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ORIGINAL ARTICLE

Lean non-alcoholic fatty liver disease (lean NAFLD): characteristics, metabolic outcomes and risk factors from a 7-year prospective, community cohort study from Sri Lanka

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Abstract

Introduction While patients with non-alcoholic fatty liver disease (NAFLD) are mostly overweight or obese, some are lean.
Methods In a community-based follow-up study (baseline and follow-up surveys performed in 2007 and 2014), we investigated and compared the clinical characteristics, body composition, metabolic associations and outcomes, and other risk factors among individuals with lean (BMI < 23 kg/m^2) NAFLD, non-lean (BMI ≥ 23 kg/m^2) NAFLD and those without NAFLD. To investigate associations of selected genetic variants, we performed a case–control study between lean NAFLD cases and lean non-NAFLD controls.
Results Of the 2985 participants in 2007, 120 (4.0%) had lean NAFLD and 816 (27.3%) had non-lean NAFLD. 1206 (40.4%) had no evidence of NAFLD (non-NAFLD). Compared to non-lean NAFLD, lean NAFLD was commoner among males ($p < 0.001$), and had a lower prevalence of hypertension ($p < 0.001$) and central obesity (WC < 90 cm for males, < 80 cm for females) ($p < 0.001$) without prominent differences in the prevalence of other metabolic comorbidities at baseline survey. Of 2142 individuals deemed as either NAFLD or non-NAFLD in 2007, 704 NAFLD individuals [84 lean NAFLD, 620 non-lean NAFLD] and 834 individuals with non-NAFLD in 2007 presented for follow-up in 2014. There was no difference in the occurrence of incident metabolic comorbidities between lean NAFLD and non-lean NAFLD. Of 294 individuals who were non-NAFLD in 2007 and lean in both 2007 and 2014, 84 (28.6%) had developed lean NAFLD, giving an annual incidence of 4.1%. Logistic regression identified the presence of diabetes at baseline, increase in weight from baseline to follow-up and a higher educational level as independent risk factors for the development of incident lean NAFLD. NAFLD association of *PNPLA3* rs738409 was more pronounced among lean individuals (one-tailed $p < 0.05$) compared to the whole cohort sample.
Conclusion Although lean NAFLD constitutes a small proportion of NAFLD, the risk of developing incident metabolic comorbidities is similar to that of non-lean NAFLD. A *PNPLA3* variant showed association with lean NAFLD in the studied population. Therefore, lean NAFLD also warrants careful evaluation and follow-up.

Keywords Fatty liver · Non-alcoholic fatty liver disease · NAFLD · Lean · Lean NAFLD · Risk factors

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]. NAFLD is probably the commonest chronic liver disease (CLD) worldwide [2]. The reported prevalence from Asia–Pacific countries ranges from 5 to 40% depending on the population studied and the method used to detect fatty liver [3].

Most patients with NAFLD are overweight (BMI ≥ 23 kg/m^2) or obese (BMI ≥ 25 kg/m^2) (non-lean NAFLD) [4].

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However, some patients with NAFLD are lean (BMI < 23 kg/m^2), and there has been an increasing clinical interest in the group [5–8]. A considerable proportion of Asians with NAFLD is described as having lean NAFLD. It was reported that present in ~ 20% of the Asian population, lean NAFLD is closely linked with insulin resistance and diabetes [8]. A recent multinational study showed an increase in mortality in lean individuals with NASH [9]. Another described its clinical characteristics and association with patatin-like phospholipase domain containing 3 (PNPLA3) risk allele carriage among Caucasians [10]. Despite these, lean NAFLD has failed to secure an unambiguous status as a unique disease entity [11].

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 Center for Global Health and Medicine (NCGM), 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% and an annual incidence of 6.6% for NAFLD in an urban, adult Sri Lankan population [12, 13]. We also reported a significant association between PNPLA3 gene rs738409 polymorphism and susceptibility to NAFLD in this population [14]. With such high rates in the community, cryptogenic (probably NAFLD related) CLD is very common in Sri Lanka [15].

