ONLINE-ONLY SUPPLEMENTARY MATERIAL

Supplementary Table 1. Clinical and biochemical characteristics of children/adolescents with biopsy-proven NAFLD, stratified by sex.

	Girls (*n*=301)	**Boys (*n*=298)**	***P* value**
Age (years)	11.5 ± 2.9	12.0 ± 3.2	0.12
Weight (kg)	60.6 ± 20.7	65.8 ± 22.8	<0.05
BMI (kg/m^2)	27.1 ± 5.6	27.3 ± 5.2	0.67
Waist circumference (cm)	84.9 ± 13.4	86.1 ± 13.7	0.31
Systolic blood pressure (mmHg)	112 ± 13.1	111 ± 12	0.55
Diastolic blood pressure (mmHg)	65 ± 10	64 ± 10	0.14
Fasting glucose (mg/dL)	82 ± 11	83 ± 11	0.32
2-hour OGTT glucose (mg/dL), *n*=499	110 ± 25	110 ± 23	0.85
HbA1c (mmol/mol), *n*=345	33 ± 5	34 ± 5	0.09
Fasting insulin (mIU/L)	16.6 ± 10.5	17.3 ± 11.1	0.52
2-hour OGTT insulin (mIU/L), *n*=499	100.4 ± 84.7	111.4 ± 91.9	0.17
HOMA-insulin resistance score	3.4 ± 2.3	3.6 ± 2.3	0.33
LDL cholesterol (mg/dL)	102 ± 28	96 ± 29	<0.05
HDL cholesterol (mg/dL)	47 ± 13	46 ± 12	0.28
Triglycerides (mg/dL)	110 ± 66	108 ± 62	0.79
AST (IU/L)	38 ± 22	37 ± 24	0.53
ALT (IU/L)	53 ± 49	52 ± 50	0.94
GGT (IU/L)	24 ± 18	23 ± 15	0.38
Glucose tolerance status			0.65
Normal glucose tolerance, % (n)	80.7 (243)	77.8 (232)	
Prediabetes, % (n)	18.6 (56)	21.1 (63)	
Diabetes, % (n)	0.7 (2)	1.1 (3)	
PNPLA3 rs738409 variant, *n*=328	*n*=167	*n*=161	0.81
CC genotype, % (n)	29.3 (49)	32.3 (52)	
GC genotype, % (n)	43.1 (72)	42.9 (69)	
GG genotype, % (n)	27.6 (46)	24.8 (40)	

Simple size, n=599 unless where indicated. Data are expressed as means±SD or relative percentages (absolute percentages are reported in parenthesis). Differences between the two groups were tested by Fisher's exact test for categorical variables, the unpaired Student's t-test for normally distributed continuous variables, and the Mann-Whitney U test for non-normally distributed continuous variables (i.e., fasting insulin, 2-hour OGTT insulin, HOMA-IR score, triglycerides and liver enzymes).

Abbreviations: ALT, alanine aminotransferase, AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1c; HOMA, homeostasis model assessment; OGTT, oral glucose tolerance test; PNPLA3, patatin-like phospholipase domain-containing protein 3.

Supplementary Table 2. Liver histology characteristics of children/adolescents with biopsy-proven NAFLD, stratified by sex.

	Girls (*n*=301)	**Boys (*n*=298)**	***P* value**
Steatosis grade, % (n)			0.37
<5%	8.6 (26)	13.1 (39)	
5% - 33%	31.5 (95)	31.2 (93)	
33% - 66%	37.2 (112)	34.6 (103)	
>66%	22.7 (68)	21.1 (63)	
Lobular inflammation, % (n)			0.65
<2 foci per 200 x field	35.2 (106)	32.6 (97)	
2-4 foci per 200 x field	48.8 (147)	52.6 (157)	
>4 foci per 200 x field	16.0 (48)	14.8 (44)	
Ballooning, % (n)			0.41
None	38.5 (116)	42.6 (127)	
Few	40.9 (123)	40.6 (121)	
Many	20.6 (62)	16.8 (50)	
Fibrosis stage, % (n)			0.39
No fibrosis	45.2 (136)	49.7 (148)	
Perisinusoidal fibrosis	47.2 (142)	43.3 (129)	
Periportal fibrosis	6.3 (19)	6.7 (20)	
Bridging fibrosis	1.3 (4)	0.3 (1)	
Definite NASH, % (n)	34.6 (104)	32.9 (98)	0.47
Borderline NASH, % (n)	26.9 (81)	23.8 (71)	
No-NASH, % (n)	38.5 (116)	43.3 (129)	

Simple size, n=599. Data are expressed as relative percentages (absolute percentages are reported in parenthesis). Differences between the groups were tested by the Fisher's exact test.

