this community cohort follow-up study, we report an annual incidence of NAFLD of 6.2%. We also found significant independent association of incident NAFLD with central and general obesity, raised TG and presence of diabetes. A tendency of association at *PNPLA3* gene polymorphisms with NAFLD was also observed after the 7-year follow-up, similarly to baseline.

In this urban, adult population in Sri Lanka, 67.9% females and 52.7% males had NAFLD in 2014. The high combined prevalence of 61.5% was coupled with an annual incidence of NAFLD of 6.2% in this ageing population. This is almost doubling of the initially reported prevalence of 32.6% among the inception cohort within a period of 7 years.[12] This highlights the growing burden of NAFLD in the community in developing economies such as Sri Lanka. With the already high prevalence, confirmed high incidence of NAFLD and predicted

TABLE 2 Factors associated with incident NAFLD on bivariate analysis among the subjects (N=778) who had no fatty liver at baseline and safe alcohol intake

Risk factors (at baseline—2007)	Total number of persons with no NAFLD at baseline (N=778)	Developed incident NAFLD (N=338)	Did not develop incident NAF LD (N=440)	*P* value*
Males	324 (41.7)	117 (34.6)	207 (47.2)	<.001
Mean age (SD)	51.6 (8.0)	50.8 (8.0)	52.2 (7.9)	.015
BMI>23 kg/m^2	310 (40.0)	199 (58.9)	111 (25.4)	<.001
Increase in weight (5%-10%)[a]	154 (19.9)	73 (21.6)	81 (18.4)	<.001
Increase in weight (>10%)[a]	124 (16.0)	75 (22.2)	49 (11.2)	<.001
Waist circumference (above sex specific cut-off)	264 (34.0)	173 (51.2)	91 (20.7)	<.001
Increase in waist (>5 cm)[a]	196 (25.4)	120 (35.8)	76 (17.4)	<.001
High total body fat[b]	561 (72.1)	316 (89.0)	245 (59.9)	<.001
High visceral fat %[b]	247 (31.7)	179 (50.4)	68 (16.6)	<.001
Diabetes or on treatment	121 (15.6)	58 (17.2)	63 (14.4)	.284
Hypertension or on treatment	375 (48.2)	181 (53.6)	194 (44.1)	<.001
Elevated TG or on treatment	197 (25.3)	107 (31.7)	90 (20.5)	<.001
Low HDL or on treatment	231 (29.7)	113 (33.4)	118 (26.9)	.096
Elevated ALT (above cut-off)	46 (5.9)	25 (7.4)	21 (4.8)	.145
Inadequate physical activity	112 (14.4)	62 (18.3)	50 (11.4)	.006
Tea consumption				
None	36	16	20	.065
1-4 cup per day	650	291	359	
>4 cups per day	88	29	59	
Coffee consumption[b]				
None	350	170	180	.396
1-4 cup per day	80	32	48	
>4 cups per day	3	1	2	
Educational level (not completed secondary education)	345 (45.2)	135 (40.7)	210 (48.6)	.091
Household income (below median)	374 (49.4)	156 (47.4)	218 (50.9)	.630

Numbers within brackets are percentages unless indicated otherwise.

**P* values based on Chi squared test comparing incident NAFLD vs no incident NAFLD.

[a]After 7 y of follow-up.

[b]Factors assessed only in 2014; Elevated TG >150 mg/L (1.7 mmol/L) or specific treatment for hypertriglyceridemia; Low HDL-cholesterol <40 mg/L (1.03 mmol/L) in males and <50 mg/L (1.29 mmol/L) in females or specific treatment for low HDL-cholesterol; Hypertension: systolic blood pressure >130 mm Hg or diastolic blood pressure >85 mm Hg or treatment for previously diagnosed hypertension; Dysglycaemia: fasting plasma glucose >100 mg/L (5.6 mmol/L) and/or 2 h post-oral glucose tolerance test glucose >7.8 mmol/L or previously diagnosed type-2 diabetes.

TABLE 3 Summary of logistic regression analysis of factors associated with NAFLD

	OR	SE	*P* value	95% Confidence Interval
BMI>23 kg/m^2	3.26	0.90	<.001	1.90-5.60
Increase in weight (5%-10%)[a]	5.70	2.28	<.001	2.61-12.47
Increase in weight (>10%)[a]	16.94	7.79	<.001	6.88-41.73
Waist circumference (above cut-off)	3.82	1.18	<.001	2.09-6.99
Increase in waist (>5%)[b]	2.46	0.90	.014	1.20-5.05
Diabetes	2.14	0.70	.019	1.13-4.06
Elevated TG or on Rx	1.96	0.52	.012	1.16-3.29

[a]Comparison group weight reduction >5%.