Features of lean NAFLD among South Asians are poorly documented in the literature. Therefore, we investigated risk factors, including genetic polymorphisms, clinical characteristics, metabolic associations and outcomes, for lean NAFLD, and compared them with non-lean NAFLD and those without NAFLD in the RHS cohort after a 7-year follow-up period.

Methods

The RHS is a community-based, prospective, cohort follow-up study. The study population was chosen by age-stratified random sampling from electoral lists, from the Ragama Medical Officer of Health area in the Gampaha district on Sri Lanka. Initial screening was done in 2007 and follow-up was after 7 years, in 2014. The population consisted adults aged 35–64 years in 2007 who were 42–71 years in 2014. On both occasions, participants were screened by structured interview to collect socio-demographic variables and lifestyle habits (with special emphasis on patterns of alcohol consumption), collection of anthropometric indices, liver ultrasonography, and biochemical and serological tests.

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 lipoproteins (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]. Genetic analysis was done at initial screening only [15]. Body composition [Total body fat (TBF) and visceral fat percentage (VFP)] was assessed by a body composition monitor using a proven bioelectrical impedance method (Omron HBF-362 body composition monitor, Omron Healthcare, Lake Forrest, Illinois, United States) only at follow-up survey in 2014. Abnormal TBF definition for females was > 32% and for males > 25% while abnormal VFP for both females and males was defined as > 10% [16].

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 standard USS criteria (two of the three following criteria: increased hepatic echogenicity compared to the spleen or the kidney, blurring of liver vasculature and deep attenuation of the ultrasonographic signal) and exclusion of alcohol overuse and other secondary causes such as hepatitis B and C. Normal cut-off values were based on the revised Adult Treatment Panel III (ATP III) criteria for metabolic syndrome for Asians [17].

At baseline and follow-up survey, lean NAFLD was defined by BMI < 23 kg/m^2 and non-lean NAFLD was defined by BMI ≥ 23 kg/m^2. It is widely accepted that the prevalent international criteria to define overweight (BMI > 25 kg/m^2) and obesity (BMI > 30 kg/m^2) are not suitable for Asian Indian and South Asian populations. Therefore, we adopted the suggested BMI cut-off for Asian Indians and defined lean or non-over-weight state as BMI < 23 kg/m^2 [17, 18]. We felt the cut-off of BMI < 23 kg/m^2, would more accurately define the lean body habitus than choosing the prevalent international cut-off for non-overweight state of BMI < 25 kg/m^2.

Individuals with lean NAFLD, non-lean NAFLD and no NAFLD (none of the three USS criteria for NAFLD and no or 'safe' alcohol consumption) in 2007 were compared for baseline differences in gender, anthropometry [weight and waist circumference (WC)] and the presence of metabolic comorbidities such as diabetes, hypertension, hypertriglyceridemia and low-HDL levels. The three groups were also compared for the development of incident diabetes, hypertension, hypertriglyceridemia, low-HDL and body composition after 7 years of follow-up in 2014.

We also looked at risk factors for the development of new onset lean NAFLD in 2014. For this the sample was restricted to those who were lean at both baseline and follow-up, in 2007 and 2014, to ensure that the confounding effect on weight changes on the outcome over the 7 years

of follow-up was limited as much as possible. Therefore, new onset lean NAFLD was defined as those developed new NAFLD in 2014 but who remained lean (BMI < 23 kg/m^2) at baseline as well as in 2014 at follow-up, with 'safe' alcohol intake throughout. Non-NAFLD lean controls were those who had none of the USS criteria for NAFLD and had a BMI < 23 kg/m^2 in both 2007 and 2014 with no or 'safe' alcohol intake throughout.