Supplementary Table 3. Clinical and biochemical characteristics of children/adolescents with biopsy-proven NAFLD, stratified by presence or absence of NASH.

	No-NASH/borderline NASH (*n*=397)	**NASH (*n*=202)**	***P* value**
Age (years)	11 ± 3.0	13 ± 3.0	<0.001
Sex (male), % (n)	50.4 (200)	48.5 (98)	0.36
Weight (kg)	55.9 ± 16	77.6 ± 24	<0.001
BMI (kg/m^2)	25.3 ± 3.6	31.0 ± 6.3	<0.001
Waist circumference (cm)	81.3 ± 12	93.8 ± 12	<0.001
Systolic blood pressure (mmHg)	110 ± 12	116 ± 13	<0.001
Diastolic blood pressure (mmHg)	63 ± 10	68 ± 10	<0.001
Fasting glucose (mg/dL)	82 ± 11	84 ± 10	0.18
2-hour OGTT glucose (mg/dL), *n*=499	109 ± 24	111 ± 23	0.47
HbA1c (mmol/mol), *n*=345	32 ± 5	35 ± 5	<0.001
Fasting insulin (mIU/L)	15.1 ± 8.6	20.5 ± 13.6	<0.001
2-hour OGTT insulin (mIU/L), *n*=499	98.6 ± 77	119.0 ± 105	<0.05
HOMA-insulin resistance score	3.1 ± 1.9	4.2 ± 2.9	<0.001
LDL cholesterol (mg/dL)	99 ± 30	100 ± 28	0.53
HDL cholesterol (mg/dL)	47 ± 13	44 ± 12	<0.01
Triglycerides (mg/dL)	100 ± 63	127 ± 64	<0.001
AST (IU/L)	37 ± 20	39 ± 27	0.17
ALT (IU/L)	47 ± 40	61 ± 58	<0.005
GGT (IU/L)	21 ± 15	27 ± 18	<0.001
Glucose tolerance status			<0.001
Normal glucose tolerance, % (n)	83.9 (333)	70.3 (142)	
Prediabetes, % (n)	15.4 (61)	28.7 (58)	
Diabetes, % (n)	0.7 (3)	1.0 (2)	
PNPLA3 rs738409 variant, *n*=328	*n*=179	*n*=149	<0.001
CC genotype, % (n)	42.5 (76)	16.8 (25)	
GC genotype, % (n)	43.0 (77)	42.9 (64)	
GG genotype, % (n)	14.5 (26)	40.3 (60)	

Simple size, n=599 unless where indicated. Data are expressed as means±SD or relative percentages (absolute percentages are reported in parenthesis). Differences between the two groups of children were tested by Fisher's exact test for categorical variables, the unpaired Student's t-test for normally distributed continuous variables, and the Mann-Whitney U test for non-normally distributed continuous variables (i.e., fasting insulin, 2-hour OGTT insulin, HOMA-IR score, triglycerides and liver enzymes).

Abbreviations: ALT, alanine aminotransferase, AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; HbA1c, hemoglobin A1c; HOMA, homeostasis model assessment; OGTT, oral glucose tolerance test; PNPLA3, patatin-like phospholipase domain-containing protein 3.

Supplementary Table 4. Association between prediabetes/diabetes status and risk of either borderline NASH or definite NASH in children/adolescents with biopsy-proven NAFLD.