[b]Comparison group those with decrease in waist >5 cm.

TABLE 4 Genetic association of selected SNPs with NAFLD at follow-up (2014) and baseline (2007)

			A case–control panel at follow-up				A case–control panel at baseline			
			Case (n=1360)	Control (n=391)			Case (n=707)	Control (n=830)		
SNP	Gene symbol	Effect/other allele	EE/EO/OO	EE/EO/OO	OR [95% CI]	*P*-value two tailed	EE/EO/OO	EE/EO/OO	OR [95% CI]	*P*-value two tailed
rs12137855	LYPLAL1	C/T	842/464/54	232/134/25	1.18 [0.93-1.49]	.1797	431/244/32	515/278/37	1.02 [0.82-1.27]	.8308
rs780094	GCKR	T/C	55/417/886	14/128/249	0.95 [0.74-1.21]	.6653	26/223/456	31/246/552	1.11 [0.89-1.39]	.3700
rs4240624[a]	PPP1R3B	A/G	1225/131/4	349/39/3	1.17 [0.76-1.78]	.4706	643/61/3	736/88/6	1.32 [0.90-1.97]	.1599
rs2854117	APOC3	T/C	373/640/347	101/208/82	1.00 [0.82-1.21]	.9692	184/338/185	245/408/177	0.92 [0.77-1.10]	.3425
rs2854116	APOC3	C/T	378/640/342	104/210/77	0.97 [0.80-1.19]	.7907	190/333/184	246/415/169	0.91 [0.77-1.09]	.3177
rs767870	ADIPOR2	A/G	916/393/51	262/112/17	1.06 [0.82-1.36]	.6668	492/190/25	555/240/35	1.23 [0.98-1.54]	.0727
rs6503695	STAT3	T/C	467/665/228	133/179/79	1.07 [0.87-1.30]	.5319	268/329/110	284/387/159	1.13 [0.94-1.35]	.1813
rs9891119	STAT3	A/C	306/678/376	91/180/120	1.04 [0.85-1.26]	.7315	178/350/179	171/407/252	1.20 [1.00-1.43]	.0451
rs2228603	NCAN	T/C	2/152/1206	2/50/339	0.76 [0.51-1.16]	.1988	0/75/632	3/98/729	0.74 [0.51-1.08]	.1233
rs738409	PNPLA3	G/C	94/471/794	22/131/238	1.22 [0.97-1.55]	.0902	56/246/405	38/271/520	1.48 [1.20-1.84]	.0003

The effect alleles are defined as those that were reported to be positively associated with NAFLD risk, as we showed previously (Kasturiratne et al.[13]).

An updated case–control panel was composed of a case group involving incident NAFLD cases at follow-up (in 2014) and those who remained NAFLD from the inception cohort (in 2007) and a control group involving subjects who remained non-NAFLD after the 7-year follow-up as well as at baseline. For evaluating longitudinal changes, participants in the case–control study were restricted to those who attended at both baseline and follow-up. Samples without genotype call data were excluded from the analysis.

Logistic regressions include BMI, age, age-squared as covariates.

Genotype classes are as follows: EE, effect/effect; EO, effect/other; OO, other/other.

[a]At *PPP1R3B*, an effect allele was defined according to the previous report (Romeo et al.[3]); the A allele of rs4240624 showed increased risk of hepatic steatosis, although it was not positively associated with NAFLD risk (OR=0.93).

increase in underlying risk factors, especially obesity and diabetes, an increased burden of chronic liver disease due NAFLD can be expected to rise exponentially in Sri Lanka in the future. Therefore, community-based strategies directed at reducing the incidence of risk factors such as obesity appears to be a potential method to resolve this public health problem. The very high prevalence of NAFLD among the ageing females may be related to menopausal transition.[18]

Although, there have been previous reports of incidence of NALFD from Asia, these have not been from community-based cohort studies. A study, based on routine health care screening of Japanese government employees, revealed an overall incidence of non-alcoholic hypertransaminasemia of 31 cases per 1000 person-years (3.1%).[14] Another Japanese study reported follow-up data on 3147 individuals without NAFLD at baseline; of these, 308 (10%) developed NAFLD over 414 days.[15] Therefore, the present study is the first report of incidence of NAFLD from a large community-based follow-up in an Asian population. The reported incidence of 6.2% here is comparable to the previous reports (3%-10% incidence).