To test associations of selected genetic variants with lean NAFLD, we performed a case–control study between lean NAFLD case and lean non-NAFLD control subjects after the 7-year follow-up. Of 10 selected SNPs, 5 SNPs had been previously identified to be associated with NAFLD and related-phenotypes at a genome-wide significance level ($p \leq 5\times10^{-8}$) in European genome-wide association studies [*PNPLA3* (rs738409), *LYPLAL1* (rs12137855), *GCKR* (rs780094), *PPP1R3B* (rs4240624) and *NCAN* (rs2228603)], and the remaining 5 SNPs were derived from 3 candidate genes previously documented for NAFLD [*APOC3* (rs2854117, rs2854116), *ADIPOR2* (rs767870) and *STAT3* (rs6503695, rs9891119)] [13]. The genotype distribution of all tested SNPs was in Hardy–Weinberg equilibrium (at $p > 10^{-3}$). Genetic association was tested between lean NAFLD cases and lean non-NAFLD controls among lean individuals (BMI < 23 kg/m^2) at each timing of survey; 84 cases vs. 461 controls at baseline ($N = 545$), where the individuals were restricted to those who also attended follow-up, and 282 cases vs. 283 controls at follow-up ($N = 565$), where part of the individuals were not in the case–control sample at baseline. Genotyping and statistical analysis were performed as previously described [13].

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, Texas, 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. $p < 0.05$ was considered as significant.

Results

There were 3012 participants in the initial study of whom 2985 (99.1%) had complete data for analysis (1636 female (54.8%), mean age (SD) 54.2 (7.8) years). Of the inception cohort, 2148 (72%) [1237 (57.6%) female, mean age (SD) 54.2 (7.7 years)] participated in the follow-up assessment in 2014. Except for fewer males attending follow-up, the other characteristics (mean BMI, percentage with diabetes, percentage with hypertension, mean TG and mean HDL) were similar among the inception and follow-up cohorts.

Of the 2985 participants in 2007, 120 (4.0%) had lean NAFLD and 816 (27.3%) had non-lean NAFLD. 1206 (40.4%) had no evidence of NAFLD (non-NAFLD) (Fig. 1). Table 1 compares the baseline characteristics of the lean NAFLD, non-lean NAFLD and no NAFLD groups. Compared to non-lean NAFLD, lean NAFLD was significantly more common among males, and had a lower prevalence of hypertension and central obesity at baseline. There was no significant difference in prevalence of other metabolic comorbidities between lean and non-lean NAFLD at baseline.

Of those who attended follow-up in 2014, 1362/2148 (63.4%) had NAFLD. Of these, 13.2% (284/2148) were lean NAFLD and 50.2% (1078/2148) were non-lean NAFLD. In 2014, the 2148 who presented for follow-up included 84 of those who had lean NAFLD in 2007, 620 who had non-lean NAFLD in 2007 and 834 who did not have features of NAFLD in 2007 (Fig. 1). Table 2 shows incident metabolic comorbidities of the three groups at follow-up after 7 years (in 2014). There were no significant differences in the occurrence of new onset metabolic comorbidities such as diabetes, hypertension and dyslipidemia, between lean and non-lean NAFLD. However, incident diabetes and low HDL were significantly higher in non-lean NAFLD compared to no NAFLD.

Of those who did not have NAFLD in 2007, 294 were lean in both 2007 and 2014, and had no or 'safe' alcohol consumption in the period of follow-up. 84 of them [male 36 (42.9%); mean age 59.4 (SD = 8.0) years] had developed NAFLD in 2014, giving an annual incidence of lean NAFLD of 4.1% (Fig. 1). The 210 lean individuals who did not develop NAFLD were considered as controls for regression analysis. On logistic regression, the presence of diabetes at baseline, an increase in weight from baseline to follow-up and an education above secondary level were independent risk factors for the development of new onset lean NAFLD (Table 3).