	No-NASH (*n*=245)	**Borderline NASH (*n*=152)**			**Definite NASH (*n*=202)**		
		Odds Ratio(s)	**95% CI**	***P* value**	**Odds Ratio(s)**	**95% CI**	***P* value**
Unadjusted model							
Normal glucose tolerance	*Ref.*	*Ref*.	*Ref*.	-	*Ref*.	*Ref*.	-
Prediabetes/diabetes status (yes *vs.* no)	*Ref.*	0.96	0.56 – 1.67	0.89	2.16	1.37 – 3.41	0.001
Adjusted model 1							
Normal glucose tolerance	*Ref.*	*Ref*.	*Ref*.	-	*Ref*.	*Ref*.	-
Prediabetes/diabetes status (yes *vs.* no)	*Ref.*	0.92	0.52 – 1.61	0.76	1.65	0.99 – 2.74	0.054
Age (years)	*Ref.*	1.05	0.97 – 1.13	0.19	1.12	1.03 – 1.22	0.01
Sex (male *vs.* female)	*Ref.*	0.74	0.49 – 1.12	0.15	0.67	0.43 – 1.03	0.07
Waist circumference (cm)	*Ref.*	1.02	0.99 – 1.04	0.06	1.09	1.07 – 1.12	<0.001
Adjusted model 2, *n*=328							
Normal glucose tolerance	*Ref.*	*Ref.*	*Ref.*	-	*Ref*.	*Ref*.	-
Prediabetes/diabetes status (yes *vs*. no)	*Ref.*	1.06	0.43 – 2.62	0.88	2.32	0.96 – 5.61	0.059
Age (years)	*Ref.*	1.12	0.99 – 1.26	0.07	1.11	0.97 – 1.26	0.12
Sex (male *vs*. female)	*Ref.*	0.78	0.41 – 1.51	0.47	0.76	0.38 – 1.52	0.44
Waist circumference (cm)	*Ref.*	1.01	0.98 – 1.04	0.59	1.11	1.07 – 1.15	<0.001
PNPLA3 rs738409 variant	*Ref.*						
CC genotype (%)	*Ref.*	*Ref.*	*Ref.*	-	*Ref*.	*Ref*.	-
GC genotype (%)	*Ref.*	5.09	2.52 – 10.3	<0.001	6.8	3.15 – 14.6	<0.001
GG genotype (%)	*Ref.*	34.8	7.43 – 163	<0.001	131.2	26.7 – 644	<0.001

Sample size, *n*=599 unless where indicated. Data are expressed as odds ratio and 95% confidence intervals (CI) as tested by logistic regression analysis. The dependent variable for all multinomial logistic regression models was the presence of histologic NASH, categorized as follows: no-NASH (the reference group), borderline NASH (group 1) and definite NASH (group 2). Presence of prediabetes and diabetes were combined into a single category, because the number of children with diabetes was small (n=5).

Abbreviations: PNPLA3, patatin-like phospholipase domain-containing protein 3; Ref., reference category.

Supplementary Table 5. Association between prediabetes status and risk of histologic NASH in children/adolescents with biopsy-proven NAFLD (*after excluding children with diabetes*).

	Odds Ratio(s)	**95% Confidence Interval(s)**	***P* value**
Unadjusted model			
Normal glucose tolerance	*Ref*.	*Ref*.	
Prediabetes status (yes *vs.* no)	2.23	1.48 – 3.36	<0.001
Adjusted model 1			
Normal glucose tolerance	*Ref*.	*Ref*.	
Prediabetes status (yes *vs.* no)	1.70	1.06 – 2.73	0.027
Age (years)	1.09	1.01 - 1.18	0.029
Sex (male *vs.* female)	0.76	0.51 – 1.12	0.17
Waist circumference (cm)	1.09	1.06 – 1.11	<0.001
Adjusted model 2, *n*=327			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	2.33	1.17 – 4.61	0.015
Age (years)	1.03	0.94 – 1.15	0.46
Sex (male *vs*. female)	0.90	0.52 – 1.54	0.69
Waist circumference (cm)	1.10	1.07 – 1.13	<0.001
PNPLA3 rs738409 variant			
CC genotype (%)	*Ref.*	*Ref.*	
GC genotype (%)	2.94	1.54 – 5.63	0.001
GG genotype (%)	12.3	5.52 – 27.3	<0.001

Sample size, *n*=594 unless where indicated. Data are expressed as odds ratio and 95% confidence intervals as tested by logistic regression analysis. The dependent variable for all logistic regression models was the presence of definite NASH on histology (*i.e*., definite NASH vs. no-NASH/borderline NASH considered together).

Abbreviations: PNPLA3, patatin-like phospholipase domain-containing protein 3; Ref., reference category.

Supplementary Table 6. Association between fasting plasma glucose concentrations and risk of NASH in children/adolescents with biopsy-proven NAFLD.

	Odds Ratio(s)	**95% Confidence Interval(s)**	***P* value**
Unadjusted model			
Fasting glucose (mg/dL)	1.01	0.99 – 1.03	0.10
Adjusted model 1			
Fasting glucose (mg/dL)	1.00	0.98 – 1.02	0.93
Age (years)	1.10	1.02 - 1.19	0.011
Sex (male *vs.* female)	0.76	0.52 – 1.12	0.17
Waist circumference (cm)	1.09	1.06 – 1.11	<0.001
Adjusted model 2, *n*=328			
Fasting glucose (mg/dL)	1.01	0.98 – 1.03	0.64
Age (years)	1.04	0.94 – 1.15	0.45
Sex (male *vs*. female)	0.86	0.51 – 1.48	0.60
Waist circumference (cm)	1.09	1.07 – 1.13	<0.001
PNPLA3 rs738409 variant			
CC genotype (%)	*Ref.*	*Ref.*	
GC genotype (%)	2.79	1.47 – 5.28	<0.005
GG genotype (%)	11.1	5.10 – 24.1	<0.001

Sample size, *n*=599, unless where indicated. Data are expressed as odds ratio and 95% confidence intervals as tested by logistic regression analysis. The dependent variable for all logistic regression models was the presence of definite NASH on histology (i.e., definite NASH vs. no-NASH/borderline NASH considered together). Presence of prediabetes and diabetes were combined into a single category, because the number of children with diabetes was small (n=5).