In the previously reported study of the inception cohort, NAFLD was associated with obesity, diastolic hypertension, insulin resistance, hyperlipidaemia, hyperglycaemia and acanthosis nigricans, which are constituents of MetS.[12] In the present study, incident NAFLD remained to be associated with some features of MetS, namely the presence of abnormal waist circumference, BMI>23 kg/m^2, waist increase >5 cm, weight gain of both >5% and >10%, raised plasma TG and diabetes detected at the initial survey. Among the identified risk factors, >5% or >10% increase in weight from baseline, general obesity (BMI>23 kg/m^2) and central obesity (WC>gender and ethnic specific cut offs) were the strongest predictors of incident NAFLD with OR>3.00. Weight gain >10% form baseline was the strongest predictor of development of incident NAFLD with OR=16.94. Although not assessed as risk factors at baseline, current abnormal TBF and VFP were strongly associated with incident NAFLD.

Although coffee consumption has been protective in earlier studies,[19,20] there was no demonstrable protective effect either coffee or tea consumption with incident NAFLD in this study. Lack of a demonstrable protective effect of coffee may have been because of the small numbers of heavy coffee consumers in this cohort. Furthermore, additional use of milk and sugar among the majority of the participants consuming coffee and tea may have off-set any potential benefit.

We have previously reported a significant association of the *PNPLA3* gene rs738409 polymorphism, and susceptibility to NAFLD in the inception cohort, after testing 10 selected SNPs in a case–control study.[13] This association remains nominally significant in the current analysis (P=.045, one-tailed) but the relationship between NAFLD and rs738409 appears weaker (OR=1.22 at follow-up and OR=1.48 at baseline). The weaker association seen in this analysis may be the result of *PNPLA3* gene rs738409 polymorphism being more strongly associated with earlier onset NAFLD. The association with genetic factors seen in the inception cohort was probably diluted/contaminated by the impact of non-genetic environmental factors (in particular, weight gain/loss) on deterioration or regression of NAFLD over the 7 years of follow-up. This is the first study to demonstrate the genetic associations of NAFLD in a prospectively followed up Asian cohort. Furthermore, these findings reconfirm the importance of *PNPLA3* gene rs738409 polymorphism in the pathogenesis of NAFLD. Other untested variants in different loci may have been involved in the development of NAFLD in individual cases.

There have been no previous large community cohort follow-up studies to report incident NAFLD. This is the first such prospective follow-up of a large community cohort to report NAFLD incidence and its risk factors. Other strengths of this study include the robust design and over 70% of the relatively large baseline population presenting for re-evaluation. One possible limitation of this study is that inter-observer reliability between the sonographers was not formally assessed before this study commenced. Another is that information on alcohol consumption was obtained only by direct questioning of the participants. This may have led to under-reporting with consequent overestimation of the prevalence and incidence of NAFLD in the inception and follow-up cohort respectively. The initial cohort comprised predominantly Sinhalese (96.2%) and only minority of other ethnicities (Tamil 1.3%, Muslim 1.3%, Burgher 1.3%).[12] Therefore, we were not able analyse the inter-ethnic variability in occurrence of incident NAFLD. We also did not look at liver-related outcomes in the follow-up cohort, as we felt that 7 years was too short a period of time.

In conclusion, the results of our study demonstrate that the incidence and prevalence of NAFLD among adults in this urban Sri Lankan community to be high. Incident NAFLD was associated with features of the MetS, especially obesity and weight gain. Despite the high incidence of NAFLD during 7-year follow-up, there remains to be a tendency of association at *PNPLA3*, reconfirming the importance of the *PNPLA3* polymorphism in the pathogenesis of NAFLD.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Higher Education of Sri Lanka and National Center for Global Health and Medicine, Tokyo, Japan. We thank those who have continuously supported the Ragama Health Study. We also thank Dr Koichi Akiyama and Ms Marika Tsuzuki, and many physicians for their assistance in collecting the DNA samples and accompanying clinical information, extracting DNA and preparing the analytical data.

CONFLICT OF INTEREST

The authors do not have any disclosures to report.