Genetic association with NAFLD was replicated for *PNPLA3* rs738409 among lean individuals (one-tailed $p < 0.05$, Table 4). The strength of association with lean NAFLD appeared to be attenuated at follow-up compared to baseline (odds ratio = 1.37 and 1.51 at follow-up and baseline, respectively), which is in accordance with our previous findings in the whole cohort sample [13]. Moreover, *PNPLA3* association was pronounced among lean individuals compared to the whole cohort sample [13]; OR = 1.51 (baseline) and 1.37 (follow-up) for lean individuals vs. OR = 1.48 (baseline) and 1.22 (follow-up) for the whole cohort sample. Although nominal ($p < 0.05$) association was observed for *LYPLAL1* rs12137855 (OR = 1.45, $p = 0.022$) and *NCAN* rs2228603 (OR = 0.57, $p = 0.049$) among 10 selected polymorphisms, careful interpretation

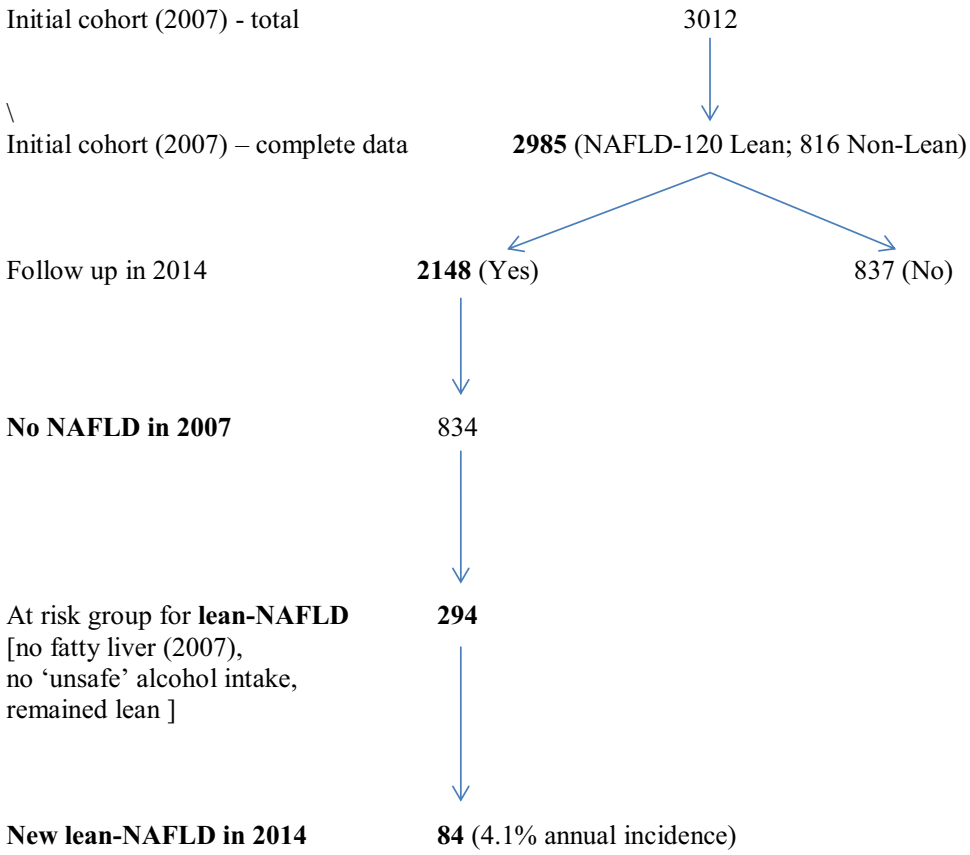

Fig. 1 Study population [at baseline (2007) and follow-up (2014)]

Table 1 Baseline characteristics of lean NAFLD, non-lean NAFLD and no NAFLD in 2007