Abbreviations: PNPLA3, patatin-like phospholipase domain-containing protein 3; Ref., reference category.

Supplementary Table 7. Association between 2-hour post-load glucose concentrations and risk of NASH in children/adolescents with biopsy-proven NAFLD.

	Odds Ratio(s)	**95% Confidence Interval(s)**	***P* value**
Unadjusted model			
2-hour OGTT glucose (mg/dL)	1.00	0.99 – 1.01	0.48
Adjusted model 1			
2-hour OGTT glucose (mg/dL)	1.00	0.99 – 1.01	0.34
Age (years)	1.12	1.02 –1.21	0.01
Sex (male *vs.* female)	0.72	0.48 – 1.09	0.13
Waist circumference (cm)	1.08	1.06 – 1.11	<0.001
Adjusted model 2, *n*=281			
2-hour OGTT glucose (mg/dL)	0.99	0.98 – 1.01	0.89
Age (years)	1.02	0.91 – 1.14	0.72
Sex (male *vs*. female)	0.76	0.43 – 1.37	0.37
Waist circumference (cm)	1.11	1.08 – 1.15	<0.001
PNPLA3 rs738409 variant			
CC genotype (%)	*Ref.*	*Ref.*	
GC genotype (%)	2.74	1.37 – 5.49	<0.005
GG genotype (%)	11.6	4.89 – 27.3	<0.001

Sample size, *n*=499, unless where indicated. Data are expressed as odds ratio and 95% confidence intervals as tested by logistic regression analysis. The dependent variable for all logistic regression models was the presence of definite NASH on histology (i.e., definite NASH vs. no-NASH/borderline NASH considered together). Presence of prediabetes and diabetes were combined into a single category, because the number of children with diabetes was small (n=5).

Abbreviations: OGTT, oral glucose tolerance test; PNPLA3, patatin-like phospholipase domain-containing protein 3; Ref., reference category.

Supplementary Table 8. Associations between prediabetes status and risk of different histological features of NAFLD in children/adolescents with biopsy-proven NAFLD (*after excluding children with established diabetes*).

	Odds Ratio(s)	**95% Confidence Interval(s)**	***P* value**
Severe hepatic steatosis			
Unadjusted model			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	2.13	1.37 – 3.33	0.001
Adjusted model 1			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	1.73	1.07 – 2.77	0.025
Age (years)	0.98	0.90 – 1.06	0.60
Sex (male *vs.* female)	0.84	0.56 – 1.27	0.40
Waist circumference (cm)	1.06	1.04 – 1.08	<0.001
Severe lobular inflammation			
Unadjusted model			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	2.19	1.33 – 3.59	0.002
Adjusted model 1			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	1.58	0.92 – 2.71	0.098
Age (years)	1.12	1.02 – 1.23	0.023
Sex (male *vs.* female)	0.78	0.48 – 1.26	0.31
Waist circumference (cm)	1.06	1.03 – 1.08	<0.001
Hepatic ballooning			
Unadjusted model			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	1.61	1.00 – 2.60	0.049
Adjusted model 1			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	1.22	0.73 – 2.04	0.45
Age (years)	1.03	0.94 –1.12	0.52
Sex (male *vs.* female)	0.69	0.45 – 1.07	0.10
Waist circumference (cm)	1.06	1.04 – 1.08	<0.001
Significant fibrosis (i.e., periportal or bridging fibrosis)			
Unadjusted model			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	1.36	0.67 – 2.79	0.39
Adjusted model 1			
Normal glucose tolerance	*Ref.*	*Ref.*	
Prediabetes status (yes *vs.* no)	1.09	0.51 – 2.32	0.82
Age (years)	0.89	0.78 – 1.02	0.10
Sex (male *vs.* female)	0.89	0.48 – 1.67	0.72
Waist circumference (cm)	1.06	1.03 – 1.09	<0.001

Sample size, *n*=594. Data are expressed as odds ratio and 95% confidence intervals as tested by logistic regression analysis.

Abbreviations: Ref., reference category.