REFERENCES

1. 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. *Hepatology*. 2012;55:2005-2023.
2. Fan JG, Saibara T, Chitturi S, et al. What are the risk factors and settings for non-alcoholic fatty liver disease in Asia-Pacific? *J Gastroenterol Hepatol*. 2007;22:794-800.

3. Romeo S, Kozlitina J, Xing C, et al. Genetic variation inPNPLA3 confers susceptibility to non-alcoholic fatty liver disease. *Nat Genet*. 2008;40:1461-1465.
4. Sayiner M, Koenig A, Henry L, Younossi ZM. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. *Clin Liver Dis*. 2016;20:205-214.
5. Amarapurkar DN, Hashimoto E, Lesmana LA, et al. Asia-Pacific working party for NAFLD: how common is non-alcoholic fatty liver disease in the Asia-Pacific region and what are the local differences? *J Gastroenterol Hepatol*. 2007;22:788-793.
6. Mahady SE, George J. The future liver of the Asia Pacific: fatter and firmer from more fructose and fortune? *J Clin Exp Hepatol*. 2013;3:106-113.
7. Niriella MA, Hapangama A, Luke HP, Pathmeswaran A, Kuruppuarachchi KA, de Silva HJ. Prevalence of hepatitis B and hepatitis C infections and their relationship to injectable drug use in a cohort of Sri Lankan prison inmates. *Ceylon Med J*. 2015;60:18-20.
8. Senanayake SM, Niriella MA, Weerasinghe SK, et al. Survival of patients with alcoholic and cryptogenic cirrhosis without liver transplantation: a single center retrospective study. *BMC Res Notes*. 2012;5:663.
9. Siriwardana RC, Niriella MA, Liyanage CA, et al. Cryptogenic cirrhosis is the leading cause for listing for liver transplantation in Sri Lanka. *Indian J Gastroenterol*. 2013;32:397-399.
10. Siriwardana RC, Niriella MA, Dassanayake AS, et al. Clinical characteristics and outcome of hepatocellular carcinoma in alcohol related and cryptogenic cirrhosis: a prospective study. *Hepatobiliary Pancreat Dis Int*. 2015;14:401-405.
11. Silva H, Siriwardana RC, Niriella MA, et al. Nonalcoholic fatty liver disease among potential live liver donors–a preliminary experience from Sri Lanka. *Indian J Gastroenterol*. 2014;33:573-574.
12. Dassanayake AS, Kasthuriratne A, Rindrajith S, et al. Prevalence and risk factors for non-alcoholic fatty liver disease among adults in an urban Sri Lankan population. *J Gastroenterol Hepatol*. 2009;24:1284-1288.
13. Kasturiratne A, Akiyama K, Niriella MA, et al. Association of genetic variants with non-alcoholic fatty liver disease in an urban Sri Lankan community. *Liver Int*. 2015;35:676-679.
14. Suzuki A, Angulo P, Lymp J, et al. Chronological development of elevated aminotransferases in a non-alcoholic population. *Hepatology*. 2005;41:64-71.
15. Hamaguchi M, Kojima T, Takeda N, et al. The metabolic syndrome as a predictor of non-alcoholic fatty liver disease. *Ann Intern Med*. 2005;143:722-728.
16. Dehghan M, Merchant AT. Is biometrical impedance accurate for use in large epidemiological studies? *Nutr J*. 2007;7:26.
17. Chitturi S, Farrell GC, Hashimoto E, et al. Non-alcoholic fatty liver disease in the Asia-Pacific region: definitions and overview of proposed guidelines. *J Gastroenterol Hepatol*. 2007;22:778-787.
18. Florentino GS, Cotrim HP, Vilar CP, Florentino AV, Guimarães GM, Barreto VS. Nonalcoholic fatty liver disease in menopausal women. *Arq Gastroenterol*. 2013;50:180-185.
19. Saab S, Mallam D, Cox G, Tong M. Impact of coffee on liver diseases: a systematic review. *Liver Int*. 2014;34:495-504.
20. Bambha K, Wilson L, Unalp A, et al. Coffee consumption in NAFLD patients with lower insulin resistance is associated with lower risk of severe fibrosis. *Liver Int*. 2014;34:1250-1258.

How to cite this article: Niriella MA, Pathmeswaran A, De Silva ST, et al. Incidence and risk factors for non-alcoholic fatty liver disease: A 7-year follow-up study among urban, adult Sri Lankans. *Liver Int*. 2017;37:1715–1722. https://doi.org/10.1111/liv.13478