	Lean NAFLD *n* = 120	Non-lean NAFLD *n* = 816	No NAFLD *n* = 1206	*p* value
Males	53 (44.2)[a]	269 (33.0)[b]	602 (49.9)[a]	< 0.001
Mean age (SD)	54.58 (6.4)[b]	52.70 (7.3)[a]	51.92 (8.2)[a]	< 0.001
Waist circumference				
>90 cm males	7 (13.2)[a]	208 (77.3)[b]	106 (17.6)[a]	< 0.001
>80 cm females	37 (55.2)[a]	531 (97.1)[b]	265 (44.0)[a]	< 0.001
Diabetes (known or newly diagnosed)	45 (37.5)[b]	266 (32.6)[b]	187 (15.5)[a]	< 0.001
Hypertension (known or newly diagnosed)	53 (44.2)[a]	486 (59.6)[b]	428 (35.5)[a]	< 0.001
Elevated TG or on treatment	59 (49.2)[b]	389 (47.7)[b]	318 (26.4)[a]	< 0.001
Low HDL or on treatment	28 (23.3)[a,b]	213 (26.1)[b]	227 (18.8)[a]	0.001

Numbers within brackets are percentages unless indicated otherwise

Different superscripts indicate significant difference between the columns in pairwise comparisons

is required without replication in an independent sample, considering the risk of multiple testing.

Discussion

In a cohort of aging adults living in an urban Sri Lankan community, the prevalence of lean NAFLD was 4% in 2007 and 13.2% in 2014. After 7 years of follow-up the annual incidence of lean NAFLD was 4.1%. Lean NAFLD was commoner among males, and had a lower prevalence of hypertension and central obesity than their non-lean NAFLD counterparts at baseline. Lean NAFLD had a similar risk to non-lean NAFLD for development of incident metabolic comorbidities. The presence of diabetes at baseline, an education above secondary level and an increase in weight from baseline were risk factors for the development of new onset lean NAFLD. To our knowledge, this is the first prospective,

Table 2 Occurrence of incident metabolic comorbidities in the three groups after 7-year follow-up (in 2014)

	Those with Lean NAFLD in 2007 *n* = 84	Those with non-Lean NAFLD in 2007 *n* = 620	Those with no NAFLD in 2007 *n* = 834	p value
Males	36 (42.9)[a,b]	200 (32.2)[b]	380 (45.6)[a]	< 0.001
Mean age (SD)	61.29 (6.3)[a]	59.4 (7.4)[b]	58.6 (8.0)[b]	< 0.01
Incident comorbidities				
Diabetes	13/54 (24.1)[a,b]	170/416 (40.9)[b]	143/675 (21.2)[a]	< 0.001
Hypertension	33/50 (66.0)[a]	173/260 (66.5)[a]	401/544 (73.7)[a]	0.079
Raised TG	14/17 (82.4)[a]	142/152 (93.4)[a]	188/206 (91.3)[a]	0.272
Low HDL	19/24 (79.2)[a,b]	184/203 (90.6)[b]	247/300 (82.3)[a]	0.024

Numbers within brackets are percentages unless indicated otherwise

Incident comorbidities: *p* values are based on Chi-square test followed by pairwise comparisons

Different superscripts indicate significant difference between the columns in pairwise comparisons

Table 3 Summary of logistic regression analysis of factors associated with incident lean NAFLD

Factor	OR	SE	*p* value	95% CI
Diabetes mellitus at baseline	5.40	0.42	< 0.001	2.39–12.18
Educational above secondary level	2.84	0.29	< 0.001	1.60–5.05
Increase in weight (< 5%)[a]	2.53	0.37	0.011	1.24–5.18
Increase in weight (5–10%)[a]	1.51	0.41	0.313	0.68–3.34
Increase in weight (> 10%)[a]	8.34	0.45	< 0.001	3.44–20.18

[a]Comparison with a group who had weight loss

OR odds ratio, *SE* standard error, *CI* confidence interval

community-based, cohort follow-up study that describes characteristics of lean NAFLD in a South-Asian population.

Previous studies on lean NAFLD have reported varying, and sometimes contradictory findings. Table 5 summarizes the studies on lean NAFLD in Western and Asian populations including the present study [6, 9, 10, 19–28]. Our reported prevalence of 13.2% (in 2014) for lean NAFLD seems to be compatible with previous studies. It is lower than the prevalence of 18.8% reported by Younossi et al. [6] and 15.2% reported by Nishioji et al. [28] but higher than 9.6% reported by Kim et al. [20] employing BMI < 25 kg/m^2 cut-off to define lean NAFLD. We found a lower prevalence of hypertension and central obesity among lean NAFLD than their non-lean NAFLD counterparts and this is comparable to the lower prevalence of metabolic syndrome (MetS) and components of MetS in lean NAFLD compared to overweight-NAFLD reported by Cruz et al. [9].

In the present study, there were no significant differences in the occurrence of incident metabolic comorbidities such as new-onset diabetes, hypertension and dyslipidemia, between lean and non-lean NAFLD after a 7-year follow-up. This highlights the need for stringent follow-up of all NAFLD patients for the development of new metabolic comorbidities, even in the absence of general obesity, and despite the lean habitus being a minority among NAFLD patients. This finding of the risk of future development of metabolic comorbidities among individuals with lean NAFLD has also been reported previously. Nishioji et al. described metabolic factors to be associated with NAFLD, even in non-obese Japanese [28]. Feng et al. reported lean NAFLD being more strongly associated with diabetes, hypertension and metabolic syndrome than overweight-obese-NAFLD in a Chinese population [27]. However, there have been previous reports of lean NAFLD being less strongly associated with MetS and its individual components. Younossi et al. reported that lean NAFLD is associated with a decreased likelihood of having insulin resistance and hypercholesterolemia [6]. Cruz et al. also reported lean NAFLD having less MetS, individual components of MetS and IR compared to overweight-NAFLD [9].

In our population, hypertension, general obesity and central obesity were less in the prevalent lean NAFLD group. This may suggest a stronger genetic predisposition for lean NAFLD than for non-lean NAFLD. However, diabetes predicted development of future lean NAFLD, and suggests an association between lean NAFLD and metabolic syndrome, and the need to develop NAFLD screening protocols for diabetic patients even in the absence of general obesity. We found that higher educational level was independently predictive of development of lean NAFLD. In previous published large population-based studies, a higher educational level was associated with lower BMI or leaner body habitus and most educated individuals displayed lower rates of obesity [29, 30]. The reason for the higher educated individuals with lean habitus to develop NAFLD compared to their lower educated lean counterparts in our study remains largely unexplained, but may be due to genetic predisposition.

PNPLA3 rs738409 showed a tendency of association with NAFLD among lean individuals (one-tailed $p < 0.05$);

Table 4 Genetic association with NAFLD among lean individuals (BMI < 23) at baseline and follow-up surveys

SNP	Gene symbol	Effect/other allele	At baseline survey (restricted to those attended follow-up)							At follow-up survey (including those not in the case–control sample at baseline)						
			EAF		Case ($n=84$)	Control ($n=461$)	OR	[95% CI]	p value	EAF		Case ($n=282$)	Control ($n=283$)	OR	[95% CI]	p value
			Case	Control	EE/EO/OO	EE/EO/OO				Case	Control	EE/EO/OO	EE/EO/OO			
rs12137855	*LYPLAL1*	C/T	0.827	0.794	55/29/0	290/152/19	1.20	[0.75–1.97]	0.4569	0.809	0.749	182/92/8	161/102/20	1.45	[1.06–1.99]	0.0223
rs780094	*GCKR*	T/C	0.175	0.191	2/25/56	15/146/300	0.98	[0.60–1.54]	0.9184	0.187	0.205	8/89/184	11/94/178	0.82	[0.59–1.15]	0.2468
rs4240624[a]	*PPP1R3B*	A/G	0.970	0.938	80/3/1	406/53/2	1.96	[0.85–5.68]	0.1553	0.945	0.949	252/29/1	255/27/1	0.88	[0.48–1.59]	0.6662
rs2854117	*APOC3*	T/C	0.500	0.543	23/38/23	131/239/91	0.83	[0.58–1.19]	0.3102	0.541	0.525	85/135/62	74/149/60	1.04	[0.80–1.36]	0.7441
rs2854116	*APOC3*	C/T	0.500	0.549	23/38/23	132/242/87	0.82	[0.57–1.18]	0.2841	0.543	0.537	85/136/61	77/150/56	1.01	[0.78–1.32]	0.9342
rs767870	*ADIPOR2*	A/G	0.857	0.823	61/22/1	316/127/18	1.50	[0.93–2.51]	0.1056	0.835	0.816	196/79/7	190/82/11	1.15	[0.81–1.62]	0.4361
rs6503695	*STAT3*	T/C	0.577	0.579	27/43/14	163/208/90	0.91	[0.64–1.31]	0.6229	0.589	0.574	95/142/45	97/131/55	1.00	[0.77–1.30]	0.9976
rs9891119	*STAT3*	A/C	0.482	0.459	20/41/23	100/223/138	1.02	[0.71–1.47]	0.9012	0.486	0.470	62/150/70	66/134/83	0.96	[0.73–1.24]	0.7409
rs2228603	*NCAN*	T/C	0.042	0.063	0/7/77	1/56/404	0.51	[0.20–1.13]	0.1220	0.050	0.072	0/28/254	1/39/243	0.57	[0.32–0.99]	0.0490
rs738409	*PNPLA3*	G/C	0.280	0.228	5/37/42	26/158/276	1.51	[0.99–2.31]	0.0539	0.270	0.223	19/114/149	15/96/172	1.37	[1.01–1.87]	0.0455

The effect alleles are defined as those that were reported to be positively associated with NAFLD risk, as we showed previously (Kastriratne et al. Liver Int. 2015). EAF, effect allele frequency

Genetic association was tested between lean NAFLD cases and lean non-NAFLD controls among lean individuals (BMI < 23 kg/m^2) at the timing of each survey

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. Nat Genet 2008); the A allele of rs4240624 showed increased risk of hepatic steatosis, although it was not positively associated with NAFLD risk (OR = 0.93)

Table 5 Lean NAFLD studies among Western and Asian populations

Study (year)	Population	Lean NAFLD (% of NAFLD)	Association	Comments
Western				
Younossi et al. (2012) [6]	Population based	2185/11,613 (18.8%)	Younger age, female gender, decreased likelihood of IR and dyslipidaemia[a]	BMI < 25 kg/m^2
Magariti et al. (2012) [19]	Hospital based (clinic patients)	19/162 (11.7%)	Higher ALT/AST[a]	BMI < 25 kg/m^2
Cruz et al. (2014) [9]	Hospital based	125/1090 (11.5%)	Male gender, non-Caucasian, less MetS, IR and components of MetS, shorter cumulative survival[a]	Multi-ethnic BMI < 25 kg/m^2
Feldman et al. (2017) [10]	Hospital based	55/116 (47.4%)	Impaired glucose tolerance, PNPLA3 CC/CG variants[b]	BMI < 25 kg/m^2
Asian				
Kim et al. (2004) [20]	Hospital based (healthy attendants)	74/180 (41.1%)	TG > 150 mg/dL Insulin resistance and central obesity[b]	Non-diabetic, BMI < 25 kg/m^2 Lean NAFLD Prevalence—9.6%
Singh et al. (2004) [21]	Hospital based (healthy attendants)	7/39 (17.9%)	–	BMI < 23 kg/m^2
Chen et al. (2006) [22]	Community-based		TG > 150 mg/dL[b]	BMI < 25 kg/m^2
Das et al. (2010) [23]	Community-based	88/164 (53.6%)	–	BMI < 23 kg/m^2 and WC < Asian cut-offs Lean NAFLD Prevalence—4.7%
Choudhary et al. (2012) [24]	Hospital based	6/21 (28.5%)	–	BMI < 23 kg/m^2
Bhat et al. (2013) [25]	Hospital based (clinic patients)	23/150 (15.3%)	80% had IR	Non-diabetic; BMI < 23 kg/m^2 and WC < Asian cut-offs
Singh et al. (2013) [26]	Hospital based	101/632 (15.9%)	–	BMI < 23 kg/m^2
Feng et al. (2014) [27]	Hospital based (healthy attendants)	134/898 (14.9%)	MetS[a]	BMI < 24 kg/m^2
Nishioji et al. (2015) [28]	Hospital based (healthy attendants)	409/805 (50.8%)	> 10 kg weight gain since age 20, increased TG and WC[a]	BMI < 25 kg/m^2 Lean NAFLD prevalence—15.2%

[a]Compared to obese NAFLD

[b]Compared to no NAFLD controls

interestingly, the strength of association was pronounced among lean individuals compared to the whole cohort sample, indicating the possibility of stronger genetic predisposition for NAFLD among lean individuals compared to non-lean (or obese) individuals. This is in accordance with the observation that the strength of association with lean NAFLD was attenuated at follow-up compared to baseline. A similar population-based study from Hong Kong demonstrated that a greater proportion of patients with non-obese NAFLD carried the variant *PNPLA3* allele than those with obese NAFLD (78.4% vs. 59.8%) and the *PNPLA3* polymorphism remained an independent risk factor for non-obese NAFLD [31].

The main strengths of our study are that it is a prospective, community-based, cohort follow-up study with a relatively large baseline population presenting for re-evaluation. Furthermore, NAFLD was diagnosed on stringent ultrasound criteria, and not on surrogate markers such as hepatic transaminases. It is the only Asian study to report the incidence of lean NAFLD in an at risk lean population. There are, however, several limitations. Inter-observer reliability between sonographers was not formally assessed before the study commenced, and information on alcohol consumption was based on self-reporting; this may have led to under-reporting with consequent overestimation of the prevalence and incidence of NAFLD. We could not account

for the possible changes and fluctuations in the BMI value of the lean individuals with NAFLD (lean NAFLD) in between the period from baseline and follow-up.

In conclusion, although lean NAFLD constitutes a small proportion of NAFLD, its impact on public health is not negligible given the high prevalence of cryptogenic CLD in Sri Lanka. The risk of developing incident metabolic comorbidities seems to be similar to non-lean NAFLD. Individuals with lean NAFLD, therefore, also warrant careful evaluation and follow-up.

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Author contributions MAN, NK, AK and HJdeS conceptualized and designed the study. AK, SdeS, MAN, ASD, APDeS, ARW and HJdeS were involved in the establishment of the Ragama Health Study cohort and its follow-up. KRP, SKCES, SKK, TACLP and KV collected data. TF and NK performed genotyping. TF, NK, AK analyzed the data assisted by MAN, AP and HJdeS. MAN, AK, NK and HJdeS prepared the manuscript. APDeS, ASD and ARW were substantially involved in revision of the manuscript. All authors checked the final manuscript before submission.

Compliance with ethical standards

Conflict of interest Madunil Anuk Niriella, A. Kasturiratne, A. Pathmeswaran, S. T. De Silva, K. R. Perera, S. K. C. E. Subasinghe, S. K. Kodisinghe, T. A. C. L. Piyaratna, K. Vithiya, A. S. Dassanayaka, A. P. De Silva, A. R. Wickramasinghe, F. Takeuchi, N. Kato and H. J. de Silva declare no conflict of interest.

Ethical approval Ethical approval for the study was obtained from the Ethical Review Committees of the Faculty of Medicine, University of Kelaniya and NCGM.

Informed consent Informed consent was obtained from all participants in RHS.

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